Feasibility of Using Mycoherbicides for Controlling Illicit Drug Crops

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Board on Agriculture and Natural Resources

Board on Environmental Studies and Toxicology

Division on Earth and Life Studies

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[1]This study was planned, overseen, and supported by the Board on Environmental Studies and Toxicology.

Preface

There is long-standing interest in developing mycoherbicides as a means of combating the production of illicit drug crops. However, efforts to develop and test mycoherbicides for that purpose have been limited by the need for special permission and requirements for growing illicit crops experimentally and for testing biological agents. Proposals to conduct field tests of mycoherbicides on drug crops in the United States and abroad have also been rejected because of scientific and political concerns. Scientific issues include concerns about the efficacy of the mycoherbicides; risks to nontarget plants, organisms, animals, and humans; and ecological effects. To address such scientific questions, Congress directed the Office of National Drug Control Policy to commission a study of the feasibility of developing mycoherbicides against illicit drug crops (PL 109-469, Sec. 1111). The agency commissioned the National Research Council to perform the study.

The National Research Council convened the Committee on Mycoherbicides for Eradicating Illicit Drug Crops, which prepared this report. The members of the committee were selected for their expertise in plant pathology; mycotoxins; fungal genetics, evolution, and ecology; mycoherbicide development, evaluation, formulation, and application; plant-disease epidemiology; soil microbiology; medical mycology; human toxicology and risk assessment; and ecological risk assessment (see Appendix A for biographic information on the members).

To help the committee in its review, public meetings were held April 20, June 23, and September 15, 2010, to gather information from relevant federal government agencies; government, academic, and industry researchers; and the general public. The committee is grateful to those who gave presentations on topics relevant to the committee's task, including Terry Zobeck, Office of National Drug Control Policy; Jay Ellenberger and Christopher Wosniak, U.S. Environmental Protection Agency; John Bargeron and Scott Harris, U.S. Department of State; Henry Appleton and Bryan Bailey, U.S. Department of Agriculture; Mark Wach, Sylvan Biosciences; David Sands, Montana State University; Christopher Eusebi, Harness, Dickey & Pierce, PLC; and Brent Nowak, University of Texas at San Antonio.

This report has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of the independent review is to provide candid and critical comments that will assist the institution in making its published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We thank the following for their review of the report: Basil Acock, Acock Info, LLP; Mary Acock, Acock Info, LLP; Karen Bailey, Agriculture and Agri-Food Canada; Michael Braverman, Rutgers University; Davie Geiser, Pennsylvania State University; Joseph Heitman, Duke University; Walter Marasas, PROMEC Unit of the South African Medical Research Council (retired); Terry Medley, E I du Pont de Nemours & Company; Louise Morin, CSIRO Entomology; and Jan Carel Zadoks, Agricultural University at Wageningen (retired).

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of the report was overseen by Laurence Madden, Ohio State University, and May Berenbaum, University of Illinois. Appointed by the National Research Council, they were responsible for making certain that an independent examination of the report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of the report rests entirely with the author committee and the institution.

The committee is grateful for the assistance of National Research Council staff in preparing the report. It particularly acknowledges the support of Susan Martel, Camilla Ables, and Janet Mulligan, who coordinated the project and contributed to the committee's report. Other staff members who contributed to this effort are Robin Schoen, director of the Board on Agriculture and Natural Resources; Tamara Dawson, program associate; and Norman Grossblatt, senior editor.

Finally, I thank all the members of the committee for their efforts throughout the development of this report.

Raghavan Charudattan, PhD, *Chair*
Committee on Mycoherbicides for
Eradicating Illicit Drug Crops

Abbreviations and Acronyms

APHIS	Animal and Plant Inspection Service
ARS	Agricultural Research Service
ATCC	American Type Culture Collection
BWC	Biological Weapons Convention
CBN	Central Bureau of Narcotics (India)
CFR	Code of Federal Regulations
CFU	colony-forming units
DAA	days after application
DAI	days after inoculation
DEA	Drug Enforcement Agency
ENACO	Empresa Nacional de Coca
EPA	U.S. Environmental Protection Agency
EIS	environmental impact statement
ESA	Endangered Species Act
EU	European Union
FFDCA	Federal Food, Drug, and Cosmetic Act
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOC	*Fusarium oxysporum* complex
f. sp.	*forma specialis*
f. spp.	*formae speciales*
IOBC	International Organization for Biological Control of Noxious Animals and Plants
IPPC	International Plant Protection Convention
ISPM	International Standards for Phytosanitary Measures
NEPA	National Environmental Policy Act
NPPO	National Plant Protection Office
ONDCP	Office of National Drug Control Policy
RAPD	random amplified polymorphic DNA
THC	delta-9-tetrahydrocannabinol
UNDCP	UN International Drug Control Programme
UNODC	UN Office of Drugs and Crime
USDA	U.S. Department of Agriculture
VCG	vegetative compatibility group

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Summary

The control of illicit-drug trafficking and drug use is a difficult and complex process that involves a variety of prevention, control, treatment, and law-enforcement strategies. Eradication strategies for controlling illicit-drug crops are used to target the beginning of the drug-supply chain by preventing or reducing crop yields. Mycoherbicides have been proposed as an eradication tool to supplement the current methods of herbicide spraying, mechanical removal, and manual destruction of illicit-drug crops. Mycoherbicides are developed from plant pathogenic fungi that occur naturally in the environment. Some people regard them as preferable to chemical herbicides for controlling illicit-drug crops because of their purported specificity to only one plant species or a few closely related species. As living microorganisms, they have the potential to provide long-term control if they can persist in the environment and affect later plantings. Research on mycoherbicides against illicit-drug crops has focused on three pathogens: *Fusarium oxysporum* f.sp. *cannabis* for cannabis (*Cannabis sativa*), *F. oxysporum* f.sp. *erythroxyli* for coca (*Erythroxylum coca* and *E. novogranatense*), and *Crivellia papaveracea* or *Brachycladium papaveris* (formerly known as *Pleospora papaveracea* and *Dendryphion penicillatum*, respectively) for opium poppy (*Papaver somniferum*).

In response to a congressional mandate (Public Law 109-469), the White House Office of National Drug Control Policy (ONDCP) requested that the National Research Council form an expert committee to examine the scientific issues associated with the feasibility of developing and implementing naturally occurring strains of the mycoherbicide fungi as a means of eradicating illicit cannabis, coca, and opium poppy crops. The study was also to evaluate the potential human health, ecological, and environmental risks associated with the use of these mycoherbicides and to identify future research and development needed to support their use. The committee was charged with addressing the following issues about the potential use of the proposed mycoherbicides: their effectiveness in eradicating their target plants; the feasibility of their large-scale industrial manufacture and delivery; their potential spread and persistence in the environment; their pathogenicity and toxicity to nontarget organisms, including other plants, fungi, animals, and humans; their potential for mutation and resulting

effects on target plants and nontarget organisms; and research and development needs.

To address its task, the committee reviewed publications and other publicly available information on the three proposed mycoherbicides and relevant publications on related fungi and other mycoherbicides that have been developed against undesirable plant species (weeds). The publications were identified through literature searches and by consulting the ONDCP, the U.S. Department of State, the U.S. Department of Agriculture, and the UN Office on Drugs and Crime. The available studies on the proposed mycoherbicides were few, were not all peer-reviewed, and were primarily greenhouse, growth-chamber, and small field studies conducted under controlled conditions. Those limitations made it difficult for the committee to draw conclusions or to make predictions about the performance of the proposed mycoherbicides in larger field settings and under natural conditions.

On the basis of its review, the committee concluded that the available data are insufficient to determine the effectiveness of the specific fungi proposed as mycoherbicides to combat illicit-drug crops or to determine their potential effects on nontarget plants, microorganisms, animals, humans, or the environment. The questions normally asked before a fungal pathogen is registered as a mycoherbicide in the United States have not been adequately addressed. The committee offers the following assessment of what can and cannot be determined at the present time regarding each of the issues raised in the statement of task.

EFFICACY

The degree of control that might be provided by the proposed mycoherbicides, the mechanisms by which they cause disease, and how control of the target plants could be maximized have not been established. Although each of the proposed mycoherbicides has been shown to cause disease in its target plant, disease severity was inconsistent and depended on biotic factors (such as age of plants and strain of fungus) and abiotic factors (such as moisture level, temperature, and ultraviolet radiation). For example, the cannabis mycoherbicide caused plant death in one study but low to moderate disease severity in a second study. For the coca mycoherbicide, published mortality ranged from 35% to 94%, but the background incidence of disease and background mortality in noninoculated plants also were high, sometimes approximating those observed in inoculated plants. Some varieties of cannabis were found to be resistant to the mycoherbicide and exhibited no effects or less severe disease.

In studies of the opium poppy mycoherbicide, a range (6-100%) of leaf necrosis in greenhouse and growth-chamber experiments was reported. Disease severity depended on the type of inoculum used (sexual or asexual spores), the age or growth stage of the plants, and environmental conditions, particularly the dew period and temperature after inoculum application. Plants in early development stages were killed or suffered foliar damage; at more mature stages,

poppy capsule number and size were reduced, and the poppy seeds had lower viability.

The types of diseases observed to be produced by the proposed mycoherbicides in their target plants are wilts in cannabis and coca and blight of the aerial parts of opium poppy. However, the mechanisms underlying the host-pathogen interactions and secondary spread of disease, which are critical determinants of mycoherbicide efficacy, have yet to be documented.

INOCULUM PRODUCTION AND DELIVERY

Large-scale production of the proposed mycoherbicides appears to be feasible, although available fermentation capacity might not suffice to combat illicit-drug crops on a global scale. The production process could be adapted from technology developed to produce microbial biomass for pharmaceutical, food, biotechnology, and other commercial uses. Large-scale production of mycoherbicides for commercial use is typically undertaken by industries that have microbial fermentation capabilities. The process involves the production of large amounts of fungal biomass by liquid fermentation, solid-substrate fermentation, or a combination of the two and has been used to produce commercial quantities of mycoherbicides.

It is difficult to estimate the quantity of mycoherbicide needed to control cannabis, coca, or opium poppy crops. Rough estimates based on the few available studies suggest that tens to hundreds of kilograms of dry formulation per hectare would be required for a single application of the cannabis and coca mycoherbicides. More accurate estimates require tests of the finished mycoherbicides under conditions that simulate field operations. Producing the amount of mycoherbicides required for global control efforts may or may not be feasible in light of cost or technical limitations; no mycoherbicide has ever been produced on such a massive scale. Studies of disease of opium poppy have used liquid spray formulations consisting of spores of the mycoherbicide fungus suspended in water or water amended with a surfactant or a vegetable oil. At the rates reported in publications, hundreds to thousands of liters of liquid-spray formulation (containing billions to trillions of spores) per hectare would be required for a single application of the opium poppy mycoherbicide.

The methods for delivering the proposed mycoherbicides to target sites would affect their performance in the field**.** The cannabis and coca mycoherbicides are soilborne and root-infecting and would have to be applied to the soil on or near plant roots for greatest efficacy. Several dry formulations (such as pellets) have been developed for this type of application. The opium poppy mycoherbicide, in contrast, would attack primarily aerial parts of the plant, so application of a liquid formulation to foliage would provide the greatest efficacy. For all three mycoherbicides, on-ground application would allow the most precise and uniform application. However, ground applications are unfeasible because of uncooperative and possibly hostile growers, who are likely to try to prevent

application of the mycoherbicides. Aerial application of mycoherbicides from airplanes as dry formulations on cannabis and coca fields could reduce their efficacy because the formulations would be subject to scattering by wind, which would lead to nonuniform, discontinuous placement of the inoculum over the target area and reduce the size of the plant-pathogen interface. A similar limitation applies to aerial application of liquid mycoherbicide formulations on opium poppy. But an even more important limiting factor is the availability of water needed for the liquid formulation and the ability to transport and apply the required quantities to the target area.

PERSISTENCE

Another issue is how long can the mycoherbicide fungi persist in the soil after application. It is important to determine whether the population density of the mycoherbicide fungi would remain high enough and for a long enough period to infect the target crops and whether they could survive in the soil and organic matter at levels necessary to affect later plantings of the crop. Another consideration is whether the mycoherbicide strains would pose any additional risks to nontarget organisms after release, in which case the prolonged persistence of these strains would be a disadvantage rather than an advantage.

Only a few studies on the long-term survival of the proposed mycoherbicides against coca and opium poppy are available, and essentially no data on the proposed cannabis mycoherbicide are available. In the available studies, the coca mycoherbicide strain survived for up to 7 months after application, and the opium poppy mycoherbicide strain survived in treated fields for two growing seasons. Those fungi are indigenous where their host plants are grown and have been linked to periodic, natural epidemics, so at least the fungal strains related to the mycoherbicide strains survive for a long time in the presence of their hosts.

Survival of the mycoherbicide fungi would also depend on the environmental conditions. Moisture (from dew, high relative humidity, or rainfall) for several hours and over several days is usually required with favorable temperatures for the fungi to become established on the target plants. In the case of the specific fungal strains studied (such as *F. oxysporum* f.sp. *erythroxyli)*, data on moisture, temperature, and other requirements for disease development and survival in soil are based on results with one or a few strains collected from relatively small areas. There is no reason to expect these strains to be adapted to any environment other than the one from which they were recovered; therefore, the strains may not be capable of attacking coca throughout its entire range. But the data are also not sufficient to conclude that strains of the mycoherbicide fungi or the diseases that they cause *cannot* occur throughout the entire geographic and climatic range where the target drug crops are grown.

The mycoherbicide strains might be able to survive on plants other than their target plants or as saprophytes on decaying organic material. Thus, it is

likely that the mycoherbicide strains would persist at some level as part of the indigenous pathogen population once they are introduced in large numbers into the environment. The pathogens also might be spread from the site of application by wind, water, insect or animal carriers, or infected seeds, soil, or plant material. Conditions that might reduce survival of the mycoherbicides include lack of adequate moisture, extreme temperatures (too high or too low), competition or suppression by soil microorganisms, and other biotic factors.

Once the mycoherbicides are applied, their persistence might be shortened through the intentional application of chemicals, such as fungicides or soil fumigants, by growers. Such a control strategy would be most effective for containing mycoherbicides in small areas but would be impractical or impossible for large areas. The chemicals also could affect indigenous microbial communities. There is some evidence that high population densities of the mycoherbicides could be maintained for several months, but the data do not support the hypothesis that the mycoherbicide strains can persist indefinitely at higher population densities than those of indigenous strains of the same fungi.

EFFECTS ON NONTARGET PLANTS AND ORGANISMS

Because of the complexity of native and agricultural ecosystems, it is difficult to predict or quantify the risks to nontarget plants and organisms accurately. Other fungi, soil organisms, plants, animals, or humans could be exposed to a mycoherbicide strain by several environmental pathways. For example, insects, reptiles, and birds might be attracted to a mycoherbicide formulation as a food source, and wind and rain can carry fungal propagules over long distances. Infested soil, plant material, and seeds can be moved to other crop sites by humans or other vectors. Such dispersal would inadvertently expose native plant species to the mycoherbicides and could pose risks to local ecosystems. The coca mycoherbicide, for example, could cause increased disease epidemics of native relatives of coca plants that could lead to adverse effects on local biodiversity or increase erosion if native coca communities on steep hillsides are reduced in size or density. Inadvertent infection of licit crops of cannabis, coca, and opium poppy could have important cultural and economic consequences.

The proposed mycoherbicide strains can cause disease on their target plants, including those grown legally and those indigenous to the area. However, the few host-range studies conducted with nonrelated species are of little value because they report only that the mycoherbicide strains did not cause disease on particular native plants and crops without providing experimental details (and in some cases even the names of the plants). Furthermore, none of the available studies used a standard, systematic process to select the most relevant plants to test in host-range studies. For example, of about 200 species of *Erythroxylum* native to South America, only two have been tested for sensitivity to the coca mycoherbicide. Thus, the data are insufficient to conclude that the proposed mycoherbicides would not pose a risk to other plants or crops.

Likewise, no data are available on the effects of the proposed mycoherbicides strains on soil microorganisms, animals, or humans. Such effects could include competition with indigenous microbial populations, diseases resulting from direct infection of animals or humans, and disorders resulting from contact with or consumption of toxins produced by the mycoherbicide strains. Although fungal species and strains related to the proposed mycoherbicides might cause such problems, there are no data to suggest that the proposed mycoherbicide strains would produce similar infections or toxins.

It will be all but impossible to control or contain the mycoherbicide strains after they are released. The strains are living organisms that interact with and adapt to their environment. Their ability to survive, propagate, and disperse beyond the target area depends on environmental factors that can be neither predicted nor controlled. The persistence of indigenous strains of these fungi throughout the native ranges of their hosts is consistent with the conclusion that the mycoherbicide strains are unlikely to be contained or eradicated once they are released.

MUTATION

The committee was asked to consider the potential of the fungi to mutate and the possible consequences, but no data on the mutability of the proposed mycoherbicide strains in particular are available. The potential for these mycoherbicide strains to mutate is expected to be similar to that of fungi in general. The genetic makeup of fungi can change by nucleotide substitution, the gain of genetic material from other fungi, the duplication of genetic material, and the loss of genetic material. Some species of *Fusarium* are well known for their spontaneous mutations to new morphologies. Gene transfer between distantly related strains of *Fusarium* occurs under laboratory conditions, and there is circumstantial evidence of such transfers under field conditions between strains that belong to different species. There are no data documenting the occurrence of such events in the *Fusarium* strains proposed for use as mycoherbicides or any strains of *C. papaveracea* or *B. papaveris*. Because indigenous strains of these fungal pathogens are present where the drug crops are grown, it is unlikely that mutations would occur in the introduced strains that have not already occurred in the indigenous population or that the mutations would pose novel risks to nontarget plants or other organisms, including humans or other animals. It is not possible to predict what types of mutations might occur, how a pathogen or target plant might be affected, or whether the mutations would be favored by natural selection.

New genetic variation results from mutation, and mutations can become established in fungal populations by natural selection or by chance. Natural selection results in adaptation to changing environments, including adaptation to new cultivars of a target species or to new host species. Adaptation might occur in fungi that reproduce sexually or asexually. Sexual reproduction allows new

genotypes to be produced more quickly through recombination than through mutation alone, as would occur in asexually reproducing organisms.

The evolutionary processes of mutation and adaptation also apply to the target plants. Plant cultivars resistant to a mycoherbicide could exist or emerge through natural selection and replace their sensitive predecessors. Thus, the development of effective mycoherbicides is a continuous rather than a one-time process.

RESEARCH AND DEVELOPMENT

The data available on the proposed mycoherbicide fungi reviewed by the committee are insufficient to determine the feasibility of their development as mycoherbicides or the risks that they might pose to nontarget plants, animals, humans, microorganisms, or the environment. Additional research is needed to address those concerns. At a minimum, a scientific team with expertise in plant disease epidemiology; plant pathology; fungal genetics; fermentation, formulation, and application technology; and nontarget risk assessment is needed to develop the proposed strains as mycoherbicides and to assess their safety and effectiveness. Initially, research is needed to study several candidate strains of each fungus and to identify strains that are the most efficacious under a broad array of environmental conditions. The resulting information would guide formulation development, the selection of a delivery method, and the scaleup required to generate enough mycoherbicide product to achieve a significant level of control.

Multiple regulatory requirements must be met before a mycoherbicide could be deployed. Many of the regulations focus on evaluating the risk to the environment posed by the introduction of the mycoherbicide. Little research of this sort has been conducted with any of the proposed mycoherbicide strains. Before the mycoherbicides could be used outside the United States, additional regulations in one or more international agreements might also need to be met, including the International Plant Protection Convention, International Standards for Phytosanitary Measures, the Biological Weapons Convention of 1972, and legal requirements in the country where the mycoherbicides are to be used.

OVERARCHING FINDINGS

Studies of the cannabis, coca, and opium poppy mycoherbicides that have been published or were made available to the panel are preliminary, exploratory, and insufficient to determine their suitability for controlling illicit-drug crops. The available data do not answer all the questions normally asked before a fungal pathogen is registered as a mycoherbicide in the United States. The rigorous, lengthy testing required by the U.S. Environmental Protection Agency has not yet begun, and conducting the research is not a guarantee that a registered mycoherbicide product will result. Mycoherbicides for the control of illicit-drug

crops will face additional difficulties in that the people cultivating the crops will be working to prevent the mycoherbicides from having their intended effects.

Potential Impediments

International Approval and Cooperation: Mycoherbicides proved to be safe and effective might not be approved for use in other countries. At least some tests of the mycoherbicide strains must be performed in the countries where the mycoherbicides might be used or in other countries that have similar climatic and environmental conditions. The testing requires the approval and cooperation of those countries and has been difficult, or impossible, to obtain. Country-specific requirements for such applications must also be satisfied.

Difficulties in Implementation: Commercial success of mycoherbicides developed to control weeds requires collaboration with the growers. Farmers who welcome attempts to control unwanted plants will tolerate aerial application from aircraft flying at low altitudes and at low speeds or from ground-based equipment, as needed, for the effective application of mycoherbicides, and they will permit or assist in the on-the-ground monitoring needed to assess the efficacy of the mycoherbicide. The proposed mycoherbicides for illicit-drug crops would not have similar cooperation from their growers, and this would constrain aerial application methods and limit on-the-ground monitoring. Technology for the effective application of mycoherbicides from high altitudes has not been developed.

Difficulty in Assessment of Effectiveness: The available data indicate that that proposed mycoherbicide strains are unlikely to kill large numbers of the target plants quickly. The combination of lack of rapid, aggressive action with little or nonexistent on-the-ground assessment would make it difficult, or even impossible, to determine the effectiveness of the mycoherbicide applications.

Development of Countermeasures: Producers of illicit-drug crops have an incentive to prevent damage to their crop yields and should be expected to develop countermeasures that reduce the efficacy of the mycoherbicides. Such countermeasures could include the use of fungicides or soil fumigants to kill the mycoherbicide strains directly or the cultivation of plant varieties that are resistant to the mycoherbicides.

Unavoidable Risks

Risks to Legal Crops and Native Plants: Cannabis, coca, and opium poppy are grown in several countries for licit uses and are part of the native flora in some regions. Plants in those settings could be vulnerable to the mycoherbicides. In addition, the mycoherbicides could spread beyond the geographic range of the illicit crops.

Risks to Nontarget Organisms: The mycoherbicide strains could have direct and indirect effects on other plants, microorganisms, animals, or the envi-

ronment. Those effects cannot be completely characterized even if research is performed to learn more about the infectivity and toxicity of the strains, if any, to nontarget plants and organisms. Mycoherbicides consist of living organisms that interact with and adapt to their environment, and it is difficult to predict how they might behave when released in substantial numbers into an ecosystem.

1

Introduction

Marijuana, cocaine, and heroin are three of the leading illicit drugs in the world. In 2008, cannabis herb production was estimated at 13,300-66,100 metric tons, and cannabis resin production at 2,200-9,900 metric tons. In 2009, global production of cocaine was estimated to range from 842 to 1,111 metric tons. Potential opium production was estimated at 7,755 metric tons, about 37% of which was projected to be available on the drug market as opium and the remainder as morphine and heroin (UNODC 2010a).

Those drugs are derived from plants: marijuana from cannabis (*Cannabis sativa*), cocaine from coca (*Erythroxylum coca* and *E. novogranatense*), and opium, morphine, and heroin from opium poppy (*Papaver somniferum*). Illicit cannabis plants are grown in most countries of the world, and it is difficult to obtain reliable estimates of cultivation because they are increasingly grown indoors. The UN Office on Drugs and Crime (UNODC) estimated that 200,000-641,800 hectares of land worldwide were used for outdoor cannabis cultivation in 2008. Afghanistan is the major cannabis producer in the world (see Table 1-1). A survey conducted by UNODC and the Afghan Ministry of Counter Narcotics in 2009 estimated 10,000-24,000 hectares of cannabis cultivation and 1,500-3,500 metric tons of cannabis resin production in Afghanistan. The extent of indoor cannabis cultivation cannot be accurately calculated, but indirect measures indicate that it is increasing because it is less likely to be detected, the yields are higher, and several crops can be grown per year (DOJ 2010; UNODC 2010a).

Illicit coca is cultivated primarily in the Andean countries of Colombia, Peru, and Bolivia. UNODC (2010a) estimated that 232,772 hectares of coca bushes were cultivated in Colombia, 69,925 hectares in Peru, and 37,241 hectares in Bolivia in 2009. Of those estimates, about 68,000, 59,900, and 30,900 hectares of coca bushes, respectively, were harvestable after eradication efforts (see Table 1-2).

The major countries that cultivate illicit opium poppy are Afghanistan, Myanmar, and Mexico. In 2009, the estimated illicit cultivation of opium poppy

was 128,351 hectares in Afghanistan and 35,787 hectares in Myanmar. After eradication efforts, about 123,000 and 31,700 hectares of opium poppy were harvestable, respectively (see Table 1-3). Estimates for Mexico in 2009 were not available, but estimates from previous years suggest that it maintained its third-place status as in 2008. Other countries in which opium poppy is cultivated include Pakistan, the Lao People's Democratic Republic, Thailand, Vietnam, and Colombia (UNODC 2010a).

The control of illicit-drug use and trafficking is difficult. It involves a variety of prevention, control, treatment, and law-enforcement strategies and the participation of local, national, and international government agencies. One approach in these endeavors, eradication of illicit crops, targets the beginning of the drug-supply chain by interfering with crop production in the fields. Crop-eradication measures include aerial application of herbicides, mechanical removal that uses tractors or other vehicles to harrow fields, and manual removal and destruction of plants.

TABLE 1-1 Major Cannabis-Cultivating and Cannabis-Producing Countries (2008, unless otherwise stated)

Country	Cultivated Area, hectares	Eradication	Harvestable Area, hectares	Production, metric tons	
				Herb	Resin
Afghanistan	10,000-24,000 (2009)	—	10,000-24,000 (2009)	1,500-3,500 (2009)	—
Bolivia	—	—	—	—	1,831
Canada	—	—	—	—	1,399-3,498 (2007)
Colombia	5,000 (2006)	—	—	—	4,000 (2006)
Mexico	—	18,562 hectares	12,000	—	21,500
Morocco	64,377	4,377 hectares	60,000	877	—
Netherlands	—	1,053,368 plants	—	—	323-766
Paraguay	6,000	1,838 hectares	—	—	16,500
South Africa	1,300	1,275 hectares	25	—	—
United States	—	7.6 million outdoor plants, 451,000 indoor plants	—	—	3,149-7,349

Source: UNODC 2010a. Reprinted with permission; copyright 2010, World Drug Report by United Nations Office on Drugs and Crime, Vienna, Austria.

TABLE 1-2 Global Illicit Cultivation of Coca and Production of Cocaine, 1995-2009

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Coca Bush Harvestable after Eradication, hectares															
Bolivia	48,600	48,100	45,800	38,000	21,800	14,600	19,900	21,600	23,600	27,700	25,400	27,500	28,900	30,500	30,900
Colombia	50,900	67,200	79,400	101,800	160,100	163,300	44,800	102,000	86,000	80,000	86,000	78,000	99,000	81,000	68,000
Peru	115,300	94,400	68,800	51,000	38,700	43,400	46,200	46,700	44,200	50,300	48,200	51,400	53,700	56,100	59,900
TOTAL	214,800	209,700	194,000	190,800	220,600	221,300	210,900	170,300	153,800	158,000	159,600	156,900	181,600	167,600	158,800
Potential Manufacture of Cocaine, metric tons[a]															
Bolivia	240	215	200	150	70	43	60	60	79	98	80	94	104	113	NA
Colombia	230	300	350	435	680	695	617	580	550	680	680	660	630	450	410
Peru	460	435	325	240	175	141	150	160	230	270	260	280	290	302	NA
TOTAL	930	950	875	825	925	879	827	800	859	1,048	1,020	1,034	1,024	865	—[b]

[a]Potential manufacture refers to the amount of 100% pure cocaine that could be produced if all coca leaves harvested in an area under coca cultivation in 1 year were processed into cocaine, on the basis of information on cocaine alkaloid content of coca leaves and efficiency of clandestine laboratories. Estimates for Bolivia and Peru take into account that not all coca leaf production is destined for cocaine production.

[b]Because of the ongoing review of conversion factors, no point estimate of cocaine production could be provided for 2009. Because of the uncertainty of total potential cocaine production, the 2009 figure was estimated as a range (842-1,111 metric tons).

Source: Adapted from UNODC 2010a. Reprinted with permission; copyright 2010, World Drug Report by United Nations Office on Drugs and Crime, Vienna, Austria.

TABLE 1-3 Global Illicit Cultivation of Opium Poppy and Production of Opiates, 1995-2009

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Opium Poppy Harvestable after Eradication, hectares															
SOUTHWEST ASIA															
Afghanistan	53,759	56,824	58,416	63,674	90,583	82,171	7,606	74,100	80,000	131,000	104,000	165,000	193,000	157,000	123,000
Pakistan	5,091	873	874	950	284	260	213	622	2,500	1,500	2,438	1,545	1,701	1,909	1,779
Subtotal	58,850	57,697	59,290	64,624	90,867	82,431	7,819	74,722	82,500	132,500	106,438	166,545	194,701	158,909	124,779
SOUTHEAST ASIA															
Lao PDR	19,650	21,601	24,082	26,837	22,543	19,052	17,255	14,000	12,000	6,600	1,800	2,500	1,500	1,600	1,900
Myanmar	154,070	163,000	155,150	130,300	89,500	108,700	105,000	81,400	62,200	44,200	32,800	21,500	27,700	28,500	31,700
Thailand	168	368	352	716	702	890	820	750	—	—	—	—	—	—	—
Vietnam	1,880	1,743	340	442	442	—	—	—	—	—	—	—	—	—	—
Subtotal	175,768	186,712	179,924	158,295	113,187	128,642	123,075	96,150	74,200	50,800	34,600	24,000	29,200	30,100	33,600
LATIN AMERICA															
Colombia	5,226	4,916	6,584	7,350	6,500	6,500	4,300	4,153	4,026	3,950	1,950	1,023	715	394	356
Mexico	5,050	5,100	4,000	5,500	3,600	1,900	4,400	2,700	4,800	3,500	3,300	5,000	6,900	15,000	NA
Subtotal	10,276	10,016	10,584	12,850	10,100	8,400	8,700	6,853	8,826	7,450	5,250	6,023	7,615	15,394	15,394
OTHER COUNTRIES															
Combined	5,025	3,190	2,050	2,050	2,050	2,479	2,500	2,500	3,074	5,190	5,212	4,432	4,184	—	—
Other	—	—	—	—	—	—	—	—	—	—	—	—	—	8,600[a]	7,600[a]
TOTAL	249,919	257,615	251,848	237,819	216,204	221,952	142,094	180,225	168,600	195,940	151,500	201,000	235,700	213,003	181,373
Potential Opium Production,[b] metric tons															
SOUTHWEST ASIA															
Afghanistan	2,335	2,248	2,804	2,693	4,565	3,276	185	3,400	3,600	4,200	4,100	6,100	8,200	7,700	6,900
Pakistan	112	24	24	26	9	8	5	5	52	40	36	39	43	48	44
Subtotal	2,447	2,272	2,828	2,719	4,574	3,284	190	3,405	3,652	4,240	4,136	6,139	8,243	7,748	6,944

(Continued)

TABLE 1-3 Continued

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
SOUTHEAST ASIA															
Lao PDR	128	140	147	124	124	167	134	112	120	43	14	20	9	10	11
Myanmar	1,664	1,760	1,676	1,303	895	1,087	1,097	828	810	370	312	315	460	410	330
Thailand	2	5	4	8	8	6	6	9	—	—	—	—	—	—	—
Vietnam	9	9	2	2	2	—	—	—	—	—	—	—	—	—	—
Subtotal	1,803	1,914	1,829	1,437	1,029	1,260	1,237	949	930	413	326	335	469	420	341
LATIN AMERICA															
Colombia	71	67	90	100	88	88	80	52	50	49	24	13	14	10	9
Mexico	53	54	46	60	43	21	91	58	101	73	71	108	149	325	NA
Subtotal	124	121	136	160	131	109	171	110	151	122	95	121	163	335	335
OTHER COUNTRIES															
Combined[c]	78	48	30	30	30	38	32	56	50	75	63	16	15	—	—
Other														139[a]	134[a]
TOTAL	4,452	4,355	4,823	4,346	5,764	4,691	1,630	4,520	4,783	4,850	4,620	6,610	8,890	8,641	7,754
Nonprocessed opium[d]	—	—	—	—	—	—	—	—	—	1,382	1,317	2,228	3,698	3,070	2,895
	Potential Manufacture of Heroin, metric tons[e]														
Outside Afghanistan	445	436	482	435	576	469	163	452	478	495	472	606	735	724	634
Total[f]	—	—	—	—	—	—	—	—	—	529	472	629	757	752	657

[a]Starting in 2008, a new method was used to estimate opium poppy cultivation and opium and heroin production. The estimates are higher than the previous estimates but of a similar order of magnitude.

[b]Potential production is the amount of oven-dry opium of unknown morphine content that could be produced if all opium poppy cultivated in an area in 1 year were harvested in the traditional method of lancing the opium capsules and collecting the opium gum or latex.

[c]In some countries, poppy straw is used to produce acetylated opium rather than opium gum. However, for reasons of comparability, it was assumed that all opium poppy cultivation is used for opium gum production.

[d]These estimates represent the amount of opium that remains opium and is not processed into morphine or heroin. It refers only to Afghan opium because the amount of opium not processed into morphine or heroin in other countries could not be estimated. For years before 2004, no such estimate was available for Afghanistan.
[e]Potential manufacture is the amount of heroin of unknown purity that could be produced if the total potential opium production were converted into heroin, excluding the opium that is consumed as opium and not processed.
[f]This series contains all heroin potentially manufactured worldwide, including heroin and morphine consumed and seized in Afghanistan. The amount of Afghan opium estimated to remain available as opium (potential opium, not processed) is not included in these figures.
Abbreviation: PDR, People's Democratic Republic; NA, not available.
Source: UNODC 2010a. Reprinted with permission; copyright 2010, World Drug Report by United Nations Office on Drugs and Crime, Vienna, Austria.

Some genotypes of parasitic fungi can infect a single plant species or a small group of closely related species, and the use of these fungal pathogens, or mycoherbicides, formulated and applied in the same ways as chemical herbicides, has been proposed as a means of controlling illicit crops. The fungi also have the potential to persist in the soil for many months or years and to affect later attempts to cultivate the crops (see Chapter 3).

FUNGAL TERMINOLOGY

The terminology of fungi used throughout this report can be confusing to those unfamiliar with the practice of taxonomy (classifying and naming organisms) and fungal characteristics. This section presents the names assigned to the fungi under consideration in this report and some of their distinguishing characteristics.

Organisms are classified with a hierarchical system of categories: kingdom, division (phylum), class, order, family, genus, and species. The Kingdom Fungi is subdivided into phyla. The proposed mycoherbicides considered in this report belong to the phylum Ascomycota. Ascomycota is the largest of the fungal phyla, containing more than 60% of described fungal species, including most species of plant-pathogenic fungi.

Fungi in Ascomycota may reproduce sexually to produce ascospores or asexually (clonally) by mitotic division to produce a different type of spore, a conidium. Most fungi in Ascomycota require a mating partner to form a zygote (cell formed by the union of male and female sex cells), which then undergoes meiosis (cell division for sexual reproduction) and produce ascospores (Figure 1-1). Some species can self-fertilize to produce a zygote which gives rise to ascospores (Figure 1-2). Ascomycota organisms that require a partner to complete the sexual cycle are termed heterothallic; those which can complete the sexual cycle without a partner are termed homothallic. The ability of fungi in Ascomycota to produce both ascospores and conidia has led to taxonomic complexity because each spore state may have its own name. Thus, a single fungus may be known by more than one name: one name when it produces sexual spores (sexual state; teleomorph), and another name when it produces mitotic spores or conidia (asexual state; anamorph). If there are two names, the teleomorph name would be synonymous with the name for the entire fungus (the holomorph). Many fungi in Ascomycota have not been observed to reproduce sexually, although they may produce abundant clonal spores (Figure 1-3).

In this report, we refer to the proposed mycoherbicides by the species names of the fungi intended for development. A species is commonly denoted by the name of a genus (a group of closely related species) and a species name that serves as an adjective or modifier of the genus name. The proposed mycoherbicides of cannabis and coca belong to the same species, *Fusarium oxysporum*. Some *F. oxysporum* names are followed by a special form name, or *forma specialis* (f. sp.) (the plural is *formae speciales* [f. spp.]), which indicates

that the fungus appears to infect a specific type of plant species or group of species. The proposed mycoherbicides against cannabis and coca are *F. oxysporum* f. sp. *cannabis* and *F. oxysporum* f. sp. *erythroxyli*. (*Cannabis* and *Erythroxylum* are the genera of cannabis and coca, respectively). *F. oxysporum* is known to produce conidia and is not known to produce ascospores, although fungi in many other species of *Fusarium* are known to reproduce sexually in both heterothallic and homothallic manners.

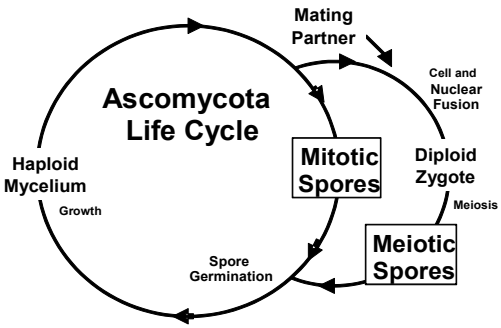

FIGURE 1-1 Life cycle of a heterothallic Ascomycota fungus. This fungus must have a partner to mate and produce meiotic progeny (ascospores). It can also make mitotic progeny (conidia). There could be two names for this fungus: one for the mitotic-spore state (anamorph name) and another for the meiotic-spore state (teleomorph name). *Crivellia papaveracea* is an example of a fungus with this type of life cycle.

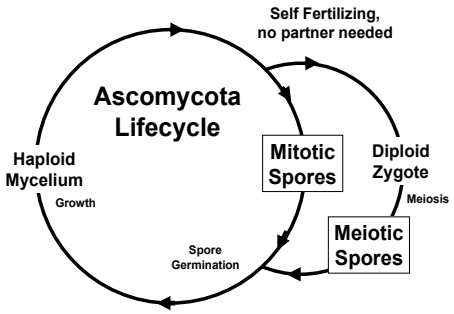

FIGURE 1-2 Life cycle for a homothallic Ascomycota fungus. This fungus does not need a partner to mate and can produce meiotic progeny (ascospores) that are genetically identical to the one parent. If a partner is available, homothallic ascomycetes can also mate to produce recombined ascospores. They also can make mitotic progeny (conidia). There could be two names for this fungus, one for the mitotic spore state (anamorph name) and another for the meiotic spore state (teleomorph name). *Brachycladum papaveris*, which is the asexual form of *Crivellia papaveracea*, has this type of life cycle.

The proposed opium poppy mycoherbicides belong to two fungal species: *Crivellia papaveracea* and *Brachycladium papaveris*. These poppy pathogens were previously named *Pleospora papaveracea* and *Dendryphion penicillatum*, respectively. The changes in names of the poppy pathogens reflect a better understanding of their biology (Inderbitzin et al. 2006); they were found to be closer relatives of *Alternaria* spp. than of *Pleospora* spp. (see Figure 1-4). To connect the new names with those of fungi used in older literature, a table is provided in Chapter 5 to document the old and new names for the fungi. When it is unclear which of the two species was studied, we refer to both fungi in this manner: *C. papaveracea/B. papaveris*.

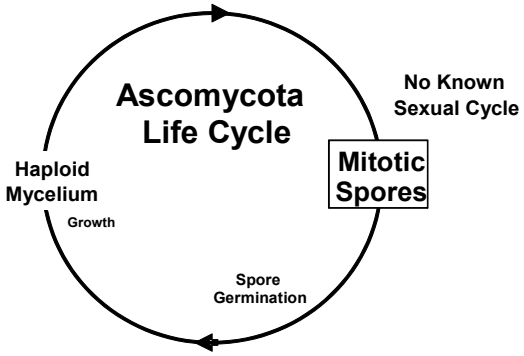

FIGURE 1-3 Life cycle for a mitosporic Ascomycota fungus. This fungus does not exhibit a sexual form and only has one name for its mitotic spore state (anamorph name). *Fusarium oxysporum* has this type of life cycle.

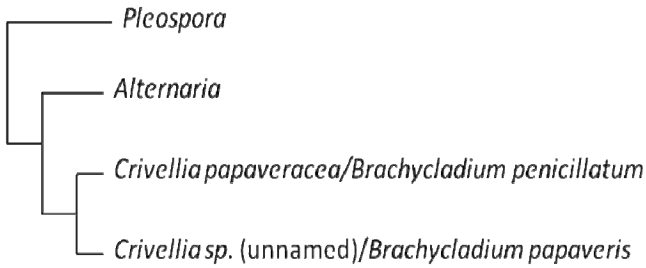

FIGURE 1-4 Phylogenetic relationships of *Crivellia* and *Brachycladium* species according to Inderbitzin et al. (2006). Until a species names is proposed for the unnamed *Crivellia* species, it must be referred to as *Brachycladium papaveris*, which, along with *Crivellia papaveracea*, are the names used for the two opium poppy fungal pathogens discussed in this report.

STATEMENT OF TASK

The Office of National Drug Control Policy Reauthorization Act of 2006 (Public Law 109-469, Section 1111) directed ONDCP to support a scientific study of the use of mycoherbicides in eradicating illicit crops. In response, ONDCP asked the National Research Council to form an expert committee to examine scientific issues associated with the feasibility of developing and implementing mycoherbicides to eradicate illicit crops of coca, cannabis, and opium poppy, including an evaluation of the potential human health, ecological, and environmental risks associated with their use and recommendations for the research and development needed for such use.

The committee was charged with addressing the following issues about the potential use of naturally occurring strains of mycoherbicides in eradicating illicit crops: (1) their effectiveness in eradicating target plants; (2) the feasibility of their large-scale industrial manufacture and delivery; (3) their potential spread and persistence in the environment; (4) their pathogenicity and toxicity to nontarget organisms, including other plants, fungi, animals, and humans; (5) their potential for mutation and resulting toxicity to nontarget organisms; and (6) future research and development needed for implementation, such as mode-of-action studies. The specific questions to be addressed are presented in Box 1-1.

The committee comprised experts in plant pathology; mycotoxins; fungal genetics, evolution, and ecology; mycoherbicide development, formulation, and application; plant-disease epidemiology; soil microbiology; medical mycology; human toxicology and risk assessment; and ecological risk assessment. This report presents the consensus findings of the committee.

COMMITTEE'S INTERPRETATION OF ITS TASK

The committee found that some elements of the statement of task were vague and open to interpretation. After discussions with the sponsor, the committee arrived at the following observations and clarifications on how to fulfill its task.

Task Clarifications

- The term *eradication* suggested to the committee that complete destruction of the target crop was the intended goal. Discussions with the sponsor made it clear that complete destruction was an unrealistic expectation and that the intended goal is substantial *control* of crop yields by causing disease or weakening a plant's ability to produce the chemical compounds used to make illicit drugs. However, the sponsor did not specify the desired extent of control of the illicit crops. It was beyond the committee's charge to determine what an appropriate extent of control would be.

BOX 1-1 Questions Posed in the Statement of Task

Effectiveness:

- Are the drug crops (cannabis, coca, and opium poppy) known to be susceptible to the proposed mycoherbicides?
- Have the mechanisms of action of the proposed mycoherbicides' toxicity to illicit-drug crops been established?
- Are the proposed mycoherbicides host-plant-specific?
- What quantities of mycoherbicides would be needed to eradicate illicit drug crops?
- How would the method of delivery affect the effectiveness of the mycoherbicides in eradicating the drug crops?

Feasibility of large-scale manufacture and delivery:

- What sort of facility would be required for the large-scale industrial manufacture of the proposed mycoherbicides?
- What sort of equipment and technology would be required for the delivery of the proposed mycoherbicides on a large scale?
- What is the overall technical feasibility of the large-scale industrial manufacture of the proposed mycoherbicides?
- What consideration would need to be made for large-scale delivery of the proposed mycoherbicides?
- What types of field trials of the proposed agents are needed? For example, is testing in tropical or arid environments required?

Persistence in the environment:

- How long are the mycoherbicides likely to persist in the environment after application?
- Do the mycoherbicides have geographic or climatic boundaries?
- What combination of environmental conditions (such as temperature, depth in soil, and pH) would favor persistence after application?
- What conditions would shorten the persistence of the proposed mycoherbicides in the environment?
- Could persistence of the mycoherbicides in the environment be controlled?

Toxicity to nontarget organisms:

- Would the proposed mycoherbicides harm licit crops or kill other soil fungi?
- Would the proposed mycoherbicides threaten biodiversity or pose other risks to the environment?
- What is the nature of the health risks to animals and humans posed by the use of the proposed mycoherbicides?
- What would be the range of transmission of the proposed mycoherbicides and what factors would influence their spread?
- Once released, would the pathogens be uncontrollable?

(Continued)

BOX 1-1 Continued

Potential for mutation and resulting toxicity to target and nontarget organisms:

- What would be the potential of the pathogens to mutate?
- Are there environmental or other conditions that would drive mutations in the mycoherbicides?
- Does the potential for mutation pose additional risks to nontarget organisms (including other plants, fungi, animals, and humans) or the environment?
- How might mutations affect the susceptibility of the target crops to the mycoherbicides?
- How might mutations affect the toxicity of mycoherbicides generally?

Research and development needs:

- Could the effectiveness of the mycoherbicides in eradicating illicit-drug crops be improved through research and development?
- What types of expertise would be most relevant for improving the effectiveness and safety of the proposed mycoherbicides?
- What types of research and technology would improve the production and delivery of the mycoherbicides?
- What type of testing would be needed before mycoherbicides could be safely and effectively used to eradicate illicit-drug crops (for example, mode-of-action studies)?
- What would be required under U.S. federal and state laws to test and approve a mycoherbicide of this type, and what guidelines of the International Organisation for Biological and Integrated Control of Noxious Animals and Plants would apply?

- *Pathogenicity* and *toxicity* are used interchangeably in the statement of task, but they have different meanings. *Pathogenicity* refers to the ability of a living agent (the pathogen) to infect and cause disease. The affected organism is termed the host. *Toxicity* is commonly used with two connotations: the capacity of any agent to cause harm to a living organism and the degree or extent of the harm caused by a *chemical*. In this report, *pathogenicity* is used to describe the former concept and *toxicity* to describe the latter concept. The distinction is important because the fungi of interest are competent plant pathogens and are capable of causing disease in the host plants that they colonize, but some also produce chemical metabolites that might adversely affect other plants, microorganisms, animals, and humans.
- Several of the task questions focus on the potential of the fungi to mutate. The term *mutation* often has a negative connotation, so it is important to define how it is used in the report. *Mutation* refers to change. In genetics, it means a sudden departure from the parental type in one or more heritable characteristics. A mutation results in a permanent, heritable alteration in the DNA sequence of a gene or the physical arrangement of a chromosome. Changes in a gene's DNA sequence can alter the phenotype (an observable physical or bio-

chemical trait) by altering its expression. Changes in the physical arrangement of a chromosome also could result in new traits even if none of the genes on the chromosome has been otherwise altered. Induced mutations are heritable genetic changes that result from the exposure to chemical and physical agents that are capable of altering DNA or chromosomal structure. Spontaneous mutations occur without known exposure to a mutagenic agent. As used in the statement of task, *mutation* appears to refer to any stable change in the fungal phenotype that might occur.

- The question "What would be required under U.S. federal and state law to test and approve a mycoherbicide of this type, and what guidelines from the International Organization for Biological Control of Noxious Animal and Plants (IOBC) would apply?" is more a legal and regulatory question than a scientific one. The committee did not feel comfortable about developing a "checklist" of testing requirements that must be met, especially inasmuch as regulations vary widely by state and by country. It also was unclear to the committee why the work of IOBC was of specific interest to ONDCP. IOBC is an international professional society that promotes the development of biological control agents. Although the organization and some of its members were involved in the development of some international standards for testing pesticides and guidelines for transport and release of biological control agents, it does not have its own set of guidelines. To address the question quoted above, the committee took a broad approach of determining what general types of information would be necessary to test or use mycoherbicides under relevant national or international laws and agreements.

Issues Outside the Committee's Task

Determining whether it is feasible to develop mycoherbicides requires defining the term *feasible*. Feasibility clearly encompasses the many scientific and technical questions in the task presented in Box 1-1. However, other issues are critical in determining the feasibility of developing and using mycoherbicides against illicit crops, such as the costs of conducting the research to support the registration of the mycoherbicides, the international procedures for getting mycoherbicides approved for testing and use in different countries, and other economic, social, and political factors. Evaluating those factors was not part of the committee's charge.

The committee is aware that some researchers have proposed modifying the proposed mycoherbicides genetically to improve their virulence and efficacy in controlling illicit drug crops. However, the committee was asked to restrict its evaluation to *naturally occurring strains* of the fungi, so genetic modification is not considered in this report.

COMMITTEE'S APPROACH

The committee held three public meetings to gather information to address its task. At the first meeting, on April 20, 2010, the committee met with officials of ONDCP to obtain background on the project and clarification on the scope of work. Representatives of the U.S. Environmental Protection Agency Office of Pesticide Programs gave a presentation on the agency's experience with mycoherbicides and its requirements for registration. At the second meeting, on June 23, 2010, representatives of the Department of State shared their perspectives on the history that led to the request for the National Research Council study. The committee also heard presentations on research conducted by the U.S. Department of Agriculture's Agricultural Research Service on mycoherbicides against coca and opium poppy; on the Forest Service's struggle to deal with illicit drug crops in national parks and other public lands; on the biological control of witchweed (*Striga hermonthica*) in sub-Saharan Africa using a strain of *Fusarium oxysporum*; and on the commercial production of mycoherbicides. At the third public meeting, on September 15, 2010, the committee reviewed a potential technique for applying mycoherbicides. The committee heard from other interested parties during the open-microphone sessions at its public meetings and in written submissions.

As noted earlier, economic, social, and political considerations in determining the feasibility of developing and using the proposed mycoherbicides were outside the committee's charge. Such considerations should be taken into account with the scientific factors by any agency or organization that might engage in the development of mycoherbicides against illicit crops.

To aid in the committee's evaluation, literature searches were performed to identify relevant research, and ONDCP, the Department of State, and the United Nations were consulted for other relevant, publicly available information. The committee found that little information on the fungi of interest that could be used to provide well-informed answers to several questions in the statement of task was available. Thus, it answered many of the questions in a broad, qualitative context, drawing from general biological principles and from experience with approved mycoherbicides and related fungi.

ORGANIZATION OF THE REPORT

The committee organized its evaluation by first providing some context for its review. Chapter 2 provides background on the biological control of undesirable plant species. All the mycoherbicides that are approved for use in the United States were developed to combat undesirable plant species. The committee considered general principles learned from developing those mycoherbicides and how they might be applied to developing mycoherbicides against illicit crops. The chapter also discusses issues that are applicable to all the proposed

mycoherbicides, including pesticide regulation in the United States, international considerations for testing mycoherbicides, and a conceptual approach to evaluating risks to nontarget plants and organisms. Chapter 3 provides a brief overview of the biology and cultivation of cannabis, coca, and the opium poppy. Chapter 4 considers the available data on the *Fusarium* mycoherbicides that target coca and cannabis and what is known about *F. oxysporum* in general. A similar review of *C. papaveracea/B. papaveris* is provided in Chapter 5. Chapter 6 provides answers to each of the questions in the statement of task.

2

Biological Control of Undesirable Plants

This chapter provides background on biological control of undesirable plants and covers general issues related to the development, use, and assessment of mycoherbicides. The concept of biological control is introduced, and examples of mycoherbicides that have been approved and registered in the United States and other countries are provided. The chapter considers key attributes of effective mycoherbicides that are in use and important challenges to their development and large-scale use. It provides a brief overview of pesticide regulation in the United States and international considerations for testing mycoherbicides. Attention also is given to a conceptual framework for considering risks to animals and humans posed by biological control agents. Information on specific mycoherbicides against illicit drug crops is provided in Chapters 4 and 5.

BACKGROUND

Biological control pertains to the deliberate use of living agents (natural enemies) to reduce populations of undesirable species (weeds and insect pests) with minimal nontarget effects. There are two main approaches to biological control of undesirable plants: the classical and bioherbicide approaches. Table 2-1 summarizes the distinguishing features of the two approaches.

The *classical (*or *inoculative) approach* is generally used against nonnative invasive species and involves the introduction of an agent from a foreign country into a population of the undesirable species to control its growth and spread. This approach does not provide instantaneous control; rather, control is achieved over months or even years after the introduction of the pathogen (Yandoc Ables et al. 2007). Under consideration since the 1960s (Inman 1971), the classical approach has been in use since the 1970s, when two rust fungi were used: one to control blackberry (*Rubus* spp.) in Chile (Oehrens 1977) and the other to control rush skeletonweed (*Chondrilla juncea*) in Australia. The latter case, control of rush skeletonweed by the rust fungus *Puccinia chondrillina,* is widely recognized as an early example of success in classical biological control

with fungi (Watson 1991; Hajek 2004). Rush skeletonweed is native to Mediterranean Europe and was a problematic weed in Australia until the deliberate introduction of the rust fungus as a control agent. Introduction of the fungus was followed by a rust disease epidemic, which caused widespread reductions in rush skeletonweed plant height, flower numbers, and viable seed production. Rush skeletonweed mortality was over 90% in areas of Australia where the climate was most favorable for infection (Cullen et al. 1973; Cullen 1985). Another successful example of the classical approach is the introduction of *Uromycladium tepperianum,* a rust fungus from Australia, into South Africa to control the invasive tree *Acacia saligna* (Morris et al. 1999). This fungal pathogen, which causes severe gall formation and tree death, has reduced *A. saligna* populations by 90-95% throughout the plant's range in South Africa; successful results were evident within 8 years after the release of the fungus (Morris 1997; Wood and Morris 2007).

In the United States, three rust fungi have been released during the last 4 decades to control invasive weeds: *Puccinia chondrillina* to control rush skeletonweed, *P. carduorum* for musk thistle (*Carduus thoermeri*), and *P. jaceae* var. *solstitialis* for yellow starthistle (*Centaurea solstitialis*) (Baudoin et al. 1993; O'Brien et al. 2010). *P. jaceae* var. *solstitialis* was found to be ineffective in controlling its target weed. *P. chondrillina* and *P. carduorum* were found to have caused damage to their target species (reduced plant density and reduced seed production), but these observations were made in field sites where insect species had also been introduced (Supkoff et al. 1988; Baudoin et al. 1993; O'Brien et al. 2010).

TABLE 2-1 Classical vs Bioherbicide Approach

Feature	Classical (Inoculative)	Bioherbicide (Inundative)
Target plants	Exotic, aggressive, widespread, generally perennials	Native or naturalized, generally annuals
Biological control agents	Exotic arthropods and pathogens	Locally occurring pathogens (a few insects)
Habitats	Marginal lands (such as rangelands) and natural systems	Intensive croplands
Host specificity	Very narrow (monospecific)	May be broader
Delivery	Small (inoculative) population released	Mass-reared and large inundative applications (releases)
Establishment	Several years to become established	Immediate effect
Control objective	Sustained long-term control	Transient nonsustainable control
Approach	Ecological	Technological

The *bioherbicide (*or *inundative) approach* involves the use of a pathogen that naturally occurs (that is, is indigenous) in a population of a native or naturalized undesirable plant species (target plant). The pathogen is grown in the laboratory, and its inoculum (infective propagules in the form of mycelium or spores of various types) is mass-produced, formulated, made into a standardized product, and applied in a large dose to inundate the target plant in a manner similar to the application of chemical herbicides. For this approach to be successful in controlling an undesirable plant species in commercial crops, a high level of control of the target plant is required within a short period to reduce economic losses (Charudattan 1990; Weston 1999). The approach might require annual application of the pathogen to cause disease or damage in the succeeding populations of the target plant. Plant pathogens or products containing the pathogens that are used in this approach are called *bioherbicides***.** When fungal pathogens are used in this approach, they are referred to as *mycoherbicides*. The earliest examples of attempts to control undesirable plants with the bioherbicide approach include the unsuccessful use of *Fusarium oxysporum* on a large scale to control the white form of the prickly pear (*Opuntia megacantha*) in Hawaii in the 1940s (Fullaway 1954; Wilson 1969) and the successful use of *Acremonium diospyri* on weedy persimmon (*Diospyros virginiana*) in the United States in the 1960s (Wilson 1965).

The first mycoherbicide to be registered in the United States by the U.S. Environmental Protection Agency (EPA) was DeVine in 1981, which is composed of *Phytophthora palmivora* strain MWV. It was developed in the 1960s and 1970s by researchers working for the Florida Department of Agriculture and Consumer Services Division of Plant Industry to control milkweed vine, or stranglervine (*Morrenia odorata*), a major nonnative weed in citrus groves (Charudattan 1991). *P. palmivora* causes root rot, wilting of the whole plant, and plant death. In field trials, a single application of DeVine was reported to reduce milkweed vine population by over 90% within 1-2 years (Woodhead 1981).

In 1982, another mycoherbicide, Collego (*Colletotrichum gloeosporioides* f.sp. *aeschynomene*), was registered in the United States. Collego was developed by the Upjohn Company in collaboration with researchers at the University of Arkansas and the U.S. Department of Agriculture Agricultural Research Service for the control of northern jointvetch (*Aeschynomene virginica*) in rice and irrigated soybean in Arkansas and neighboring rice-growing states (TeBeest 1985). The mycoherbicide was reregistered by EPA in 1992 and again in 2006, the latter time under the label Lockdown (Yandoc Ables et al. 2007).

Bioherbicides can constitute an alternative to mechanical methods and chemical herbicides in the control of undesirable plants. They can help to mitigate the adverse effects of chemical herbicides, such as ground and water contamination, nontarget effects, and development of herbicide resistance. They could be a cheaper alternative to expensive mechanical controls and help to reduce some undesirable side effects, such as acceleration of soil erosion from frequent cultivation. The cancellation of registration of several chemical herbicides, the lack of registered herbicides for some nonagricultural and nonnative

invasive weeds, the high cost of controlling undesirable plants in natural areas, and the need for weed-control methods for organic production systems are other reasons for developing bioherbicides (Yandoc Ables et al. 2007).

In the United States, overt interest in using mycoherbicides to control illicit-drug crops dates from the late 1970s, when Hildebrand and McCain (1978) suggested using *Fusarium oxysporum* f.sp. *cannabis* to control cannabis. In the 1990s, the feasibility of using three fungi—two specialized forms of *F. oxysporum* and *Crivellia papaveracea* (formerly called *Pleospora papaveracea*)—as mycoherbicides for the control of illicit narcotic crops (coca, cannabis, and opium poppy) was further investigated by scientists in the United States, Kazakhstan, and Uzbekistan (see Chapters 4 and 5 for details). The use of those pathogens was regarded as an option for eradicating illicit-drug crops because of their purported ability to cause serious damage to the crops, their specificity and narrow host range (they would have minimal nontarget effects), and the likelihood that they would survive in the soil for an extended period and cause disease in succeeding crops of the target plant species (Hildebrand and McCain 1978; Tiourebaev et al. 2001). Mycoherbicides were also promoted as a safer alternative to chemical herbicides (UNODC 2002). Another reason for considering the use of biological control is the need to replace the commonly used eradication methods for illicit crops (mechanical and manual removal and aerial and ground application of chemical herbicides) or to augment the level of control achieved with these methods.

KEY ATTRIBUTES OF EFFECTIVE MYCOHERBICIDES IN USE

Registered mycoherbicides, their current status, and the reasons for discontinued production and use are presented in Table 2-2. Although 12 plant pathogens were registered worldwide as mycoherbicides during the last 3 decades, only four—Chontrol, Collego (now Lockdown), DeVine, and Sarritor—have been used with measurable success. An important reason for that small number is mainly economic (economically nonsustainability in the marketplace), although lack of consistent efficacy is also a major reason. Five of 62 bioherbicide programs (involving fungi, bacteria, and a virus) have produced successful products that are used to a certain degree (Charudattan 2005). The remainder, about 92% of the projects, were abandoned because of lack of efficacy, because of inconsistency of performance, or because they were not profitable. Synergy between research and industry has been instrumental in the successful development of registered mycoherbicides. As pointed out by Bailey and Falk (in press), the registered mycoherbicides are the result of a linking of research developments to a series of key management decisions based on a "stage and gate" process used in business models. To be successful, the proposed mycoherbicides for drug crops should follow a similar research-industry partnership.

TABLE 2-2 Status of Registered Mycoherbicides (2010)

Pathogen	Target Plants	Trade Name	Status	Reference
Alternaria destruens strain 059	*Cuscuta* spp., such as dodder, swamp dodder, large-seed dodder, field dodder, and small-seed dodder	Smolder	Registered to Sylvan Biosciences in 2005 (United States) Not currently registered and marketed by the company	EPA 2005a Personal communication, Mark Wach, Sylvan Biosciences, Kittanning, PA (August 16, 2010)
Chondrostereum purpureum strain PFC2139	Sitka, red alder, and other hardwood species in rights-of-way and forests (used as a stump treatment)	Chontrol™	Registered in 2004 (Canada); 2004 (United States)	Health Canada 2011a; EPA2004a, b
Chondrostereum purpureum strain HQ1	Birch, pin-cherry, poplar/aspen, maples, and speckled alder growing in rights-of-way, wood lots, and conifer plantations (stump treatment)	MycoTech™ Paste	Registered in 2002 (Canada); canceled in 2008	Health Canada 2011b
		MycoTech strain HQ1 Concentrate	Registered in 2005 (United States) Conditional registration in 2005 (United States)	EPA 2005b,c
Colletotrichum acutatum	Silky hakea (*Hakea sericea*)	Hakatak	Registered in 1990 (South Africa); registration was allowed to lapse in 1991 because of budget cuts by the main user and the limited market for the product	Morris et al. 1999
			Currently, the Agricultural Research Council Plant Protection Research Institute (South Africa) is supplying a small number of farmers and biocontrol implementation officers with dried spore preparation of the fungus	Personal communication, Andries Fourie, ARC-PPRI, Stellenbosch, South Africa (June 2010)

(Continued)

TABLE 2-2 Continued

Pathogen	Target Plants	Trade Name	Status	Reference
Colletotrichum gloeosporioides f.sp. *aschynomene* ATCC 20358	Northern jointvetch (*Aeschynomene virginica*) in rice	Collego (1982) Lockdown (2006)	Registered in 1982; 1993 data call-in required additional product data; reregistered as Lockdown in 2006 (United States)	EPA 1997
Colletotrichum gloeosporioides f.sp. *cuscutae*	Dodder (*Cuscuta* spp.)	Luboa	Registered in 1963 (China); not available commercially in China since 1980	Personal communication, Sheng Qiang, Nanjing Agricultural University, Nanjing, China (July 7, 2010)
Colletotrichum gloeosporioides f.sp. *malvae*	Round-leaved mallow (*Malva pusilla*) in field crops	BioMal	Registered in 1992 (Canada); registration is currently inactive because it is not being manufactured and sold	Personal communication, Karen Bailey, Agriculture and Agri-Food Canada, Saskatchewan, Canada (July 2010)
			Commercialization plans for BioMal were discontinued because of unfavorable market conditions and high production costs	Bioline International 1995
Cylindrobasidium laeve	Black wattle (*Acacia mearnsii*); golden wattle (*A. pycnantha*)	Stumpout	Registered in 1997 (South Africa) as a fungal inoculant; registration allowed to lapse in 2005 because of problems with annual registration process; inoculum is still produced and supplied on demand (a public-good service)	ARC 2010 Personal communication, Alan Wood, ARC-PPRI, Stellenbosch, South Africa (June 2010)
Phytophthora palmivora MWV	Strangler vine or milkweed (*Morrenia odorata*) in citrus	DeVine	First mycoherbicide to be registered and marketed in United States (1981); still registered as of 2006 to Valent Biosciences Corporation	EPA 2006a,b

			No longer available, because of small market niche	Ridings 1986
			Not included in the Valent Biosciences Corp. online agricultural products list	Valent 2010
Puccinia canaliculata	Yellow nutsedge (*Cyperus esculentus*)	Dr. BioSedge	First rust fungus to be registered as a bioherbicide although it cannot be mass-produced on nonliving, artificial substrates	Phatak et al. 1983
			The product was not offered for sale within 1-2 years after registration, because of marketing considerations	Personal communication, Sharad Phatak, University of Georgia (to R. Charudattan)
			The product is not available commercially, because of difficulty in mass production	Hajek 2004
Puccinia thlaspeos strain woad	Dyer's woad (*Isatis tinctoria*)	Woad Warrior	Registered in 2002 (United States)	EPA 2002
Sclerotinia minor IMI 3144141	Dandelion (*Taraxacum officinale*) in turf	Sarritor	Registered in 2007 (Canada)	Health Canada 2011c
			U.S. registration pending	
			Currently manufactured by Sylvan Biosciences	Sylvan Bio 2009

A brief review of a successful mycoherbicide, Collego (Lockdown), sheds some light on the attributes of a successful mycoherbicide and provides lessons to guide the debate on mycoherbicides for drug crops. The Collego fungus (*Colletotrichum gloeosporioides* f.sp. *aeschynomene*) causes a disease characterized by lesions on stems, petioles, and leaves of northern jointvetch, an annual plant and a weed of rice and irrigated soybean in Arkansas, Mississippi, and Louisiana. Lesions expand rapidly, and the fungus colonizes tissues beneath the lesions and grows laterally and vertically on the hollow stems. The coalescence of lesions results in the girdling of the stem and the death of plant parts above the lesions (TeBeest et al. 1978). In the field, Collego is applied shortly after northern jointvetch emerges above the crop canopy, when it is applied from a fixed-wing, crop-sprayer aircraft. The product is formulated to permit appliers to simply add each unit of the dry product to an appropriate number of gallons of water (10 gal/acre). The spray suspension is adjusted to provide 2 million spores per milliliter (94 billion spores/acre). The final product consists of 15% fungus and 85% inert diluents. On the average, the amount of material required for an application is often only 20-40 g/acre (TeBeest and Templeton 1985). The application is intended not to control (kill) northern jointvetch but to establish the pathogen on the weed so that it can produce a new crop of several million spores (secondary inoculum) in each lesion. Pathogens that lack the ability to multiply from an initial application are generally not suitable as mycoherbicides.

C. gloeosporioides f.sp. *aeschynomene* can persist from year to year on infected northern jointvetch stubble (TeBeest 1982). It infects seeds, and seed infections can give rise to seedling infections (TeBeest and Brumley 1978). However, it does not persist as spores in soil or rice irrigation water in the absence of the host plant for more than several months, so annual applications of Collego are necessary to provide acceptable levels of northern jointvetch control.

An initial study suggested that the host range of *C. gloeosporioides* f.sp. *aeschynomene* was limited to northern jointvetch and Indian jointvetch (*Aeschynomene indica*), of which the latter was highly resistant to infection by the fungus (Daniel et al. 1973). However, later tests showed that seven genera of Fabaceae were susceptible (TeBeest 1988; Weidemann et al. 1988; Weidemann and TeBeest 1990). Because those genera were not found in the area or fields to be inoculated, the newly recognized broader host range did not affect registration or its requirements.

Studies show that the commercial strain (ATCC 20358) of *C. gloeosporioides* f.sp. *aeschynomene* is not necessarily competitive with other strains of the fungus that occur naturally in the field (Yang and TeBeest 1995; Luo and TeBeest 1997, 1998, 1999). Another virulent isolate of *C. gloeosporioides* f.sp. *aeschynomene* easily replaces the commercial strain on northern jointvetch after coinoculation (Ditmore et al. 2008); the replacement occurs within several weeks of inoculation and might account for the inability to find the commercial strain in the field after discontinuation of its use.

Thus, the success of Collego can be attributed to a combination of host specificity, the virulence and aggressiveness of the strain and the susceptibility

of its host, the conducive environment in which it is used, the prodigious amounts of secondary inoculum produced on lesions that result from primary infections, increase and the greater dispersal in the host population relative to other strains, the ease of formulation and reasonable application requirements, and the rapid decline from the environment after the host is destroyed or Collego use is discontinued.

CHALLENGES TO AND CONSTRAINTS IN THE DEVELOPMENT AND LARGE-SCALE USE OF MYCOHERBICIDES

Collego exemplifies a highly successful mycoherbicide, but an analysis of the examples of failed mycoherbicides reveals a number of issues in the development and use of mycoherbicides, including the cost and feasibility of mass production, shelf-life, and feasibility of application of the mycoherbicide (Weaver et al. 2007). Many market considerations—such as the cost of the product, competing products, market size, and user preferences—that impinge on the commercialization of mycoherbicides might not apply if policy rather than market considerations dictated the development and use of mycoherbicides for illicit-drug crops.

Moreover, under the existing regulatory guidelines, scientific data in support of efficacy, safety, and statement of formula claimed in the application for EPA registration should be gathered on a strain of the pathogen identified as the mycoherbicide active ingredient. It is also necessary to characterize this strain for environmental-monitoring purposes and to deposit a voucher specimen in a leading culture collection, such as the American Type Culture Collection. For those reasons, the process of gathering data for EPA registration begins with a designated isolate or strain and typically requires years of intensive research and development efforts after the initial demonstration of the potential of a candidate pathogen. For example, Collego underwent 13 years of precommercial studies of proof of concept, small-scale efficacy assessment, and host-range determination in addition to 3-5 years of industrial research and development, trials of efficacy on a large scale for an experimental-use permit, gathering of registration data, and registration.

One constraint on the development of mycoherbicides for large-scale use is the need to find strains that will reliably infect the target plant under a variety of moisture and temperature conditions. Most fungal plant pathogens, including those used as mycoherbicides, require a combination of adequate moisture on plant surfaces (humidity and free water) and appropriate temperature (TeBeest and Templeton 1985; Greaves et al. 1989; Heale et al. 1989) for germination, infection, and disease development. Several fungal pathogens evaluated as potential mycoherbicides have been found to require at least 10 hours of moisture (generally from overnight dew formation) to cause disease at magnitudes that sufficiently damage the target plant (Boyette and Walker 1985; Makowski 1993; McRae and Auld 1988; Wymore et al. 1988; Morin et al. 1990; Zhang et al.

2002; Peng et al. 2004; Stewart-Wade and Boland 2004). Inadequate moisture and high temperature can prevent fungi from infecting plants; many mycoherbicide agents are ineffective when used in areas that have infrequent or minimal rainfall and low relative humidity. Studies done in controlled environments have shown that mycoherbicides can be less effective at some temperatures. *C. gloeosporioides* f.sp. *aeschynomene* causes rapid infection on northern jointvetch at 20-32°C and optimally near 28°C; disease development is restricted at 36°C, and this indicates the crucial role of temperature in disease development (Te-Beest 1991). Solar radiation can also affect the efficacy of mycoherbicides. A study by Stojanovic et al. (1999) showed that germination and germ-tube length of *C. gloeosporioides* were reduced by exposure to the sun. Ghajar et al. (2006) reported that UV-B exposure at some levels can deactivate spores or delay spore germination of the fungi *Colletotrichum orbiculare* and *Plectosporium alismatis*. The adverse effects of low moisture, suboptimal temperature, and solar radiation are generally mitigated by using formulations (e.g., liquid concentrates, emulsions, pellets).

Another important consideration in the development of a mycoherbicide is the possible constraint imposed by the target plant. Over time, the use of a biological control agent might select for plant genotypes that are resistant to the agent. The genotypes may be present in the existing plant population, they may emerge through mutation of existing plant genotypes, or they may be imported by agriculturists who are invested in the cultivation of the plants. There has been no report of a plant exhibiting resistance to a mycoherbicide over time, as happens with chemical herbicides, but it is known that naturally resistant plant varieties or biotypes exist. For example, three leaf forms of rush skeletonweed (*C. juncea*) occur in Australia, each with a different susceptibility to the classical biological control agent *P. chondrillina.* After the introduction of the biocontrol fungus, the prevalence of the most susceptible form A was reduced, but the more tolerant forms B and C became more widespread (Cullen and Groves 1977; Cullen 1978; Burdon et al. 1981). *P. chondrillina* was also released in western U.S. states to control *C. juncea* (Adams and Line 1984; Emge et al. 1981; Lee 1986; Supkoff et al 1988); as in Australia, the differences in the susceptibility of *C. juncea* biotypes required the introduction of more than one strain of the rust pathogen (Emge et al. 1981; Lee 1986).

Other challenges to effective control by a mycoherbicide are the effects of plant age and vigor on disease development. In some plant species, the seedling stage is the most susceptible to pathogen attack; in others, the mature plant is more susceptible (Graham et al. 2007; Holcomb 1982; Rosskopf et al. 2005; Watson and Wymore 1990). A vigorous target plant may recover quickly from nonlethal foliar infection (Auld and Morin 1995); two weeds targeted for mycoherbicidal control, velvetleaf (*Abutilon theophrasti*) and round-leafed mallow (*Malva pusilla*), reportedly overcame infection on the leaves and upper stem by growing axillary shoots (Makowski 1993; Wymore et al. 1988).

Finally, producers of illicit-drug crops have an incentive to prevent damage to their crop yields and should be expected to develop countermeasures that

reduce the efficacy of mycoherbicides. Such countermeasures could include the use of fungicides or soil fumigants to kill the mycoherbicide strains directly or the cultivation of plant varieties that are resistant to the mycoherbicides.

PESTICIDE REGULATION IN THE UNITED STATES

In the United States, the regulation of pesticides and their use is administered by EPA under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA). EPA registers mycoherbicides as biopesticides, and the proposed mycoherbicides for illicit-drug crops would fall under the EPA registration requirement. By EPA's definition, "[b]iopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants that contain added genetic material (plant-incorporated protectants)" (EPA 2011). Microbial pesticides consist of microorganisms (including fungi) that are somewhat specific to their target pests.

EPA may require an experimental-use permit for field-testing of microbial pest-control agents, including ones that are genetically altered or nonindigenous. Before field trials of such an agent are initiated, the research organization, company, or individual developing it must submit a notification to EPA so that the agency can determine whether an experimental-use permit is required.

The U.S. Department of Agriculture's Animal and Plant Health Inspection Service (APHIS) regulates the import of organisms that are genetically engineered or are considered as plant pests. The latter category includes nonnative fungi that are intended for development as mycoherbicides. An APHIS permit is required to introduce such regulated organisms into the United States and for their interstate transport.

FIFRA authorizes EPA to review and register pesticides for specific uses and to suspend or cancel registrations when a use would pose an unreasonable risk. FFDCA authorizes EPA to establish tolerances, the maximum pesticide-residue concentrations allowed to remain in or on foods or animal feeds. Under the Food Quality Protection Act as amended in 1996, the evaluation of risk includes provisions for protecting infants and children to ensure reasonable certainty of no harm. Consideration is given to aggregate exposure from multiple routes, cumulative risk from related pesticides, and the potential for endocrine-disruption effects.

Data required for the registration of biochemical and microbial pesticides by EPA are published in the *Code of Federal Regulations* (40 *CFR* 158, Subpart V). EPA's requirements and guidelines for testing (Series 885) are provided in Appendix B of the present report. The requirements include:

- Product analysis, including product chemistry and composition, analysis and certified limits, and physical and chemical characteristics.

- Pesticide-residue analysis, including chemical identity, residue nature and analytic methods in plants and animals, storage stability, and magnitudes in plants, food, and water.
- Toxicity, which involves three tiers of testing (see Box 2-1).
- Toxicity and pathogenicity to nontarget organisms and environmental fate, which involves four tiers of testing (see Box 2-1).

In compliance with the Endangered Species Act of 1973, EPA must also assess potential harm to endangered or threatened species from pesticides that it registers.

BOX 2-1 Requirements for Tiered Toxicity Testing and Tiered Toxicity and Pathogenicity Testing under FIFRA (40 CFR 158, Subpart V)

Toxicity Testing:

- Tier I: includes acute oral and pulmonary toxicity and pathogenicity tests, cell culture for the technical grade of the active ingredient, five types of acute toxicity tests (oral, dermal, and inhalation toxicity and dermal and eye irritation), and hypersensitivity tests.
- Tier II: consists of acute toxicity and subchronic toxicity-pathogenicity tests; these are required on the basis of the substantial route-specific toxicity, infectivity, or unusual persistence found in Tier I.
- Tier III: consists of tests for reproductive fertility, carcinogenicity, immunotoxicity, and infectivity-pathogenicity analysis; tests are conditionally required on the basis of findings in Tier I and Tier II.

Toxicity and Pathogenicity Testing:

- Tier I: generally includes studies of effects on birds, freshwater fish, invertebrates, and honeybees; other tests might be required on the basis of potential exposure and use; nontarget-plant testing is required if the microbial pesticide is taxonomically related to a known plant pathogen; nontarget-insect testing is not required unless the mechanism of pesticidal action on the target insect pest is through infectivity; depending on the results of Tier I, Tiers II-IV might be required.
- Tier II: includes environmental expression.
- Tier III: includes avian chronic pathogenicity and reproduction tests, aquatic-invertebrate range and ecosystem tests, and fish life-cycle tests.
- Tier IV: includes terrestrial wildlife and aquatic field tests and simulated or field tests for birds, mammals, aquatic animals, insect predators, parasites, insect pollinators, and plants; Tier IV testing might be required either for registration or for postregistration monitoring.

See Appendix B for more details.

Although the EPA requirements for pesticide registration (under which the requirements for biopesticides fall) do not include product-performance data (efficacy data), EPA reserves the right to request performance data case by case during the registration process (Part 158 – Data Requirements for Pesticides, Subpart E – Product Performance). In other countries, such as Canada and the United Kingdom, agencies that regulate pesticides (including biopesticides) require submission of product-performance data as part of the registration process.

Pesticides must be registered by both EPA and states before use. States may place more restrictive requirements on pesticides than EPA. Most states conduct a review of pesticide labels to ensure compliance with federal labeling requirements and any additional state restrictions on use. States that have adopted and are implementing pesticide-use regulations may be delegated primary enforcement responsibility for use violations. Enforcement may also be delegated to states under a cooperative agreement with EPA in connection with specific pesticides.

In the United States, the National Environmental Policy Act (NEPA) requires all federal agencies to consider the environmental impact of any proposed agency action before taking such action and to prepare an environmental-impact statement for any proposed action that is expected to affect the environment substantially (42 *USC* § 4332(2)(C)). Although NEPA applies to domestic federal activities, it also has extraterritorial applications in some instances, through Executive Order 12114, related to environmental effects abroad of major federal actions (44 FR 1957, 3 *CFR*, 1979 Comp., p. 356). Executive Order 12114 requires that responsible officials of federal agencies be informed of environmental considerations and take those considerations into account when making decisions on major federal actions that could have environmental impacts anywhere beyond the borders of the United States. NEPA case law has reinforced the need to analyze environmental impacts of federal actions, including the decision-making process, regardless of geographic boundaries (Eccleston 2008).

CONSIDERATIONS FOR INTERNATIONAL TESTING AND USE OF MYCOHERBICIDES

The International Organization for Biological Control of Noxious Animals and Plants (IOBC) is a professional organization that promotes the development of biological control and its application in integrated pest management. IOBC serves as a clearinghouse for information on biological control, organizes conferences and symposia, and publishes a journal. It has worked with various organizations in developing standards for testing of pesticides and guidelines for transport and release of biological control agents.

At the international level, applicable guidelines and requirements for testing, approval, and application of mycoherbicides against illicit crops fall under the International Plant Protection Convention (IPPC), the International Standards for Phytosanitary Measures (ISPM), and possibly the Biological Weapons

Convention of 1972 (BWC). The IPPC (ratified by the United States in 1972 and amended in 1987) creates an international framework to prevent the spread and introduction of plant and plant-product pests. It is based on exchange of phytosanitary certificates between importing and exporting countries' national plant protection offices (NPPOs). The provisions of the IPPC extend to any organism capable of harboring or spreading plant pests, particularly where international transportation is involved (Article I of the IPPC, 1997). NPPOs established according to the IPPC have authority in relation to quarantine control, risk analysis, and other measures to prevent the establishment and spread of invasive alien species that, directly or indirectly, are pests of plants. The IPPC further provides that NPPOs have authority to prohibit or restrict the movement of biological control agents and other organisms that might pose a threat to plants.

Specific guidelines are found in the ISPM, promulgated by the UN Food and Agriculture Organization. ISPM No. 3 "provides guidelines for risk management related to the export, shipment, import and release of biological control agents and other beneficial organisms. . . . The standard addresses biological control agents capable of self-replication (including . . . pathogens such as fungi . . .) and includes those packaged or formulated as commercial products. Provisions are also included for import for research in quarantine facilities of non-indigenous biological control agents and other beneficial organisms" (FAO 2006a, p. 25). However, the standard does not address genetically modified organisms or issues specifically related to biopesticide registration.

Responsibilities relating to ISPM No. 3 (2005) are held by contracting parties, NPPOs, or other responsible authorities and by importers and exporters. The standard delineates the responsibilities of the NPPOs of the exporting and importing countries to implement and document adherence to the standard. Those responsibilities include carrying out pest risk analysis of biological control agents before import or release; ensuring compliance with phytosanitary import requirements; overseeing appropriate documentation relevant to the export, shipment, import, or release of biocontrol agents; ensuring that biocontrol agents are transported appropriately; and encouraging the monitoring of released agents (Vapner and Manzella 2007).

The 2005 ISPM revision also provides recommended guidelines for safe use of biological control agents and other beneficial organisms. In addition to phyosanitary concerns, the standard includes guidelines concerning potential effects on nontarget organisms and possible consequences to habitats or ecosystems (FAO 2006a). ISPM No. 3 refers to other standards on pest risk analysis (ISPM No. 2, *Guidelines for Pest Risk Analysis*; and ISPM No. 11, *Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Living Modified Organisms*) (FAO 2006b, c), which provide the fundamental processes for carrying out the relevant pest risk assessments, including determination of environmental risks.

The 1972 BWC, as ratified by the United States in 1975, provides that state parties to the convention undertake "never . . . to develop, produce, stockpile or otherwise acquire or retain: (1) Microbial or other biological agents . . .

of types and in quantities that have no justification for . . . protective or other peaceful purposes; (2) Weapons, equipment or means of delivery designed to use such agents . . . for hostile purposes or in armed conflict." However, the BWC does allow for "international exchange of bacteriological (biological) agents and toxins and equipment for the processing, use or production of bacteriological (biological) agents and toxins for peaceful purposes." That provision has been interpreted as excluding biological control agents used in agriculture, presumably even mycoherbicides used by consenting governments against illicit crops.

However, possible legal implications of using mycoherbicides in border regions, where the potential for drift into nonconsenting countries exists, need to be considered. For example, Ecuador recently filed a lawsuit against Colombia in the International Court of Justice in The Hague, Netherlands, seeking to end Colombia's application of chemical herbicide against coca crops growing along the border between the two countries (Ecuador v Colombia, pending). Ecuador claimed that herbicide drift had killed legal crops in Ecuador and resulted in illness of Ecuadoreans living near the border. It is likely that use of mycoherbicides in similar circumstances could provoke a similar suit. There are potential international legal implications, which should be explored, about whether intentional application of a mycoherbicide that drifts into an unwilling country would still fall under the "peaceful purposes" exemption of the BWC.

RISK TO NONTARGET PLANTS AND ORGANISMS

This section reviews some aspects of the scientific literature regarding potential exposure pathways and risks to nontarget plants and organisms (ecological receptors) posed by the introduction of biological control agents and provides a conceptual model to describe the pathways at a specific site (insofar as that is possible given current data constraints).

Review of Ecological Pathways and Risks

Evaluations of potential ecological effects of biological control agents usually recognize that information on host specificity alone is inadequate for identifying and addressing risks because the risks are often propagated through the food chain or express themselves in other indirect effects (such as changes in competition or displacement of prey). Although much of this work addresses risk from classical control approaches (e.g., Wright et al. 2005; Seymour and Veldtman 2010), many of the conclusions are applicable to the bioherbicide approach. Some information is specific to mycoherbicide application and includes assessment of its effects on community levels of organization, such as changes in fungal community structure in the soil as a result of the introduction of a fungal biocontrol agent (e.g., Schwarzenbach 2008).

Some general observations on the potential for ecological exposures and risks posed by the application of biological control agents to control weeds or plant pathogens or pests are emerging:

- *The potential for indirect risks to nontarget species even if the biological control agent is highly host-specific.* Pearson and Calloway (2005) reviewed case studies demonstrating the nontarget effects of biological control agents and evaluated them in the context of theoretical work in community ecology. Their analysis suggests that the magnitude of the indirect effects on nontarget organisms is indirectly proportional to the abundance of the biological control agent; this suggests that the more efficacious agents are less likely to cause indirect effects. They conclude that "[b]iological control agents that greatly reduce their target species while remaining host-specific will reduce their own populations through density-dependent feedbacks that minimize risks to nontarget species" (Pearson and Calloway 2005, p. 288).
- *The classical biological-control literature suggests that a high level of sophistication is available for assessing risks.* The methods include population models (e.g., Johnson 1994) and the application of probabilistic techniques (e.g., Wright et al. 2005). Such techniques have not been explicitly used to assess the risks associated with the introduction of mycoherbicides, but the principles derived from the classical control literature could be transferred to such assessments. The application of such techniques requires a detailed understanding of the toxicology, environmental fate, and biological interactions of the proposed strains of mycoherbicides. For example, using a probabilistic risk assessment of the application of mycoherbicides would require detailed statistical knowledge of the range and type of distribution of measurements that described the biology and ecological interactions of the mycoherbicides (for example, the range and distribution of values that describe toxic responses of nontarget organisms, temporal persistence, and environmental dispersal).
- *The construction of food webs helps in assessing the safety of biological control agents.* Willis and Memmott (2005, p. 299), working with examples of classical biological control, recommend "that constructing and analyzing food webs may be a valuable addition to standard biological control research techniques, as they offer a means of assessing the post-release safety of control agents. . . . [F]ood webs can be used to generate testable hypotheses regarding indirect interactions between introduced agents and non-target species."
- *Establishment and dispersal of a biological control agent are critical properties that contribute to the potential for indirect ecological effects.* Van Lenteren et al. (2003), in their development of an environmental risk assessment of inundative biological control, note that the critical issues are the probability of attack on nontarget organisms, the dispersal of the biological control agent, and the establishment capacity of the agent. They state that the time scales for establishment are critical and might range from one generation (no reproduction) to seasonal or long-term survival and reproduction. Evaluation of the potential

for establishment requires knowledge of the abiotic and biotic requirements of the control agent's life cycle and knowledge of the capacity of the area of release to meet the requirements. They recognize that it might be necessary to conduct laboratory and field tests to determine and prove whether a control agent is capable of establishing itself in a particular area. They also describe the range of potential indirect effects of changes in competition and energy flow due to the introduction of a biological control agent.

- *Modeling and field studies provide site-specific methods for assessing risk, especially risk of increased load of mycoherbicide inoculum, to nontarget areas.* De Jong et al. (1990) describes a risk-analysis method for biocontrol with mycoherbicides that incorporates field studies, surveys, and air-dispersal model simulations for estimating the dispersal of spores to nontarget areas and some preliminary assessment of the relative inoculum load to these areas. The method was applied to assess the risk of a mycoherbicide application to downwind market garden crops by calculating safety zones on the basis of the ratio of added to naturally present spores (De Jong et al. 1999, 2002). Bourdôt et al. (2000) described field experiments to assess the survival of the mycoheribicde in soils in such downwind areas. The studies demonstrate the feasibility and the level of effort necessary for analyzing risks associated with the application of a particular mycoherbicide in a specific area and risks to specific, local nontarget organisms.
- *Recent reviews identify and categorize the information necessary for conducting risk assessments of biological control agents.* McCoy and Frank (2010) address the question of how risks associated with biological control agents should be estimated (largely on the basis of the introduction of such agents into Florida). They concluded that the ability to assess risks associated with the introduction of biological control agents is related primarily to the understanding of how an agent will behave after application. They note that this understanding is often based on inadequate knowledge derived from inadequate testing of the specificity of the biological control agent, inadequate appreciation of basic ecological relationships, and a rush to solve acute pest problems without proper consideration of the consequences (for example, the consequences of using only moderately effective agents or of releasing highly mobile agents).

Conceptual Model

Risk assessments generally involve four major steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization. Hazard identification is an assessment of whether an agent causes an adverse effect and is typically based on laboratory and field observations of adverse effects and exposures to the agent. Dose-response assessment involves evaluating the relationship between the dose of the agent and the incidence of the adverse effect in the population of concern. Exposure assessment involves the prediction of route and level of exposure that might be experienced under different conditions. The

final step of risk characterization involves the integration and synthesis of the information and analysis in the first three steps to describe the estimated incidence of an adverse effect in a given population (NRC 1994).

This section focuses on exposure assessment. Exposures to stressors (such as chemical agents, pathogens, introduction of invasive species, and physical stresses) are generally defined by a conceptual model that describes which ecological and human receptors have the potential to be exposed. It typically includes a narrative description and diagram of:

- General ecological conditions and potential human exposure scenarios.
- The stressor in question.
- Potential sources of the stressor, transport pathways, and affected media.
- Ecological exposure routes between affected media and representative receptors.
- Identification of representative ecological receptors.
- Human exposure routes between affected media and likely human receptors.

Conceptual exposure models are integral to assessing exposure and risk within various federal and state agency frameworks in the United States. Guidance on the development of conceptual models is available from several agencies and departments, such as EPA (1995, 2005d), the U.S. Army Corps of Engineers (Cura et al. 1999), and the U.S. Navy (DeGrandchamps and Barron 2005). The conceptual model gives or allows for the following:

- Format for summarizing exposure pathways that are familiar to regulators and scientific reviewers.
- Discussion of data gaps and recommended further work (field work and risk assessment approach).
- An explicit understanding of the site-specific fate and transport mechanisms upon which regulators and stakeholders can reach agreement.
- Framed recommendations of potential exposures that are site specific.

Figure 2-1 is an example of a conceptual model that might be used for the proposed mycoherbicides. It allows one to trace the physical exposure pathways (independent of geography) of toxins, spores, and vegetative bodies from a particular application method through potential physical transport mechanisms to potentially affected environments. It generally defines the exposure routes and the general classes of ecological and human receptors. The dotted lines and question marks in the figure show where there is particular uncertainty about a pathway. As will be shown in later chapters, there is little information on the proposed mycoherbicides and the specifics of their behavior in the environment

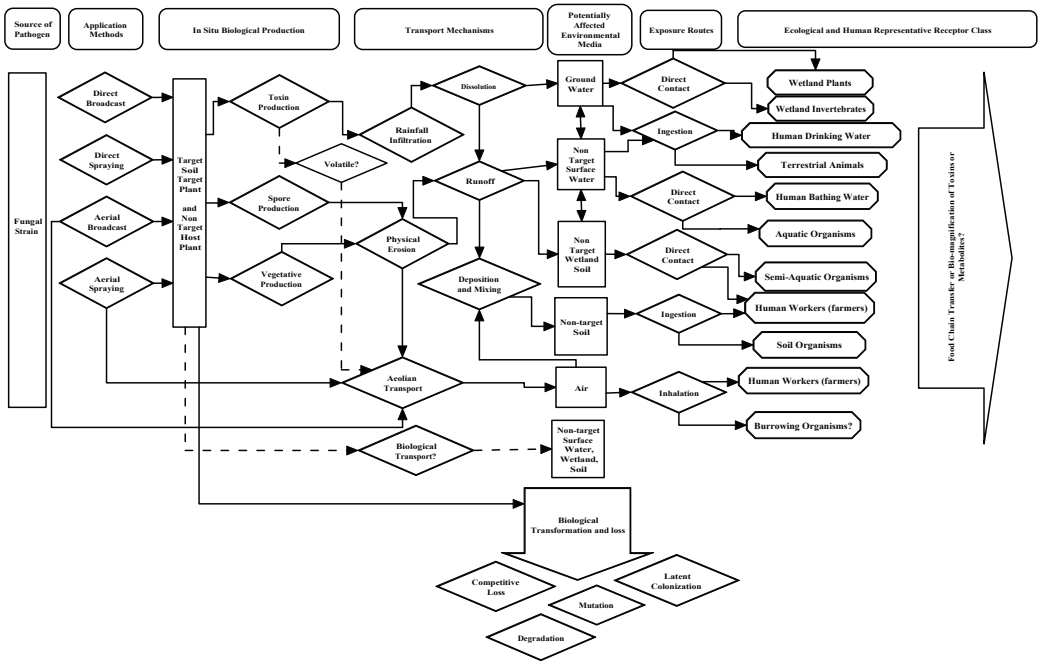

FIGURE 2-1 An example of a generalized conceptual model for mycoherbicide exposure that traces the potential physical exposure pathways of toxins, spores, and vegetative bodies from a particular application method to the general classes of ecological and human receptors. Dotted lines and question marks show where there is likely to be a particular lack of information about a pathway. There is a lack of strain-specific information to provide detailed description of the potential exposures, but the figure provides a guide to help in identifying the type of information needed to characterize the potential exposures.

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that would be needed to describe the potential exposures beyond generalizations. If a concerted effort to develop the mycoherbicides is embarked on, Figure 2-1 could be used as a guide to identify where the data gaps are and the types of information that are needed to characterize potential exposures.

Classical or Bioherbicide Approach

The registered mycoherbicides discussed earlier (Table 2-2) were all developed from pathogens indigenous to the regions where they were proposed to be used. Unless a decision is made to develop only strains indigenous to the relevant crop-producing areas, a mycoherbicide based on a nonindigenous strain may require more comprehensive testing to assess the risk to nontarget plant species than would a mycoherbicide based on indigenous strains. The higher level of testing would be comparable with the current testing requirement for the release of a classical weed-biocontrol agent. Such extensive testing may not be required in regions where the mycoherbicide pathogen is indigenous and has never been found to pose a risk to nontarget plant species. It is presumed that the proposed mycoherbicide strains would come from the population of fungi found in the geographic area where the target crop is being grown. If the mycoherbicide were released in areas where the pathogen is not native, estimating the potential for disease in nontarget crops caused by the released mycoherbicide strain or strains descended from it would be far more complicated.

3

Target Illicit Crops

This chapter examines crops of cannabis (*Cannabis sativa*), coca (*Erythroxylum* spp.), and opium poppy (*Papaver somniferum*) grown for production of illicit drugs. Cultivation of these plants for drug use has made them targets for interdiction, but their licit cultivation and use as part of cultural and spiritual heritages or for culinary, industrial, medicinal, and ornamental purposes go far back into human history. Whereas cultivation of coca is restricted largely to its native phytogeographic region in South America, opium poppy and cannabis are grown worldwide as licit crops or for illicit drug trade (UNODC 2010a). The biology, cultivation practices, growth conditions, uses, and other information relevant to this report are discussed in this chapter.

CANNABIS: *CANNABIS SATIVA*

Biology

Cannabis sativa (including industrial hemp and cannabis herb or marijuana) is a dioecious (having male and female parts in separate plants) annual dicot with distinctly palmate leaves (Figure 3-1). It is frequently found alongside cultivated fields because of the high soil nitrogen content and in disturbed soil. Depending on the cultivar and growing conditions, the number of leaflets per leaf can range from three to 13, and the leaflets are up to 10 cm long. The first pair of leaves (bottom, in opposite arrangement) and the last pair of leaves (top, in alternate arrangement) usually have only one leaflet each.

The height of a mature plant can range from 1 to 5 m, depending on environmental and genetic factors. Male plants are more visibly flowered and are usually taller than female plants; the male flowers have five anthers to disperse pollen and five yellowish tepals. Hanging nearly 30 cm long, the inflorescence of male flowers are multibranched loose clusters (UNODC 2006). The pollen from male flowers is released, and the male plant dies soon after (UNODC 2006). Female plants have smaller leaves, and the flowers are more tightly

packed than flowers on the male plants. Flowering is induced by lengthening dark periods to 11-13 hours to simulate the end of summer and the beginning of autumn (UNODC 2006).

Throughout human history, cannabis has been used for textiles, paper, medicine, and food (Abel 1980). It was originally native to central Asia and historically was cultivated in China, the rest of Asia, and Europe. It has become more widespread, growing in temperate, tropical, and subarctic climates (Duke 1983). Most botanists consider cannabis as a single species, *Cannabis sativa,* but some (see McPartland and West 1999) consider *C. sativa* as one of three species in the Cannabaceae family that is used to produce marijuana.

Cultivars of *C. sativa* that grow more than 6 m in height produce a durable fiber called hemp. The production of hemp predates written history and is thought to have begun in China. Early Chinese texts indicate that hemp was cultivated to make cloth, the stalks were used as fuel, and the seeds were used for oil and food. In the present report, *cannabis* refers to the plant species, *hemp* refers to industrial hemp, and *marijuana* refers to the psychotropic drug (recreational or medical).

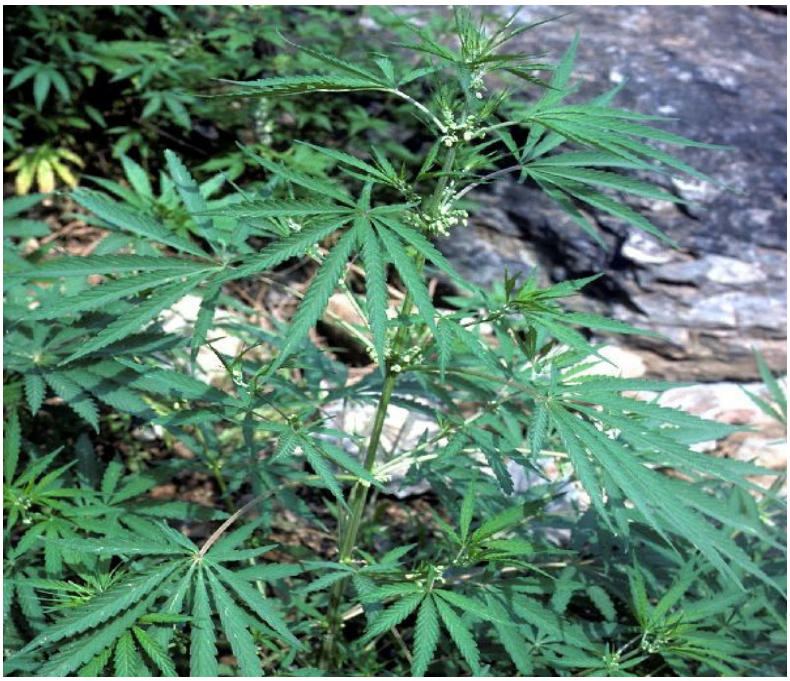

FIGURE 3-1 *Cannabis sativa.* Source: Richard A. Howard Image Collection, courtesy of Smithsonian Institution.

By the 16th century, hemp was being cultivated and widely used in Europe for the fiber and for the seeds, which were cooked with grains and eaten. During the 16th and 17th centuries, hemp production made its way to North America and South America. In the United States, hemp production provided weaving fiber for New England Puritans but could not displace the flax already in use. Cultivation spread to Kentucky and other states but did not take a strong hold, particularly as cotton production increased in the South. The Marijuana Tax Act of 1937 required all hemp producers to register with the U.S. Department of the Treasury Department; hemp production since then has been negligible in the United States.

Over 400 compounds are found in cannabis, 60 of which are peculiar to cannabis and are called cannabinoids (Turner et al. 1980; UNODC 2006). Cannabinoids exist in the form of carboxylic acids that readily decarboxylate when heated (De Zeeuw et al. 1972; Kimura and Okamoto 1970), in alkaline environments (Grlic and Andrec 1961; Masoud and Doorenboos 1973), and over time (Masoud and Doorenboos 1973; Turner et al. 1973). Δ-9-Tetrahydrocannabinol (THC) is the main cannabinoid that produces the psychoactive effects of cannabis-based products (UNODC 2006).

Cannabis produces a variety of secondary metabolic compounds, including flavonoids (Gellert et al. 1974; Paris et al. 1975), alkanes (Adams and Jones 1973; De Zeeuw et al. 1973; Mobarak et al. 1974a, 1974b), and nitrogenous compounds (Hanus 1975a, 1975b). Terpenes add to the characteristic odor of cannabis (Hood et al. 1973) and are abundant in the plant (Hanus 1975a; Hendricks et al. 1975) and in some of its preparations (such as hashish). Ecological factors and heredity are thought to contribute heavily to the production of these compounds (Fetterman et al. 1971; Small and Beckstead 1973).

Illicit Cultivation

There are two major products of illicit cannabis cultivation: resin and herb (marijuana). Resin is the pressed secretion of the cannabis plant, also commonly called hashish or charas in India, whereas the herb refers to the leaves and flowers of the plant (UNODC 2006).

Afghanistan is the largest producer of cannabis resin in the world (UNODC 2010a; see Table 1-1). Morocco has at least 3 times as much land in cultivation, but Afghanistan's resin yields are 4 times as high per hectare (UNODC 2010a). Cannabis cultivation occurs in 17 of the 34 provinces of Afghanistan on an estimated 10,000-24,000 hectares. Mexico, Paraguay, Colombia, the Netherlands, Bolivia, Canada, and the United States are producers of cannabis herb; Mexico and Paraguay produce the majority (UNODC 2010a).

Cannabis can be cultivated nearly anywhere, including indoors. Seeds are readily obtained from marijuana samples or even by mail order in some countries. Cannabis plants can tolerate suboptimal soils (such as sand) but flourish in

loams that have high nitrogen content (UNODC 2006). The plants need soil that has good drainage; otherwise, the roots will rot. Hardy varieties can withstand freezing temperatures for brief periods, but ideal growing temperatures are 14-27°C (UNODC 2006). The most important environmental factor in the cultivation of cannabis plants is the length and intensity of light to which they are exposed (UNODC 2006). Outdoor growers rely on sunlight, which, depending on location, can vary in length and intensity. Indoor growers, however, have precise control over the light wavelength and intensity to which their plants are exposed; this is particularly important for the first phases of germination and growth.

Cannabis is cultivated in Afghanistan as an annual summer crop. It is usually planted after the winter crops (wheat and opium poppy) are harvested (UNODC 2010b). In the southern region of Afghanistan, there is a large overlap of opium poppy and cannabis cultivation. Mixed cropping is not extremely common, but some farmers plant cannabis alongside other crops, including maize, barley, cotton, peanuts, tobacco, sesame, and vegetables (carrots, cucumbers, tomatoes, potatoes, and eggplant). Cannabis provides effective protection from predatory insects, and mixed cropping helps to shield the cannabis cultivation from detection (UNODC 2010b).

Cannabis can be cultivated in several ways. Outdoor cannabis cultivation is usually irrigated and can vary in density of plants; in areas where eradication is a concern, plants are less densely cultivated (UNODC 2006). "Guerilla" cultivation involves growing cannabis on land that does not belong to the grower. This land includes private property and public lands, such as parks (UNODC 2006). In 2003, 200,000 cannabis plants were eradicated from Daniel Boone National Park in Kentucky, and another 400,000 plants are destroyed each year in California parks (DOJ 2005). Public lands in Canada and Colombia also experience illicit cannabis cultivation.

Among the three drug crops considered in the present report, cannabis might be the most difficult to control with mycoherbicides because it is grown more widely in more regions and under wider geographic conditions than coca or opium poppy. A cannabis mycoherbicide should be capable of performing under varied environmental conditions and against several host genotypes. A large amount of cannabis is cultivated indoors, where use of a mycoherbicide would be irrelevant.

Germination is the first step in cultivating cannabis plants. Seeds are sown generally from March to May, and the plants need long daylight hours during this growth period. Germination takes 2-3 weeks, and roughly 36 plants can fit within 1 m^2 (UNODC 2006). Cloning is used as an alternative to germinating new plants and has several advantages: duplicating a known productive individual, reducing flowering because a near-mature individual is being planted, and guaranteeing a female plant. The next phase is vegetative growth, during which the cannabis plants will continue to grow and reach mature size (UNODC 2006). During this time, the plants continue to require long daylight hours. As they grow, the plants need more space; once mature, only nine plants will fit within 1 m^2 (UNODC 2006). Indoor growers have the advantage of being able to ma-

nipulate the photoperiod to provide longer periods of darkness, which can accelerate the plants' progress to the flowering stage (UNODC 2006).

High concentrations of cannabinoids build up in the small, mushroom-shaped glands called trichomes on the flowers. The trichomes are made up of cellular sacs and must be harvested at the correct time to prevent a drop in the THC concentrations. The harvested flowers are then hung upside down to dry for several days. For resin production, the secretions will be collected from the trichomes when they are wet or after they have dried on the plant (UNODC 2006).

Licit Cultivation

Commercial cultivation of cannabis herb is generally not permitted in most countries. Several countries have a "medical" designation available for cannabis use (but do not provide for commercial production). A patient must carry a medical card or prescription and may possess no more than a specified quantity of the herb. Health Canada, for example, contracts with Prairie Plant Systems Inc. to produce medical marijuana for people licensed to consume it. Alternatively, eligible patients may apply to grow medical marijuana for their own use (Health Canada 2010). The use of mycoherbicides to control the production of cannabis in the United States would not affect any federally legal production, but it would potentially be detrimental to production sites that are licensed by individual states.

Industrial hemp is cultivated in 30 countries. It is harvested for the fiber, seeds, and oil, which can then be made into a variety of products, including clothing, paper, food, and cosmetics. Detailed production data are not readily available on all countries. China is estimated to be one of the largest producers and exporters of hemp products. Most member countries in the European Union (EU) participate in cultivation of industrial hemp on a total of roughly 18,500 hectares (EIHA 2009). France, the United Kingdom, Romania, and Hungary are the largest EU producers, but cultivation also occurs in Austria, Denmark, Finland, Germany, Italy, the Netherlands, Poland, Portugal, Slovenia, and Spain (Johnson 2010). Non-EU producers include Russia, Ukraine, Switzerland, Australia, New Zealand, India, Japan, Korea, Turkey, Egypt, Chile, and Thailand (Johnson 2010). Canada has licensed 10,856 hectares for hemp cultivation; 90% of the yield is exported to the United States (Brook 2008). Hemp is commonly grown in rotation with other crops, such as corn, soybeans, and wheat (Bosca and Karus 1998; Lotz et. al. 1991).

Hemp cultivation is not illegal in the United States, but it requires a permit from the Drug Enforcement Administration (DEA), given oversight of all cannabis cultivation under the Controlled Substance Act of 1970. Thus far, only research plots have been permitted; no commercial growers have been granted permits. Despite the federal law, nine states have legalized hemp production (Hawaii, Kentucky, Maine, Maryland, Montana, North Dakota, Oregon, Ver-

mont, and West Virginia). North Dakota's law does not require growers to obtain the DEA permit to gain the state permit (Johnson 2010).

COCA: *ERYTHROXYLUM* SPECIES

Biology

There are as many as 250 species of *Erythroxylum* in the family Erythroxylaceae, of which about 200 are native to the American tropics (Plowman and Hensold 2004). Two species, *E. coca* and *E. novogranatense,* and a number of varieties within them are used for the production of cocaine (Plowman 1979). *E. coca* is cultivated most commonly and has two varieties, *E. coca* var. *coca* and *E. coca* var. *ipadu*, which are nearly impossible to distinguish by physical characteristics. *E. novogranatense* has two subspecies, *E. novogranatense* var. *novogranatense* and *E. novogranatense* var. *truxillense* (Plowman and Rivier 1983).

Coca plants are small, evergreen shrubs that have reddish-brown bark and small branchlets featuring alternate leaves that are elliptical-obovate (Figure 3-2), 10-18 cm long, and 7.5-10.5 cm wide (Rottman 1998). There are regional variations in leaf structure: the Bolivian plants have larger and more pointed leaves than the Colombian plants. The flowers are small and greenish yellow, and the fruits are red drupes. Although *E. novogranatense* var. *novogranatense* and *E. novogranatense* var. *truxillense* have higher cocaine alkaloid content than *E. coca* varieties, they are not the preferred varieties for illicit coca production, because of their high content of cinnamoylcocaines, also referred to as "uncrystallizable cocaine" (Johnson et al. 2003). In addition to varietal differences, coca grown in low-lying areas, such as the Amazon region, tends to produce less cocaine than that grown at higher elevations, on the upper slopes of the Andes (Plowman and Rivier 1983). Since 2004, cultivation of coca in the lower-lying Amazon areas of Colombia has decreased while production on the eastern slopes of the Andes in Peru has increased (UNODC 2010a).

FIGURE 3-2 *Erythroxylum coca.* Source: Richard A. Howard Image Collection, courtesy of Smithsonian Institution.

Historically, coca grown in the Andean valleys was for personal or local consumption; during the Inca period, the use of coca was seen as a privilege and was associated with spiritual rites (Gootenberg 2008). The coca leaf is chewed in South America for its nutrients in addition to its stimulatory and hunger-suppressing properties (Plowman 1979). During early European colonization (16th century), Spanish land-grant holders took control of coca plantations (Karch 1998).

Pure cocaine alkaloid was first isolated in 1859 (Bennett 1873). The concentration of cocaine in coca leaves ranges from 0.13 to 1.5% (Acock et al. 1996). Cocaine stimulates the central nervous system and can act as an appetite suppressant. A variety of other alkaloids are also found in coca leaves, including methylecgonine cinnamate, tropinone, tropacocaine, cuscohygrine, hygrine, and nicotine (Novak et al. 1984; Johnson 1996). Tropinone is a precursor of atropine, a muscle relaxer and antispasmodic drug. Nicotine is an addictive alkaloid found in tobacco that acts as both a stimulant and a depressant.

Illicit Cultivation

Large-scale cultivation of coca for the drug trade appears to have started after World War II (Gootenberg 2008). Today, coca is cultivated in its greatest quantity in Colombia, Peru, and Bolivia (UNODC 2010a; see Table 1-2). Nearly 90% of the cocaine that enters the United States comes from Colombia (U.S. Department of State 2010). Production has decreased in Colombia and increased in Peru over the last 3 years, reducing the gap between the two countries in cultivated hectares (UNODC 2010a). The main growing areas in Colombia are in the departments of Putumayo, Caqueta, Guaviare, and Vaupes. The main growing regions in Bolivia are Yungas de La Paz, Chapare, and Apolo. Cultivation in Peru takes place in northern Peru, including the Upper Huallaga Valley region, and in the valleys of La Convencion and Lares (DEA 1993).

The northern and central regions of the Andes mountain range feature areas with sudden changes in altitude, and the accompanying ecozones vary greatly in topography and environment (OTA 1993). The latitudinal and longitudinal expanse encompassed in these regions—from the low-lying rain forest and desert areas to the uppermost mountain tundra (with snow and ice at the highest altitudes)—provides diverse conditions in which land use, climate, soil, and vegetation vary greatly (OTA 1993; UNODC 2010a). Coca bushes are cultivated in many of these settings in Colombia, Peru, and Bolivia (Figure 3-3).

The northern Andes encompass the region from coastal Colombia south to northern Peru; the coldest areas reach 4,500 m above sea level (OTA 1993). In general, the northern Andes region differs from other regions in that it has higher relative humidity and greater similarity of climate in its western and eastern portions. In the upper altitudes, precipitation is heavy, averaging 400 cm a year. The central Andes extend south from northern Peru into Chile and Argentina. This region features agricultural zones that have diverse environmental

conditions and plateaus at high altitudes (over 3,500 m above sea level), which are not present in the northern Andes (OTA 1993). The western part of the Central Andes has desert-like soils and a relatively dry climate. The eastern regions of the Central Andes feature varied soils (Abruzzesse 1989) and are more similar to the moist woodlands of the northern Andes (with the addition of a dry season). Valleys cut through the high plateaus of this region, descending thousands of meters in relatively short distances.

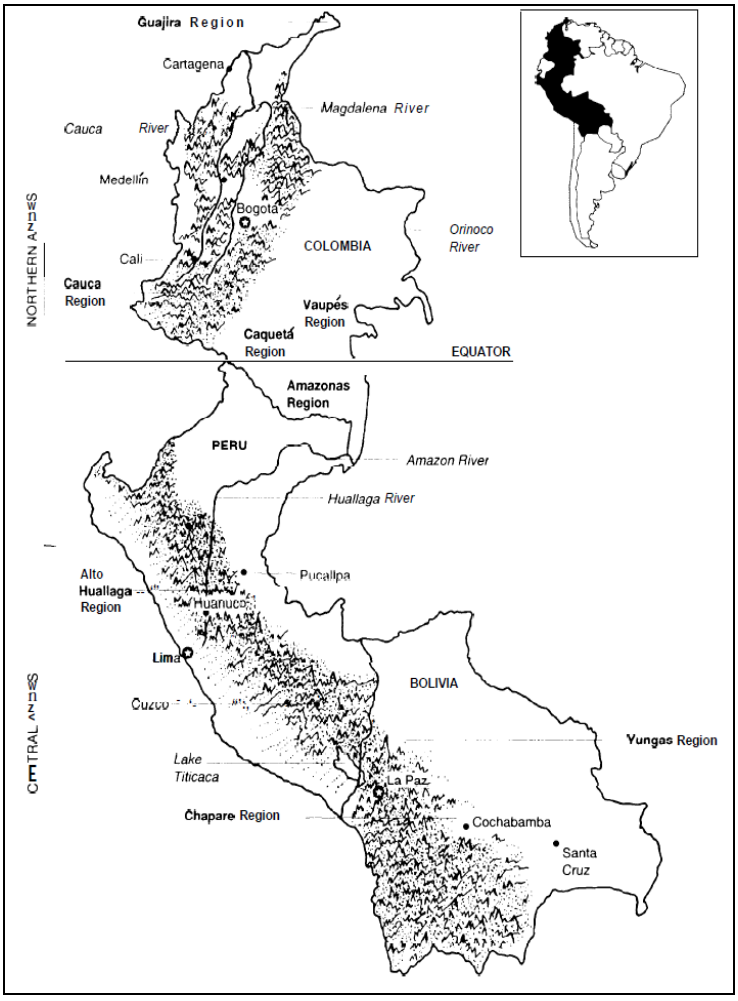

FIGURE 3-3 Major coca-producing countries in the Andean region. Source: Office of Technology Assessment 1993.

The production of coca can be detrimental to the surrounding environments. Coca farmers frequently use slash-and-burn techniques, which gravely, perhaps irreparably, damage the environment and ecosystem (UNODC 2008), particularly in the humid Amazonia and the Andes regions, which are rich in biodiversity (Fjeldsa et al. 2005). Deforestation of land contributes to soil erosion and the accompanying loss of soil fertility (OTA 1993). Coca growers also use large quantities of pesticide, and these chemicals are leached from the plants and soil into the surface water and the groundwater (OTA 1990). Processing of coca into cocaine also produces chemical wastes (including sulfuric acid, toluene, hydrochloric acid, and kerosene) that can end up in the water system and cause reduced oxygen, increased pH, and poisoning of fish (OTA 1990).

There is evidence of cultivation in the national parks and protected areas of those nations, which puts vulnerable ecosystems at increased risk (UNODC 2008). In 2007, coca was found in 16 of Colombia's 51 national parks, where it accounted for 4% of the total coca cultivation in Colombia (UNODC 2008). Some 21% of the total coca cultivation in Bolivia in 2007 occurred in national parks and protected areas, but that was down by 21% from the previous year (UNODC 2008). Eradication has effectively reduced cultivation.

The cultivation of coca often takes place in an agricultural matrix. Fields of coca are interspersed with food crops and scrub habitats (grasses, bushes, and stunted trees), which provide an environment in which the growers can hunt animals to supplement their diets (Fjeldsa et al. 2005). In the Apolo region (Bolivia), cassava (*Manihot esculenta*) is commonly grown alongside coca so that the same furrows can be used for both crops (UNODC 2008). Alternatively, farmers in Chapare (Bolivia) grow coca under the cover of leguminous trees, taking advantage of the ability of the trees to disguise the coca field and its ability to improve nitrogen content in the soil (UNODC 2008). More advanced technology and agricultural techniques are being used by coca farmers to improve yield, including the use of irrigation, pesticides, and fertilizer (UNODC 2008).

Coca is grown from seeds, which are gathered in December-March from young, 2- to 3-year-old coca plants (DEA 1993). The seeds can be directly planted in the prepared plots, where they are protected from direct sunlight. Once the plants have reached at least 30 cm in height, they are transplanted into a prepared field, usually in the rainy season. Farmers often use cuttings (cloning) to start their growing fields to save time and resources. Plants mature and the leaves are ready to harvest 12-24 months after being transplanted. Coca plants can grow several meters tall but are usually kept pruned down to 1-2 meters for ease of harvesting (DEA 1993). Plants are harvested two to six times a year (DEA 1993; OTA 1993, UNODC 2008). The yield depends on environmental factors (particularly precipitation) and anthropogenic factors (such as farming practices and interdiction efforts).

Processing methods vary by locale. In Colombia, the fresh leaves are immediately processed and the alkaloids extracted, usually by the farmers. In Peru and Bolivia, the leaves are dried (either by sun or by warm air), and the alka-

loids are extracted from the dried leaves. Great care is taken during the drying process to ensure that wet weather does not keep the leaves from drying. Once dried, the leaves are much lighter, and this makes their transport easier. The cocaine content of the dried leaves is fairly stable, but improper handling (for example, in excessive temperature or humidity) can result in the rapid decomposition of cocaine in the leaf.

Licit Cultivation

Coca may be legally cultivated in some countries for traditional purposes. For example, in Bolivia, Law 1008 provides that 12,000 hectares of coca may be cultivated for traditional use, such as chewing, tea, and medicines (UNODC 2008). The law does not define geographic limits for legal cultivation. In 2004, the Chapare region was temporarily authorized to grow 3,200 hectares of coca (UNODC 2008). It has been estimated that under Law 1008, roughly 14,520 metric tons of sun-dried coca leaves were produced in 2007 (UNODC 2008). In contrast with Peru, farmers in Bolivia are permitted to market a specified quantity of coca directly themselves (UNODC 2008).

In 1978, Peru's government enacted Law 22,095 with the goal of limiting "the traffic of dependence creating drugs, to prevent their inappropriate use, to socially and physically rehabilitate addicts and to reduce coca plantings" (Cotler 1996). Soon after, the Empresa Nacional de Coca (ENACO) was formed. Its main purpose at the time was to secure coca leaves from Peruvian farmers to market to Coca-Cola; as of 2000, the company has stopped using coca to flavor its products (Thoumi 2005). When the law was enacted, 25,148 coca growers registered nearly 18,000 hectares with ENACO; in a 2002 census, only 7,910 active growers were registered (ENACO 2002). ENACO has overseen the licit cultivation of coca in Peru for nearly 35 years. Although detailed estimates of production and demand have not been made available, ENACO assumes 12,000 hectares of licit cultivation in Peru (Thoumi 2005). ENACO purchases coca leaves from farmers and sells them to domestic retailers for resale, as tea or for chewing, and for export to produce pharmaceuticals.

OPIUM POPPY: *PAPAVER SOMNIFERUM*

Biology

The poppy family, Papaveraceae, contains some 23 genera and roughly 250 species (Grey-Wilson 2000). The *Papaver* genus, of which opium poppy (*Papaver somniferum*) is a member, comprises about 70-80 species (Carolan et al. 2006) that are considered "true poppies." A recent study of the genus *Papaver* in Kashmir lists nine species, including *P. somniferum* (Dar et al. 2010). *P. somniferum* (Figure 3-4) is an annual herb and has pinnately lobed leaves that can appear gray-green or blue-green (Grey-Wilson 2000). Opium poppy is usu-

ally erect and hairless and can stand 50-150 cm tall (Duke 1983; Grey-Wilson 2000). The flowers are white to red to pink-purple and are large and cup-shaped (up to 18 cm across in some cultivated varieties). The capsule is smooth and has various sizes and shapes, depending on cultivar and geography (Veselovskaya 1976). Indian varieties tend to be more oval, and capsules of European poppy are more orbicular, flat, and conical (Levy and Milo 1998). The capsule also features a stigmatic disk that is deeply lobed and has five to 12 (or more) rays (Grey-Wilson 2000). The seeds can range from dark gray, black, or bluish to white and are oily (Duke 1983). When the immature capsule is injured (for example, by lancing or scratching), sap oozes from the capsule. Opium is the gum (dried sap or resin) that contain narcotic alkaloids.

Three poppy varieties are grown for opium production: *P. somniferum* var. *nigrum* has oblong, roundish capsules and purple-red flowers and is a wild form of the poppy; *P. somniferum* var. *album* is a wild form that has white flowers and roundish ovate capsules; and *P. somniferum* var. *abnormale* has oblong or roundish capsules and small red flowers (Finetto 2008).

The cultivation and use of opium poppy date back to the Neolithic Age (Grey-Wilson 2000). Opium has been used since the third millennium BCE as a painkiller and tranquilizer and for social, religious, health, and other purposes in many cultures, countries, and regions (Roman et al. 2005). Recreational use of opium also dates back over 10 centuries; the current worldwide epidemic in the use of opiates began around the beginning of the 20th century (Roman et al. 2005).

FIGURE 3-4 *Papaver somniferum.* Note: See horizontal marks on capsules, indicating recent harvest. Source: U.S. DEA 2007 Microgram Journal. Reprinted with permission; copyright 2007, U.S. DEA.

The total alkaloid content in opium is 10-20%, and more than 40 alkaloids have been isolated (Schiff 2002). Codeine, morphine, and thebaine are the major narcotic components. Morphine concentration is greatest (8-17%), followed by codeine (0.7-5%) and thebaine (0.1-2.5%) (Schiff 2002). Poppy straw (the dried remains of poppy capsules and upper stalks) accounts for 80% of the morphine and 93% of the thebaine produced globally (Finetto 2008). Manufactured codeine is obtained largely through a semisynthetic process involving morphine and is used to treat mild or moderate pain (Finetto 2008). Although not used directly in any medical therapy, thebaine is a critical starting material for producing other opioids (Finetto 2008). Heroin is synthesized from the natural plant alkaloid morphine. More deaths are related to the use of heroin than to the use of any other illicit narcotic (UNODC 2010c).

Illicit Cultivation

Afghanistan leads the world in cultivation of illicit poppy crop and production of opiates (nearly 90% of world production from roughly 123,000 hectares), followed by Myanmar, Mexico, and Colombia (UNODC 2010a, c; see Table 1-3). A major increase in production was seen during the 1970s and was due to the combination of war and increased international demand. Cultivation has continued as farmers in Afghanistan face limited access to markets and have few alternative sources of income (UNODC 2000).

Opium poppy can be cultivated in a variety of environments, although morphine concentration depends on the environmental conditions (Finetto 2008). For maximum yields, the opium poppy must have the required amount of moisture during the appropriate time in the growing cycle and higher temperatures once flowering has occurred (Finetto 2008). Heavy precipitation during the spring or after plants have flowered can lead to decreased yields and to the development of production-inhibiting fungal pathogens (Finetto 2008). In areas that experience heavy rainfall (monsoons), farmers change the growing season to include the winter months and thus avoid excess water (Chouvy 2009).

Alternatively, drought is damaging to opium-poppy production, so moisture must be balanced by precipitation or irrigation (Finetto 2008). Genetic improvement through selective breeding of opium poppy has led to plants that have more morphine, codeine, and thebaine. However, cultivation and production methods have not changed dramatically in hundreds of years. Opium poppy grows best in loamy sand that is high in organic matter. Soils that retain too much water or drain too quickly will affect the production of opium in the capsule and the time it takes for the capsule to mature (Merlin 1984).

Land is commonly cleared to prepare an area for opium-poppy planting. Slash-and-burn is a common practice used to remove vegetation from the land to plant poppy on relatively small plots, generally 0.1-10 hectares (Finetto 2008). During the portion of the year when poppy is not grown, the plots are often used

for food crops (Afghanistan), used for tobacco (Turkey), or alternated with maize (Southeast Asia) by the farmers (Finetto 2008).

Plants are propagated from seed, which is often sown by broadcasting seeds mixed with soil or sand to ensure even distribution. In Thailand, poppy seeds are mixed with lettuce, bean, mustard, and parsley seeds (Finetto 2008). Poppy grown in Lao PDR is most commonly grown at high elevations in mountainous regions (UNODC 2010c). After germination, plants are thinned to optimize plant health.

Opium poppy is usually a spring or winter crop, having been sown in September-March (UNODC 2010c). Multistage cropping—growing poppy in different periods in a single field—is often seen in areas where eradication efforts have occurred (UNODC 2010c). Seeds are sown multiple times in the same field so that harvest of the poppies is staggered. That not only protects the farmer from potential eradication—interdiction sweeps are rarely performed twice on the same plot—but makes harvest more manageable for areas where there is a shortage of labor (UNODC 2010c). Depending on the cultivar, climate, elevation, and sowing date, the crop will be harvestable 120-250 days later. Moisture is a key element in opium production; crops that are irrigated are found to produce more of the desired alkaloids (Finetto 2008). Poppy that is harvested during warm, dry weather is likely to have higher morphine content than that harvested during cool, wet weather. In areas where snowfall occurs, it is most beneficial if the first snow falls before the first frost. The snow protects the poppy; if frost gets to the plants, the farmers risk losing the crop (Finetto 2008).

About 3 months after the seeds are sown, the plants (now waist-high) begin to blossom (Kapoor 1995). The petals fall from the flower in about 3 days; after an additional 10-14 days, the capsules are ready to lance, and harvesting of opium begins (Kapoor 1995). The morphine content decreases as the capsule ripens (Kapoor 1995). Timing and tools vary, but all collection of gum from opium poppies involves using a sharpened, knife-like tool to incise the capsule. The opium sap oozes from the lacerated capsules and is left to dry and harden. The laceration must be precise: if it is too shallow, only a small amount of sap will exude; if it is too deep, the sap will seep into the inner compartment of the capsule (Kapoor 1995). The capsules are scored in this manner several times every 2-3 days. After each cutting, the milky sap that oozes from the capsule is allowed to dry to a sticky, dark gum, which is harvested on the next day. Harvesting of a single plot can take 14-18 days but can take up to 3 weeks if production is exceptionally high. The largest determinant of harvesting time is the available labor force.

Once the capsules have been lanced and all the gum has been collected, they are left in the fields to mature over the next 20-25 days. The fully matured capsules are removed, spread out in plots to dry, and processed to obtain the seeds for the next planting (Kapoor 1995). A 1-hectare plot can produce 60,000-120,000 poppy plants, or 120,000-250,000 capsules. The labor involved in the cultivation of opium poppy is immense; the time and physical effort required to

prepare the fields, weed the plants, and harvest the opium are much greater than for other cash crops or food crops.

Licit Cultivation

About 20 countries cultivate poppy for pharmaceutical use (Chouvy 2009). They include Australia (Tasmania), Austria, China, the Czech Republic, Estonia, France, Germany, Hungary, Japan, India, Macedonia, the Netherlands, Poland, Romania, Slovakia, South Korea, Spain, Turkey, and the United Kingdom. China, India, South Korea, and Japan produce raw opium; India is the only country that exports raw opium (Mansfield 2001; Bhattacharji 2007; Chouvy 2008), and the other three maintain their supply for domestic medicinal use (Chouvy 2009). The remaining countries cultivate opium poppies that they harvest and dry, producing "poppy straw." The poppy straw is then processed to produce concentrate of poppy straw, which can be used to extract opioid alkaloids (Chouvy 2009). Tasmanian production fell from 20,000 hectares in 2000 to 10,000 hectares in 2007 (Berkoff 2007); as of 2010, 25,000 hectares of poppy production had been contracted to meet world demand (Sanders 2010).

The production of opium in India is regulated by the Central Bureau of Narcotics (CBN), which licenses growers (Bhattacharji 2007). For the year 2009-2010, roughly 60,800 farmers were licensed to grow opium poppy on about 23,400 hectares (12,200 hectares was actually harvested). The farmers must meet a minimum qualifying yield determined by CBN to be eligible for a license to grow opium poppy in the next year (Bhattacharji 2007). Most of the farmers use plots of 0.1 hectare, but some high-performing growers are permitted to plant on 0.2-hectare plots (Bhattacharji 2007).

Varieties of *P. somniferum* that are low in opiates are widely grown in Europe and Eurasia for poppy-seed production. Poppy seeds are used in specialty breads and cakes. The major producers of poppy seeds are the Czech Republic (53,623 hectares), Turkey (48,893 hectares), France (10,000 hectares), Hungary (3,928 hectares), Romania (3,100 hectares), Austria (2,186 hectares), Slovakia (1,904 hectares), Croatia (1,642 hectares), and Spain (1,400 hectares) (FAOSTAT 2009). Under the U.S. Controlled Substances Act, Schedule II (2005), *P. somniferum* is illegal to possess or grow in the United States, except as poppy seed used for culinary purposes. However, cultivation of opium poppy for nonnarcotic use is common in the United States, including for use of dried seed capsules in flower arrangements and for selling *Papaver* seeds to grow poppies in gardens.

4

Fusarium oxysporum formae speciales as Candidate Biological Control Agents for Cannabis and Coca

The genus *Fusarium* is one of the largest genera of fungi and includes species that reproduce clonally and by recombination and species that reproduce only clonally (Leslie and Summerell 2006). *F. oxysporum* is a species complex (O'Donnell et al. 2004) that is pathogenic to a wide variety of plant species, including several economically important vegetable and ornamental crops (Nelson et al. 1981; Michielse and Rep 2009). Sexual reproduction by *F. oxysporum* has not been observed. However, genetic variation is significant in populations of nonpathogenic strains, and strains of some form species (forma specialis, abbreviated f.sp.; plural formae speciales, abbreviated f.spp.) have few clonal genotypes and large amounts of genetic variation (Leslie and Summerell 2006). As Baayen et al. (2000) state, "although a teleomorph has not yet been found, the sexual cycle may still be active in FOC [*F. oxysporum* complex]." *F. oxysporum* occurs in all types of soils worldwide and causes severe vascular wilts, damping-off, and crown and root rot in its hosts (Jarvis and Shoemaker 1978; Nelson et al. 1981; Summerell et al. 2001; Di Pietro et al. 2003). In the absence of a plant host, it can exist as a saprophyte (an organism that lives on dead organic matter) in soil for extended periods (Burgess 1981).

F. oxysporum has at least three and probably more species-level clades (O'Donnell et al. 2004). Isolates are placed in form species on the basis of the host-plant species that is attacked (Armstrong and Armstrong 1981; Kistler 1997). More than 150 form species of *F. oxysporum* have been characterized (Armstrong and Armstrong 1981; Baayen et al. 2000). Nonpathogenic strains of *F. oxysporum* also exist in soil and are distinguished from pathogenic strains through pathogenicity testing (Edel et al. 2001). In some instances, a *F. oxysporum* form species can have a wide host range and a single genotype is capable of parasitizing several different plants. For example, *F. oxysporum* f.sp. *vasinfectum* can be isolated from cotton, alfalfa, and tobacco (Assigbetse et al.

1994). Other forms show evidence of convergent evolution: several genotypes have independently evolved the ability to parasitize the same plant species, for example, *F. oxysporum* f.sp. *cubense* (O'Donnell et al. 1998).

BACKGROUND ON THE SPECIFIC FUNGI

Biological Control of Cannabis with *Fusarium oxysporum* f.sp. *cannabis*

Plants in the genus *Cannabis,* including *C. sativa* (fiber hemp and cannabis), are attacked by a number of pathogens, some of which may cause serious damage or plant death. A review of scientific literature on cannabis pathogens by McPartland (1992) identified at least 88 fungal species that attack cannabis. Among those fungi are several *Fusarium* species that cause damping off, stem canker, root rot, and wilt (McPartland 1996). *Fusarium* wilt of hemp, caused by *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *vasinfectum*, was first noted in eastern Europe (Russia, the Czech Republic, Poland, and Romania) more than 50 years ago; it also reportedly occurs in western Europe (Italy and France), central Asia (Kazakhstan and Pakistan), and North America (Canada and the United States) (McPartland and Hillig 2004).

F. oxysporum f.sp. *cannabis* and *F. oxysporum* f.sp. *vasinfectum* are morphologically similar in culture, but, according to McPartland and Hillig (2004), the two can be differentiated on the basis of their host range. *F. oxysporum* f.sp. *cannabis* is reported to infect only cannabis, whereas the host range of *F. oxysporum* f.sp. *vasinfectum* includes (in addition to cannabis) cotton, mungbean, pigeon pea, rubber tree, alfalfa, soybean, coffee, tobacco, and other plants (McPartland and Hillig 2004). The study of genetic variability with DNA polymorphisms may provide an alternative route for identification of these two formae speciales.

F. oxysporum f.sp. *cannabis* was considered a potential control agent for cannabis as early as the 1970s on the basis of its purported specificity to members of the genus *Cannabis*, its ability to survive in the soil for extended periods, and the likelihood of infecting new plantings of the crop. Hildebrand and McCain (1978) conducted laboratory experiments to develop a suitable technique for the production of *F. oxysporum* f.sp. *cannabis* inoculum consisting of chlamydospores, which are suitable for soil application. Later, McCain and Noviello (1985) explored the feasibility of using *F. oxysporum* f.sp. *cannabis* (isolated in Italy) as a biological control agent against *C. sativa* and claimed that the fungus caused disease only on *C. sativa* and that it was able to survive in the soil for at least one growing season.

In the late 1990s, Tiourebaev et al. (2001) conducted experiments to test the pathogenicity of *F. oxysporum* f.sp. *cannabis* isolates obtained from diseased cannabis plants collected in various regions of Kazakhstan and to determine their virulence, their host range, and the formulation best suited for field application. The findings led them to conclude that the disease caused by *F. oxysporum*

f.sp. *cannabis* was not severe enough to cause "permanent and lasting control" of cannabis plants and that there was a need for an improved formulation and improved delivery systems to enhance the pathogen's efficacy as a mycoherbicide (Tiourebaev et al. 2001). The results of those studies are reviewed below in the context of efficacy, inoculum production and delivery, and persistence in the environment. No studies of *F. oxysporum* f.sp. *cannabis* for the control of cannabis have been published since 2001.

Biological Control of Coca with *Fusarium oxysporum* f.sp. *erythroxyli*

Sands et al. (1997) isolated *F. oxysporum* from severely diseased coca plants (*Erythroxylum coca* and *E. novogranatense*) grown by the U.S. Department of Agriculture (USDA) on a secure experimental site on the island of Kauai, Hawaii. This site was originally a coca plantation of a U.S. beverage (soda) manufacturer in the 1960s and is now maintained by USDA for its herbicide studies on coca. Coca plants grown on the site (grown from seeds imported mainly from Peru) in the 1960s and 1970s exhibited damping off and wilt symptoms (Darlington 1996). Pathogenicity and host-range experiments led the investigators to identify the *F. oxysporum* as a unique forma specialis that attacks members of the family Erythroxylaceae and to name it *F. oxysporum* f.sp. *erythroxyli* (Sands et al. 1997).

In Peru, a similar disease of coca plants was observed in the 1930s (El Comercio 1995), in the 1980s (Stevenson 1991), and in the 1990s (Arévalo et al. 1994). *F. oxysporum* was the causal agent of an epidemic in coca in the Huallaga Valley of Peru, and the disease was considered a threat to coca production in that region (OTA 1993; Arévalo et al. 1994). In 1997, with the aid of random amplified polymorphic DNA (RAPD) analysis, Nelson et al. identified two subpopulations of *F. oxysporum* f.sp. *erythroxyli* in the Huallaga Valley (Nelson et al. 1997). Later, Gracia-Garza et al. (1999) used RAPD and vegetative compatibility group (VCG) analyses to determine that *F. oxysporum* f.sp. *erythroxyli* isolates from Peru and Hawaii were genetically similar. They speculated that the pathogen might have been introduced into Hawaii in plant material from Peru (Gracia-Garza et al. 1999). The known distribution of *F. oxysporum* f.sp. *erythroxyli* today includes Peru, Hawaii, and possibly Colombia (El Comercio 1995; Nelson et al. 1997). On the basis of its ability to cause the observed natural wilt disease in coca plantings in Hawaii and Peru and its purported narrow host range, it is considered a potential mycoherbicide for coca (Bailey et al. 1997; Sands et al. 1997).

Several studies of *F. oxysporum* f.sp. *erythroxyli* were conducted in the United States in the middle to late 1990s to develop methods for inoculum production and formulation for the purpose of evaluating its efficacy against coca (Bailey et al. 1997; Hebbar et al. 1997; Connick et al. 1998) and to study its dispersal in the field (Bailey et al. 1997; Bailey et al. 1998; Gracia-Garza et al. 1998, 1999). The results of the studies are reviewed below in the context of effi-

cacy, inoculum production and delivery, and persistence in the environment. No studies of *F. oxysporum* f.sp. *erythroxyli* for the control of coca have been published since 1999.

EFFICACY AND IMPLEMENTATION

The overarching consideration in determining the feasibility of the proposed mycoherbicides is the ability of *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* to inflict severe damage or death on their target plants. The committee reviewed the available data for evidence of such high levels of efficacy as a prerequisite for the use of the identified strains as mycoherbicides. In the case of registered mycoherbicides—such as Chontrol, Collego (Lockdown), DeVine, and Sarritor—the most commonly used measure of efficacy is reduction in weed numbers due to complete and fairly rapid killing of the target weed after application. Accordingly, in the following analysis, efficacy is viewed as the ability to yield an acceptable level of control of the target crops within a short period after application, namely, a few days to a few weeks for the annual crops of cannabis and a few months in the case of coca, a long-lived tree crop. As mentioned in Chapter 1, eradication of the crops is not a realistic goal, because biological agents rarely, if ever, kill 100% of their hosts. Therefore, the committee looked for data on different steps in efficacy measurements, such as greenhouse and field evaluations, with emphasis on the latter; appraisal at relevant growth stages of the target crops; and suitable measures of control, such as plant death and reduction in plant population density, plant growth rate, or crop yield (as harvestable biomass of cannabis and coca). Disease assessments reported in the literature, including visual ratings and direct measurement of disease on individual plants and populations, help to quantify the destructiveness of the pathogens in controlled experiments, but they provide only an indirect measure of the potential efficacy of the proposed mycoherbicides under field conditions.

Fusarium oxysporum f.sp. *cannabis*

Only two publications shed light on the efficacy of *F. oxysporum* f.sp. *cannabis* as a mycoherbicide for cannabis. McCain and Noviello (1985) in conference proceedings reported on the effectiveness of *F. oxysporum* f.sp. *cannabis* against cannabis (industrial hemp) plants in the greenhouse, growth chamber, and fields in Italy. Tiourebaev et al. (2001) in a short communication described greenhouse and field studies to evaluate the pathogenicity and virulence of *F. oxysporum* f.sp. *cannabis* isolates obtained from diseased cannabis plants collected in various regions of Kazakhstan. The details of the experimental methods used in those studies are presented in Tables 4-1 and 4-2.

TABLE 4-1 Greenhouse and Field Studies in Italy by McCain and Noviello (1985)

Greenhouse Study	
Inoculum	*F. oxysporum* f.sp. *cannabis* isolate recovered from industrial hemp plants collected from fields in Italy in 1972 Inoculum (composed of mycelium, conidia, and chlamydospores) produced on wheat straw and soybean meal Five inoculum levels tested: 7, 70, 700, 1,400, and 7,000 propagules/g of soil
Test plants	*C. sativa* cultivar Iran (susceptible variety)
Assessment method	Efficacy based on percentage of plants killed
Field Studies	
Location	Field experiments conducted in Vitulazio, Alvignano, and Portici, Italy, in 1974
Inoculum	Air-dried straw-soybean inoculum used; applied by hand and mixed with top 10 cm of soil
Application rate and cultivars used	Vitulazio field trial: 10 g/m^2; inoculated plots seeded with *C. sativa* cultivars CS and SF Alvignano field trial: 10 g/m^2; inoculated plots seeded with *C. sativa* cultivars Iran and SF Portici field trial: 1, 10, and 30 g/m^2; inoculated plots seeded with *C. sativa* cultivars Iran, CS, and SF; trial repeated with field soil in large ceramic pots
Assessment method	Efficacy based on plant mortality

In their greenhouse study, McCain and Noviello noted that the time required to kill all the seedlings was proportional to the inoculum level. At the highest inoculum level (7,000 propagules/g of soil), 100% of the plants were killed within 9 days after planting, whereas it took 47 days for all the plants to die at the inoculum level of 70 propagules/g of soil. Only 50% of the plants were killed at the lowest inoculum level (7 propagules/g of soil) at 47 days after planting.

McCain and Noviello reported that in field plots at Vitulazio, no *Fusarium*-infected CS or SF hemp plants were observed during the study, but they did not provide an explanation for the apparent failure of this trial. At the Alvignano field trials, 4 months after the cultivars were planted in the fungus-infested soil, 71% of the Iran plants had died and the ones that survived were shorter than the plants in the noninoculated control plots. Only two SF plants became infected—an indication that this cultivar is resistant to the disease. In the Portici trial, 50%, 94%, and 94% of the Iran plants had died 4 months after the cultivars were planted in the soil treated at 1, 10, and 30 g/m^2, respectively. The Iran plants that survived were stunted by 26 and 45% at the 1-g/m^2 and 10-g/m^2 inoculum levels, respectively, compared with the Iran plants in the non-inoculated control plots. At the highest inoculum level, 30 g/m^2, only 4% and 14% of the cultivars CS and SF died.

TABLE 4-2 Greenhouse and Field Studies in Kazakhstan by Tiourebaev et al. (2001)

Greenhouse Study	
Inoculum	125 isolates of *F. oxysporum* obtained from diseased *C. sativa* plants collected in various regions of Kazakhstan 125 isolates of *F. oxysporum* "evaluated for pathogenicity" to *C. sativa* All isolates formulated with wheat or oat seeds or birch sawdust and applied at 0.5 g per 2-cm^2 pot of soil
Test plants	*C. sativa* (cultivar not stated)
Environmental conditions	28°C; 19-h photoperiod
Assessment method	Disease severity rating: 1 = healthy plant; 2 = wilting of lower leaves; 3 = wilting of 25-50% of leaves; 4 = over 50% of leaves wilted; 5 = dead plant
Field Studies	
Location	Kazakhstan field naturally infested with *C. sativa* plants (cultivar not stated)
Inoculum	25 *F. oxysporum* isolates "that showed virulence and host specificity towards *C. sativa*" in the greenhouse Trial 1: 25 isolates formulated with wheat seed, oat seed, and birch sawdust Trial 2: 25 isolates formulated in birch sawdust
Application rate	12.5 g/m^2 (plot size, 2 m^2); inocula of the 25 isolates applied twice: when *C. sativa* seedlings were 2-3 wk old and when the plants were 6-7 wk old
Assessment method	Estimation of "percentage of infected plants within treated plots" done on 2-m transect laid across plot; disease severity calculated with the equation D = a/b(100), where D = disease severity, a = number of *C. sativa* plants with disease severity rating of 3-5 (see rating scale in greenhouse studies), and b = total number of *C. sativa* along the transect with 1-5 disease severity rating

There are a few important shortcomings in the experiments and paper by McCain and Noviello (1985): the greenhouse trial of the inoculum rate study was done only once, and the number of replications per treatment was not stated; the temperature and humidity conditions during the greenhouse and field trials were not stated; and the isolates used were not specifically identified. Thus, the experiments are not readily repeatable, and the results cannot be accepted as critical evidence.

McCain and Noviello screened several other unnamed *C. sativa* cultivars besides those in Table 4-1 and seed collections and discovered that some industrial hemp varieties were resistant to *F. oxysporum* f.sp. *cannabis.* That led them to conclude that the presence of resistant varieties might limit the effectiveness of the mycoherbicide.

The results of the study by McCain and Noviello confirm that *F. oxysporum* f.sp. *cannabis* is a pathogen of *C. sativa,* but the pathogen's efficacy as a mycoherbicide agent is low. By the authors' own admission, the time required for disease development and death of cannabis (at least 4 months) is a limitation (McCain and Noviello 1985). The decline of populations of *F. oxysporum* f.sp. *cannabis* in the soil in the absence of the host plant suggests that the fungus may not persist unless the fields are continuously cropped with cannabis. The authors' claim that complete coverage of a field is unnecessary because natural spread would occur is not backed up by the data that they presented. Therefore, the hypothesis that *F. oxysporum* f.sp. *cannabis* will persist in treated soil and can infect new crops of cannabis remains untested.

Tiourebaev et al. (2001) conducted greenhouse and field experiments with a fairly large collection of *F. oxysporum* f.sp. *cannabis* isolates. In the greenhouse study that was conducted only once, 25 of the 125 isolates tested were found to cause disease in cannabis. Plant mortality for the wheat-seed, oat-seed, and birch-sawdust formulations was 64%, 61%, and 59%, respectively. Tiourebaev et al. noted that cannabis mortality varied according to the fungal isolate.

In the first field experiment, 12 of the 25 isolates that were previously screened in the greenhouse study were found to be pathogenic to cannabis. Isolate CR-21 caused the most damage, namely, wilting of the plant within 2 weeks. Other symptoms on the treated plants were stunting, leaf curling, root discoloration, and loss of structural integrity in the upper part of the plant. At the end of the first field experiment (5 months after the initial application of the formulations), disease incidence ranged from 12% to 67% for the isolates tested. Disease severity ranged from 1.27 to 2.00 on a scale of 1-5 (see Table 4-2), in which a rating of 2.00 corresponds to wilting of lower leaves. In the second field trial, disease incidence ranged from 6.8% to 39% and disease severity from 1.4 to 2.7 for the isolates tested. Plot size was 2 m^2, and the number of replicates was not mentioned (Tiourebaev et al. 2001).

Like McCain and Noviello, Tiourebaev et al. (2001) confirmed *F. oxysporum* f.sp. *cannabis* as a pathogen of cannabis. However, the levels of disease, based on disease-severity and disease-incidence estimates, were low to moderate and led the authors to conclude that under field conditions "the infection rate was still too low to affect permanent and lasting control of the weed." Thus, the Kazakh isolates of *F. oxysporum* f.sp. *cannabis* tested by Tiourebaev et al. (2001) do not appear to be efficacious or suitable for development as a mycoherbicide.

According to Tiourebaev et al. (2001), the "pathogenicity of the isolates varied greatly" between greenhouse and field trials. One explanation for the variation could be temperature: the greenhouse temperature during the experiment was 28°C with 19 hours of daylight, whereas the temperature in the field ranged from 20°C to 30°C during May or June, the time when infection began to appear. The disease levels were highest in late August and September and, according to the authors, the "disease tapered off as cooler conditions prevailed"

(Tiourebaev et al. 2001). Another factor might be the genetic composition of the cannabis plants in the field; inasmuch as the trial took place in a field with a natural cannabis infestation, the hosts' genetic variability could have affected the isolates' field performance.

The identification of the isolates as *F. oxysporum* was based on "microscopic analysis and colony morphology" (Tiourebaev et al. 2001). The authors assigned the name *F. oxysporum* f.sp. *cannabis* without completing Koch's postulates to prove their pathogenicity toward cannabis. Without additional support from molecular or VCG analysis, this identification is unlikely to be acceptable for mycoherbicide-registration purposes. It cannot be ruled out that this large collection of *F. oxysporum* isolates from the wild included several formae speciales that are cross-infective on cannabis and one or more additional hosts, with cannabis being an alternative rather than a primary host.

Overall, the data on *F. oxysporum* f.sp. *cannabis* gleaned from the publications of McCain and Noviello (1985) and Tiourebaev et al. (2001) are not sufficient to draw conclusions about the feasibility of *F. oxysporum* f.sp. *cannabis* as a mycoherbicide.

Fusarium oxysporum f.sp. *erythroxyli*

The committee reviewed the available data on the efficacy of *F. oxysporum* f.sp. *erythroxyli* in three publications: Sands et al. (1997), Bailey et al. (1997), and Bailey et al. (1998). The study by Sands et al. consisted of experiments in the field and in a growth chamber to assess the virulence of *F. oxysporum* f.sp. *erythroxyli* isolated from an *Erythroxylum* population growing in Hawaii. Bailey et al. (1997) performed three field trials in Hawaii with a rice-alginate prill formulation of strain EN-4 of *F. oxysporum* f.sp. *erythroxyli,* which was one of the Hawaiian isolates studied by Sands et al. (1997), to test the feasibility of enhancing the pathogen populations in the soil and to cause disease in coca. Later, Bailey et al. (1998) examined six formulations, including the rice-alginate prill, for their ability to enhance the pathogen's populations and cause disease on coca in the field. The experimental methods used in the studies are summarized in Tables 4-3 and 4-4.

In the growth-chamber study by Sands et al. (1997), severe disease was not observed with any of the *F. oxysporum* f.sp. *erythroxyli* strains tested when the inoculum was placed in 10-cm holes around the plant. Better results were obtained by first infesting the soil with the fungus and then transplanting coca plants into the soil. Wilting was observed 3 weeks after the plants were transplanted, and the plants eventually died; however, no quantitative data were provided.

In the field study, the initial disease symptoms were leaf drop and death of a "few lower stems"; plant death was observed 7 weeks after the plants were transplanted into the fungus-infested soil. The time from the appearance

TABLE 4-3 Growth-Chamber and Field Studies by Sands et al. (1997)

Growth-Chamber Study	
Inoculum	*F. oxysporum* strains tested: Ec1-3 and EN1-4 (from Hawaii) and SA1 (from South America)
	Soil in pots planted with test plants infested with millet seeds colonized by *F. oxysporum* by placing seeds in three 10-cm holes (10 seeds/hole)
Test plants	6- to 12-mo *E. coca* plants
Environmental conditions	30°C/28°C day/night temperature; 12-h light/dark periods Test plants watered daily (80-100 mL)
Assessment method	Test plants evaluated for disease weekly over 3.5-mo period
Field Study	
Location	Conducted in 1989 on field plots transplanted with *E. coca* and *E. novogranatense* in the previous fall (1988)
Inoculum	Field plots infested with millet seeds colonized by *F. oxysporum* Seven Hawaiian isolates of *F. oxysporum* evaluated for pathogenicity on coca
Environmental conditions	All infested plots received trickle irrigation
Application method and rate	Subsurface method: inoculum (5.0 g /plot) deposited in 2-cm-deep V-shaped trench and covered with 2-3 cm of soil
	Surface method: inoculum applied directly to the soil surface and covered with mesh screen to protect from predation
Assessment method	Plants in treated and nontreated soil evaluated for severity and mortality after 7, 8, 9, 10, and 15 mo
	Efficacy assessed with a disease-severity rating scale of 0-2: 0 = no disease, 1 = wilt, 2 = plant death

of initial disease symptoms to plant death ranged from days to months. The average disease severity for all seven isolates tested was 1.1 (on a scale of 0-2) in the subsurface-applied plots and 1.03 in the surface-applied plots. Plants in the control plots had an average disease severity rating of 0.71, that is, plants were symptomatic but not dead. Some control plants also developed disease, presumably from naturally occurring inoculum of *F. oxysporum* f.sp. *erythroxyli. E. coca* plants were more severely diseased (rating, 1.22) than *E. novogranatense* plants (0.93).

Apparently on the basis of a combination of the data from all seven isolates and two methods of inoculum application, Sands et al. (1997) reported that 94% of *E. coca* and 49% of *E. novogranatense* plants (42 per species) in the fungus-infested soil were killed at 15 months after inoculation, whereas about 95% of *E. coca* and 43% of *E. novogranatense* plants (28 plants per species) in the noninfested control soil (values read off the graph in Figure 1 of Sands et al. [1997]) had died. *F. oxysporum* was isolated from the vascular tissues of symptomatic and asymptomatic plants from the fungus-infested soil and from symp-

tomatic plants in the noninfested soil; this implied nonpathogenic colonization of coca plants by *F. oxysporum* f.sp. *erythroxyli* and fairly high levels of natural incidence of wilt at the test site.

TABLE 4-4 Field Studies in Hawaii by Bailey et al. (1997, 1998)

First Set of Field Trials	
Location	Three experiments conducted from 1995 to 1996 in Kauai, Hawaii
	Two trials in fields continuously planted with coca for at least 7 years; one trial in a field not previously planted to coca
Environmental conditions	Fields irrigated daily for 14 d after *F. oxysporum* f.sp. *erythroxyli* strain EN-4 formulations were incorporated in soil
	Temperature 17-21°C minimum and 23-27°C maximum; regular afternoon rains (3.9-57.9 cm)
Inoculum	Alginate prill formulation *F. oxysporum* f.sp. *erythroxyli* strain EN-4
Application rate	33.6 kg/ha
Assessment method	*F. oxysporum* f.sp. *erythroxyli* strain EN-4 efficacy assessed with disease-severity rating scale of 0-2: 0 = asymptomatic, 1 = symptomatic (plant defoliating), 2 = dead
Second Set of Field Trials	
Location	Three experiments in 1995-1996 in Kauai, Hawaii
	Two experiments in fields continuously planted with coca for at least 7 years; one trial in a field not previously planted to coca
	Low percentage of diseased coca plants present in all field plots before the experiment was conducted
Environmental conditions	Fields irrigated daily for 14 d after the *F. oxysporum* f.sp. *erythroxyli* strain EN-4 formulations were incorporated in soil
	For experiments 2 and 3, average low air temperature: 19.5°C and 21.3°C; average low soil temperature: 22.6°C and 24.2°C; relative humidity for both trials from less than 80% to over 97% during the first 10 d after application of formulations
Inoculum	Six formulations (rice-alginate, C6, Pesta, canola-alginate, rice-alginate + canola oil, corn cob-alginate) and biomass alone of *F. oxysporum* f.sp. *erythroxyli* strain EN-4
Application rate	33.6 kg/ha
Assessment method	*F. oxysporum* f.sp. *erythroxyli* strain EN-4 efficacy assessed with disease-severity rating scale of 0-2: 0 = asymptomatic, 1 = symptomatic (plant defoliating), 2 = dead

These results confirm *F. oxysporum* f.sp. *erythroxyli* as a virulent pathogen of *E. coca* and *E. novogranatense;* with respect to efficacy, however, the data are equivocal. No quantitative data from the growth-chamber study were provided, so these data are of limited value in assessing efficacy. The data in Figure 1 of the paper shows that the percentages of dead control and fungal-treated *E. coca* plants were nearly equal at the end of the experiment. In the case of *E. novogranatense*, nearly 60% (not 49% as stated in the publication) of the inoculated plants and nearly 50% of the control plants appear to have died. Without the benefit of statistical analysis of data in Figure 1, the small number of plants tested (42 and 28 plants per species in the fungus-infested and fungus-free soils, respectively) raises the question whether the naturally prevalent inoculum at the field site rather than the experimentally applied inoculum was responsible for the observed results. Furthermore, the lack of information on climatic conditions (average daily temperature, relative humidity, soil moisture) during the study and the use of 6- to 12-month-old seedling transplants instead of older and established coca plants are additional limitations.

In two later studies, Bailey et al. (1997, 1998) reported an increase in the rate of disease development in plots treated with different formulations compared with the rate in untreated plots, which had only background levels of *Fusarium* wilt. However, the mortality of coca plants was variable, ranging from 35% to 85% among all seven formulations tested (Bailey et al. 1998).

The results obtained by Bailey et al. are consistent with the finding by Sands et al. (1997) that *F. oxysporum* f.sp. *erythroxyli* is a virulent pathogen of coca plants. However, as in the study by Sands et al., the results with respect to efficacy are ambivalent. Bailey et al. (1997) claimed that "the 33.6 kg/ha rate of the rice-alginate prill formulation enhanced the killing of coca plants in three different field experiments over 7-9 months," but the data in the paper do not report plant mortality; the maximum disease rating for up to 350 days was under 1; that is, the plants were symptomatic, not dead. Bailey et al. (1997) also reported that some plants "appear to seal off the infected areas and resume normal growth patterns," and this could indicate the plants' ability to overcome the disease.

The incidence of natural wilt of about 58% at the experimental site in Hawaii where Bailey et al. (1997) performed the first set of field studies and the 17% mortality in the second set of field studies (Bailey et al. 1998) are problematic. Against such background disease levels, the increase in disease incidence resulting from the added inoculum cannot be ascertained on the basis of the collective data of Bailey et al. (1997, 1998) and Sands et al. (1997).

Bailey et al. (1997) also state that by "maintaining soil surface moisture with drip irrigation, overhead watering, and/or rain, the rice-alginate prill formulation germinated relatively uniformly in two greenhouse experiments and at least two field experiments when applied to the soil surface." Thus, the rice-alginate prill formulation appears to be a feasible and effective way to deliver the inoculum if it is deployed under conditions where adequate moisture is present.

Bailey et al. (1997), referring to *Fusarium* wilts in general, make the observation that the disease "becomes more severe with continuous cropping, and the pathogen often persists in infested fields for years after the crop is removed." However, in the case of coca, anecdotal evidence from the Huallaga Valley of Peru, where a natural wilt epidemic caused by *F. oxysporum* f.sp. *erythroxyli* occurred in the 1990s, indicates that disease has not increased in severity during the last decade despite its sporadic outbreaks (Arévalo et al. 1994).

Mechanisms of Pathogenicity

There is no information on the mechanisms by which *F. oxysporum* f.sp. *cannabis* or *F. oxysporum* f.sp. *erythroxyli* causes disease in its host plants, but some generalizations can be drawn from what is known about vascular *Fusarium* wilts. *F. oxysporum* causes vascular wilt, as exhibited by leaf yellowing, loss of turgidity, necrosis, wilt, and plant death. Infection occurs when mycelium or germinating spores penetrate the roots of the plant, enter the xylem, and produce microconidia. Vascular vessels become clogged by the accumulation of mycelium, spores, and oxidation of breakdown products from enzymatic lysis. Toxins may be produced that cause vein clearing (loss of chlorophyll production along the veins), a reduction in photosynthesis, and tissue damage that leads to excessive water loss through transpiration. Symptoms typically start at the lower leaves and progress upward. Roots may also become infected and become stunted and rot (Desjardins 2006; Leslie and Summerell 2006; Garibaldi et al. in press).

Wilt-inducing *F. oxysporum* f. spp. are primarily soilborne, and epidemic development is predominantly monocyclic (only one disease cycle in a cropping season). Epidemics start from inoculum originating in plant debris in the soil in the form of chlamydospores (asexual, one-celled, thick-walled spores that can persist for a long time), macroconidia (the larger type of conidia or asexual spores formed by *Fusarium* spp.), and microconidia (the smaller type of conidia formed by *Fusarium* spp.). To a lesser extent, there is evidence that aerial inoculum (in the form of macroconidia and microconidia) is produced on infected stems and that more than one cycle of infection might be possible in a season (Timmer 1982; Gamliel et al. 1996; Katan et al. 1997; Rekah et al. 2000; Garibaldi et al. in press).

INOCULUM PRODUCTION AND DELIVERY

Facility, Equipment, and Technology for Large-Scale Manufacture

Large-scale production of mycoherbicides for commercial use is typically undertaken by industries that have microbial fermentation capability. Depending on the fungus, production would require submerged liquid fermentation, solid-substrate fermentation, or a biphasic system in which the fungal biomass is first

produced in a liquid culture and then transferred to an organic or inorganic solid matrix for the production of spores or other desired propagules. If necessary, sophisticated approaches based on those principal systems could be developed (such as airlift fermentation). In general, the feasibility of adapting existing, readily available technology has been a strong incentive for industries to invest in producing mycoherbicides. The ability to produce the required amounts of mycoherbicides without major new investments or disruption of other commercial manufacturing commitments is an additional incentive. Several U.S. companies that specialize in the production of microbial biomass for pharmaceutical, food, biotechnology, and other industrial uses have the necessary technological capability and expertise to produce the proposed mycoherbicides (Bowers 1982; Churchill 1982).

Two mycoherbicides registered for use in North America (Collego and Sarritor) and an experimental mycoherbicide (CASST) are good examples for reviewing the industrial production processes that could be adapted to produce the required amounts of the proposed mycoherbicides. Collego (now registered as Lockdown and composed of *Colletotrichum gloeosporioides* f.sp. *aeschynomene*) is produced by submerged liquid fermentation. Sarritor (*Sclerotinia minor*) is produced by solid-substrate fermentation. CASST (*Alternaria cassiae*), a mycoherbicide that was industrially developed but not registered, for business reasons, was produced by using a biphasic method. The processes illustrated in Figure 4-1 can be modified in several ways to suit particular needs.

The proposed mycoherbicide agents *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* could be grown on a variety of solid substrates and liquid media to produce infective propagules, with emphasis on thick-walled chlamydospores that are more durable than conidia. Fermentation methods have been developed that specifically promote the production of chlamydospores (Hildebrand and McCain 1978; Hebbar et al. 1997; Connick et al. 1998). Although the available data on the proposed mycoherbicide strains provide useful leads, they are exploratory; any large-scale attempt at production must start with basic studies in an industrial setting to develop and optimize the production process. A few *Fusarium* species are currently mass-produced by industrial fermentation processes for production of various commercial products, such as gibberellic acid, zearalenone, and edible mycoprotein (Waites et al. 2001).

In addition, Elzein and Kroschel (2004) and Watson et al. (2007) have developed laboratory-scale production methods for *F. oxysporum* strains for the control of the parasitic weed striga (*Striga hermonthica*). Collectively, experience with these fermentation methods could be used to guide the development of the proposed *Fusarium* mycoherbicides. Typically, industrial studies are needed to determine the choice of fermentation method (liquid, solid, or biphasic), the type of propagules (mycelium, asexual spore [conidium], or resting spore or structure [chlamydospore or microsclerotia]), the formulation (aqueous liquid concentrate, emulsion, dust, pellet, food-grain-based, seed-based, and so on), the intended delivery method (aerial or ground-based; directed or dispersed), and expected efficacy, shelf-life, and handling specifications of the product.

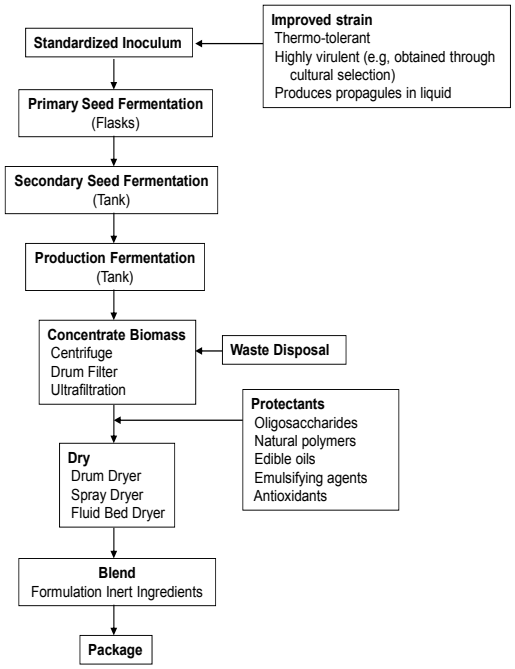

FIGURE 4-1 A simplified diagram for commercial production of a bioherbicide using standard fermentation and downstream processing equipment. Source: Stowell 1991. Reprinted with permission; copyright 1991, Springer Science + Business Media B.V.

The committee reviewed studies by Hildebrand and McCain (1978), McCain and Noviello (1985), and Tiourebaev et al. (2001) to assess the materials and methods used for the production of *F. oxysporum* f.sp. *cannabis* inoculum for field trials. Studies by Sands et al. (1997) and Hebbar et al. (1997) were reviewed to assess the production of *F. oxysporum* f.sp. *erythroxyli* inoculum for field trials. The available data on the media and methods used and the amounts of inoculum produced are given in Table 4-5. These data were used to estimate the amount of inoculum that might be needed to treat illicit cannabis and coca crops.

Feasibility of Large-Scale Production

To estimate the likely amounts of the mycoherbicide products that might be required for a hypothetical operational drug-crop control program worldwide, the committee made calculations (Table 4-6) based on published data on the

amounts of inoculum produced for use in field trials (see Table 4-5). However, the committee cautions that the published data are at best a guide because they are rudimentary in scope and based on arbitrary choices of materials and methods. Therefore, the actual amounts needed can only be determined by testing different formulations under field conditions. Such testing is best done in collaboration with an industrial producer of fungal-based products. The actual amounts required to control the drug crops may or may not be feasible to produce due to cost and/or technical limitations.

Adjuvants and Formulation

Although there are exceptions, the registered mycoherbicides are generally applied without adjuvants, substances added with but separate in function from the mycoherbicide formulation and intended to improve the mycoherbicide's effects, such as spreaders-stickers, infection aids, and virulence enhancers. For example, a sugar (high-fructose corn syrup) is added to the preparation of Collego (Lockdown) for regulating rehydration of dry spores. The sugar enables a slow and gradual hydration of the spores, which is critical for maintaining spore viability during application (Churchill 1982).

Formulation is crucial not only to guarantee mycoherbicide performance but also to improve its efficacy, shelf life, ease of handling, and application. A range of formulations—from wettable powder (Collego/Lockdown) to liquid concentrate (DeVine) to paste (Chontrol) and granules (Sarritor), have been used commercially. To avoid drift and unintentional exposure to a harsh environment, it is unlikely that the proposed *F. oxysporum* mycoherbicides would be sprayed in water or dusted as a fungal powder. Solid media, such as translucent or light-colored alginate formulations, would help to protect the live pathogen propagules from desiccation and ultraviolet radiation. Such formulations could include a nutrient base—such as amino acids, sugars, and some fatty acids—for initial growth, survival, and establishment of the pathogens at the target site (Hildebrand and McCain 1978; Gracia-Garza et al. 1998; Hebbar et al. 1999).

A review of published data on the proposed mycoherbicide strains indicates that several formulations were developed and tested in the field although only on a small scale. The committee reviewed studies by Hildebrand and McCain (1978), McCain and Noviello (1985), and Tiourebaev et al. (2001) on *F. oxysporum* f.sp. *cannabis* and by Hebbar et al. (1997, 1999), Sands et al (1997), Bailey et al. (1997, 1998), and Connick et al. (1998) on *F. oxysporum* f.sp. *erythroxyli*. The formulations tested and their viability or efficacy are summarized in Table 4-7.

The study by McCain and Noviello (1985) evaluated the feasibility of producing stable and efficacious inoculum composed of straw and soybean meal colonized by the fungus, but the inoculum was not characterized in terms of

TABLE 4-5 Media and Methods Used for Production of *Fusarium oxysporum* f.sp. *cannabis* and *Fusarium oxysporum* f.sp. *erythroxyli* for Field Trials

Method	Inoculum Produced
F. oxysporum f.sp. *cannabis*	
Hildebrand and McCain 1978	
Extracts of various plant products (prepared by autoclaving 75 g of a product in 50 mL of distilled water at 212°C for 15 min and decanting supernatant liquid) inoculated with 0.1 mL of aqueous conidia + mycelium suspension and incubated for 2 wk	The following amounts of chlamydospores produced in each substrate tested: Extract of alfalfa straw: 192×10^4 or $\sim 3.8 \times 10^4$/mL of medium Extract of cottonseed meal: 90×10^4 or $\sim 1.8 \times 10^4$/mL of medium Extract of soybean meal: 24×10^4 or $\sim 0.5 \times 10^4$/mL of medium
Barley straw (800 g), 2 L distilled water plus 160 g of soybean meal, alfalfa straw, or cottonseed meal or glycine-succinate-$NaNO_3$ solution inoculated with the fungus, incubated for 3 wk in 2- to 3-L glass flasks at room temperature, removed from the flasks, air dried for 2 wk, and added to the soil at 10 g/1,000 g of soil; number of propagules in soil determined after 5 wk	Per gram of fungus-colonized soil: Barley straw plus cottonseed meal: 3.5×10^4 Barley straw plus alfalfa: 2.7×10^4 Barely straw plus succinate-glycine-$NaNO_3$ mixture: 2.5×10^4 Barley straw plus soybean meal: 1.46×10^4
McCain and Noviello 1985	
F. oxysporum f.sp. *cannabis* cultured on twice-autoclaved barley straw and soybean meal (oil extracted) mixture for 2-4 wk and air dried for 5-7 d	Inoculum composed of particles of straw and soybean meal with fungal mycelium, conidia, and chlamydospores Number of fungal propagules per gram of colonized and air-dried straw and soybean meal not stated in paper
Tiourebaev et al. 2001	
Fungus cultured in 100 mL of potato dextrose broth in 250-mL flasks for 2-3 d with constant agitation at 200 rpm Cultures homogenized by stirring and then suspended in a membrane-stabilizing solution composed of 1,000 mM sucrose, 500 mM sorbitol, and 10% tryptone Homogenized culture mixed with birch sawdust, wheat seeds, or oat seeds and then used as inoculum for experiments	Amount of conidia per gram of formulations not stated in paper

F. oxysporum f.sp. *erythroxyli*	
Sands et al. 1997	
Potato dextrose broth culture of *F. oxysporum* f.sp. *erythroxyli* mixed with sterilized, dehulled, milled rice grains and inoculated with 3.3×10^5 conidia/g of rice Treated rice air-dried for 12-24 h and then mixed with steamed greenhouse soil (1:100, wt/wt); rice-soil mixture moistened and fungus allowed to grow throughout the soil for 1-3 wk	Type and amount of infective propagules produced on rice-soil mixture not stated in paper
Hebbar et al. 1997	
Used a bench-top fermentation with an aqueous extract of soybean-hull fiber that had been autoclaved, filtered, and poured into a 2.5-L fermentor vessel; three fermentation conditions tested: (1) T1 = sparged (bubbled) from 0 to 14 d, (2) T2 = nonsparged from 0 to 14 d, (3) T3 = nonsparged from 0 to 14 d and pH raised after 4 d and maintained at 9.0-10.0 For fermentation in 20-L carboys, autoclaved nonfiltered soybean-hull fiber used as medium	Liquid-fermentation method increased chlamydospore yield (6.3×10^6 chlamydospores/mL) after fermentation for 14 d, with biomass viable counts of 1×10^8 propagules/g of air-dried biomass Methods increased chlamydospore yields and reduced time needed for their production from 5 wk to 2 wk
Connick et al. 1998	
Biomass of *F. oxysporum* f.sp. *erythroxyli* strain EN-4 produced by method of Hebbar et al. (1997) with aqueous extract of soybean fiber in medium	3×10^7 CFU/mL produced with this method Conidia then air-dried and used in laboratory-scale Pesta formulations (containing 5.5×10^7 CFU/g) and twin-screw extrusion formulation (containing 1.3×10^8 CFU/g); biomass used in Pesta formulation composed mostly of chlamydospores

TABLE 4-6 Estimated Amounts of Proposed *Fusarium oxysporum* Mycoherbicides Needed for Single Application Against Illicit Cannabis and Coca Crops Worldwide

Mycoherbicide—Target Drug Crop	Reference	Amount of Inoculum Reportedly Used in Field Trials[a]	Amount of Mycoherbicide Needed *for Each Application* over potential worldwide area,[b] metric tons	Amount of Mycoherbicide Needed *for Each Application,* kg/ha
F. oxysporum f.sp. *cannabis*—cannabis	McCain and Noviello 1985	1-30 g/m^2, inoculum composed of fungus-colonized straw and soybean meal[c].	2,000-192,540[d]	10-300
F. oxysporum f.sp. *cannabis*—cannabis	Tiourebaev et al. 2001	12.5 g/m^2, inoculum composed of liquid fungal culture at ~33% by weight mixed with birch sawdust, wheat seeds, or oat seeds and air-dried; two applications of inoculum 1 mo apart	25-80.2 million	125
F. oxysporum f.sp. *erythroxyli*—coca	Sands et al. 1997	5 g/0.3 m^2, inoculum composed of fungus-colonized millet seeds; proportion of fungus to millet seed not specified	26,996	170
F. oxysporum f.sp. *erythroxyli*—coca	Bailey et al. 1997	33.6 kg/ha, inoculum composed of alginate prill formulation containing 1×10^6 to 5.3×10^6 CFU	5,336	34
F. oxysporum f.sp. *erythroxyli*—coca	Bailey et al. 1998	33.6 kg/ha, inoculum composed of rice-alginate prill, wheat flour-kaolin (Pesta), and rice flour-wheat flour (C6), each containing 1×10^6 to 5.3×10^6 CFU	5,336	34

[a]Amounts of inoculum used in field experiments were not aimed at defining minimum inoculum quantity needed for effectiveness; this remains to be determined with actual mycoherbicide products.

[b]Estimated potential worldwide target area for mycoherbicide use in hectares is based on UN Office on Drugs and Crime's most recent estimate of total area under cannabis and coca cultivation worldwide (UNODC 2010a): 200,000-641,800 ha and 158,800 ha, respectively. Calculations provided on the basis of potential worldwide target area to indicate industrial production capacity that might be needed. The committee regards simultaneous worldwide application or even a worldwide application within a growing season as logistically unrealistic.

[c]Lower inoculum level reported to be less effective than higher level; nonetheless, both figures are used to indicate low and high estimates of inoculum needed.

[d]For a perspective, a familiar automobile, such as Toyota Prius, weighs 1.33 metric tons. Low-end amount of inoculum needed for single application would approximate the weight of 1,504 Prius cars.

TABLE 4-7 Developed and Tested Formulations of *Fusarium oxysporum* f.sp. *cannabis* and *Fusarium oxysporum* f.sp. *erythroxyli*

Formulation	Viability and Efficacy
Fusarium oxysporum f.sp. *cannabis*	
Hildebrand and McCain (1978)	
Fungus + barley straw + soybean meal; fungus + barley straw + alfalfa straw; fungus + barley straw + cottonseed meal; fungus + barley straw + glycine-succinate-$NaNO_3$ solution; all inoculated straw-substrate mixtures air-dried for 2 wk)	Air-dried inoculum produced on all straw-substrate mixtures and stored in plastic bags at room temperature reportedly remained efficacious for 6 mo; seedlings inoculated with formulations stored for 6 mo died within 18-20 d after inoculation; loss in efficacy noted for formulations stored for 9 mo and 12 m; seedlings inoculated with formulations stored for 9 mo and 12 mo died after 24 and 30 d after inoculation, respectively
McCain and Noviello (1985)	
Fungus + barley straw and soybean meal (oil-extracted), incubated for 2-4 wk and air-dried for 5-7 d	Inoculum (particles of straw and soybean meal with fungal mycelium, conidia, and chlamydospores) stored at 20-22°C; no "appreciable loss" of viability observed after a year of storage at 20-22°C or -10°C
Tiourebaev et al. (2001)	
Fungus suspended in solution composed of 1,000 mM sucrose, 500 mM sorbitol, and 10% tryptone + carrier (wheat seed, oat seed, or birch sawdust soaked in yeast extract plus 1 M citric acid (pH, 5.5) solution for 30 min and then autoclaved); inoculated carrier incubated at room temperature for 2 d (to allow mycelial growth) and then air-dried at ambient temperature for about 5 h and stored at 5°C	Wheat seed, oat seed, and birch-sawdust formulations used in greenhouse and field experiments; wheat and oat formulations “heavily predated” by insects, lizards, and rodents, and this reduced their efficacy in the field
Fusarium oxysporum f.sp. *erythroxyli*	
Hebbar et al. (1997)	
Bench-top fermentation: fungus + aqueous extract of soybean-hull fiber (autoclaved, filtered, and poured into a 2.5-L fermentor vessel); fermented for 14 d; fermentation in 20-L carboys; fungus + soybean-hull fiber (autoclaved, nonfiltered)	After 14 d of fermentation (bench-top), mostly microconidia and chlamydospores produced; average number of viable propagules (determined on potato-dextrose agar plates) was 2.5×10^7 propagules/mL; inoculum produced with this fermentation method not tested in greenhouse or field for efficacy against coca plants

(Continued)

TABLE 4-7 Continued

Formulation	Viability and Efficacy
Sands et al. (1997)	
Fungal conidia (from potato-dextrose broth culture) + rice grains (autoclaved, dehulled, milled); conidia + rice grain mixture air-dried for 12-24 h and mixed uniformly with steamed greenhouse soil (1:100, w/w)	Rice grain-soil formulation used in greenhouse and field trials where disease symptoms (leaf drop and plant death) were observed

Formulation	Viability and Efficacy
Bailey et al. (1998)	
Rice alginate prill; C6 (rice flour + wheat flour); Pesta (wheat flour + kaolin)	All three formulations enhanced *F. oxysporum* f.sp. *erythroxyli* EN-4 strain population in soil (greenhouse and field experiments)
Bailey et al. (1997)	
Rice alginate prill formulation	In greenhouse tests, application of viable rice alginate prill formulation at 33.6 kg/ha significantly increased soil fungal population (four soil types tested); population remained 1-2 log units higher in top 5.1 cm of soil than in lower 5.1-10.2 cm throughout 7-wk experiment
Connick et al. (1998)	
Pesta formulation (32 g semolina, 5 g kaolin, 3 g fungal biomass, 23 and mL deionized water); twin-screw extrusion formulation (930 g semolina, 232 g kaolin, 112-150 g dried fungal biomass, and 500 mL deionized water, blended together in a food processor and then dried to water activity of 0.24-0.38)	Viability of Pesta formulation tested in laboratory with water agar plates; percentage germination of *F. oxysporum* f.sp. *erythroxyli* determined at 4- or 8-wk intervals for 52 wk; *F. oxysporum* f.sp. *erythroxyli* in Pesta granules had shelf life of at least 1 yr when stored at 35°C (0.12 a_w) and 2 yr if stored at 25°C (0.12 and 0.33 a_w); granules made with twin-screw extrusion method retained 100% of its CFU/g after extrusion and drying at 35°C, and 77% of CFU/g after drying at 50°C

a_w= water activity.

colony-forming units per gram of soil or the proportion of fungal biomass relative to the substrate. Such information is essential for standardization and assurance of the efficacy of the formulation. McCain and Noviello also mentioned the use of other animal feeds to produce the inoculum but did not provide further details. Finally, the application method that they used to infest the field soil, distribution of the inoculum by hand and its incorporation into the top 10 cm of the soil, is not practical for field operations to control drug crops.

There are two problems with the food-based formulations used by Tiourebaev et al. (2001). First, no data were presented on the characteristics of the formulations, such as the number of colony-forming units. Second, the amount of mycoherbicide needed, 125 kg/ha, applied twice in field trials, is too large to be feasible (see Table 4-6). Another serious limitation was the loss of inoculum through predation by fauna, which raises the prospect that formulation with food materials might not be an effective method of delivery of mycoherbicides.

Delivery

The method of delivery of a mycoherbicide is dictated by the biology of the pathogen (whether soilborne or aerial); its mode of action, for example, whether it causes a foliar blight or vascular wilt or infects through wounds; formulation type (liquid or dry); the feasibility of the delivery method; and the desired objective, for example, to kill the target plant population or only to establish disease in it. How the target plant is grown also is likely to dictate the method of delivery; *F. oxysporum* f.sp. *erythroxyli* would probably be applied from the air, while *F. oxysporum* f.sp. *cannabis* might be applied with a ground rig because the plantings often are placed under the canopy of trees, for example, in the southwestern United States. The currently registered mycoherbicides are applied aerially from fixed-wing aircraft (Collego/Lockdown) or backpack or tractor-based sprayer (DeVine), by painting cut stumps (Chontrol), by spreading with a fertilizer spreader or by hand (Sarritor), and by other methods. With mycoherbicides used in agriculture and forestry, the prevalent application and cropping practices largely dictate the method of delivery, and any extra cost and effort needed to apply mycoherbicides with novel tools or methods would be a disincentive.

Although on-ground application of mycoherbicides for cannabis and coca is possible, from a tactical standpoint only aerial application seems feasible for application over areas where growers are uncooperative and possibly hostile. However, aerial application of dry formulations—that is, in powder or pellet forms—might not be effective with a soilborne pathogen, such as *F. oxysporum*. Furthermore, aerial application of a dry formulation is likely to result in a nonuniform, discontinuous placement of the inoculum over the target area and thus limit opportunities for plant-pathogen contacts.

Sands et al. (2002) developed a patented technology to deliver a soilborne fungus, such as *F. oxysporum,* by aerial dispersion. By dispersing live seeds of

cannabis, tall fescue (*Festuca arundinacea*), bluebunch wheatgrass (*Agropyron spicatum*), and tomato treated with a coating agent (carboxymethyl cellulose, methyl cellulose, Mycotech oil, or potato-dextrose broth) and fungal spores, they have demonstrated soil penetration of the fungus and its establishment in the root zone. The germinating seed not only carries the fungal inoculum into the soil but enables the active concentration of a mycoherbicide fungus, such as *F. oxysporum* f.sp. *cannabis,* to increase in the root zone of the carrier plant. This method of delivering the inoculum has been tested in two small-scale field trials in Kazakhstan, where the formulation of cannabis seeds coated with *F. oxysporum* f.sp. *cannabis* yielded higher disease incidence than did a birch-sawdust formulation or a control without inoculum. This novel method offers an intriguing possibility for delivering mycoherbicides that contain soilborne pathogens, but it requires further testing on a large scale to determine its utility as a method for delivering the proposed mycoherbicides.

The committee reviewed published methods for applying *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* used in greenhouse and field tests conducted by McCain and Noviello (1985), Tiourebaev et al. (2001), Sands et al. (1997, 2002), and Bailey et al. (1997, 1998). It also considered an innovative method of mycoherbicide delivery: deploying (from a cargo aircraft) seeds that have been colonized by the mycoherbicide fungus and coated with controlled-release polymers, which are meant to protect the biological agent until optimal environmental conditions occur (Nowak and Eusebi 2010). This method is interesting but so far has not been tested in the field with any mycoherbicide fungi. As for tools or implements for delivery of mycoherbicides, little has been done; dry formulations were spread by hand over the soil surface or incorporated into the soil below the surface, presumably with an implement like a hoe. The rates of application varied from one study to another; in some studies, the method, equipment, or tools used for delivery of inoculum to the soil were not described in enough detail to draw conclusions. Details of the delivery methods tested are summarized in Table 4-8.

Assessment of Performance

The performance of registered mycoherbicides is usually assessed as a percentage of control of the target weed (based on the number of plants in a population killed); as reduction in competition for space, nutrients, water, and light from the weed (collectively termed reduction in weed interference); or as alleviation of yield loss due to the weed. Assessment of efficacy of *F. oxysporum* f.sp. *cannabis* was based on plant mortality (McCain and Noviello 1985) or on a disease-severity scale of 1-5, in which 1 = healthy plant, 2 = wilting of lower leaves, 3 = wilting of 25-50% of leaves, 4 = wilting of over 50% of leaves, and 5 = plant death (Tiourebaev et al. 2001). Assessment of efficacy of

F. oxysporum f.sp. *erythroxyli* was based on a disease-severity scale of 0-2, where 0 = no disease, 1 = plant wilting, and 2 = plant death (Sands et al. 1997). In field trials, Bailey et al. (1997, 1998) used a disease-severity scale of 0-2, where 0 = plant asymptomatic, 1 = symptomatic plant that was defoliating, and 2 = plant death. Such assessments are typically used in academic research and are possible on a small scale when growers are cooperative; that is unlikely to be the case with illicit-drug crops.

TABLE 4-8 Methods of Delivering *Fusarium oxysporum* f.sp. *cannabis* and *Fusarium oxysporum* f.sp. *erythroxyli* in Greenhouse and Field Experiments

Reference	Delivery Method
F. oxysporum f.sp. *cannabis*	
McCain and Noviello 1985	In field experiments: inoculum (barley straw and soybean meal particles with fungal mycelium, conidia, and chlamydospores) distributed by hand and mixed with top 10 cm of soil
	In soil survival experiments (in the field): inoculum incorporated into the top 7.5 cm of soil at 1 g/m^2 and 10 g/m^2
Tiourebaev et al. 2001	In greenhouse experiments: 0.5 g of carrier (inoculated with the fungus) scattered on the surface of soil in a 10-cm^2 pot; equipment or tools used to scatter the inoculum not stated in the paper
	In field experiments: inoculum (mixed with carrier) broadcast at 12.5 g/m^2
F. oxysporum f.sp. *erythroxyli*	
Sands et al. 1997	In growth-chamber experiments: millet seeds colonized by fungus inserted into 10-cm-deep holes in potted soil
	In field experiments: (1) millet seeds colonized by fungus deposited into a 2-cm-deep V-shaped trench and covered with 2-3 cm of soil (5.0 g millet seeds per plot) or (2) millet seeds colonized by fungus applied onto soil surface at 5.0 g/plot
Bailey et al. 1997	In greenhouse and field experiments: Rice alginate prill formulation applied to soil surface at 33.6 kg/ha; method of applying to soil surface not specified
Bailey et al. 1998	Various formulations applied to soil surface at 33.6 kg/ha; method of applying to soil surface not specified

In the case of the proposed mycoherbicides, "acceptable performance" is inadequately defined. As noted earlier, complete eradication (100% of the plants killed) is an unrealistic objective, but high disease incidence that results in high yield losses might be possible, such as over 50% loss, as has been reported in the natural epidemics of *F. oxysporum* f.sp. *erythroxyli* in Peru in the 1990s. In the absence of quick, clear-cut, and measurable results, it might be impossible to assess the performance of the mycoherbicides in operational drug-control programs. Inconsistency of performance by the mycoherbicide strains due to unfavorable climatic conditions, improper timing of application, incorrect method of application, and other factors could render assessments difficult, subjective, and unreliable. On-the-ground scouting and grower surveys could provide reliable estimates, but the safety of the personnel assigned to this task must be considered. Estimates from aerial surveillance probably are not feasible for cannabis and coca, which often are grown as an understory crop or in the midst of natural vegetation, such as rain forests, woodlands, and parklands. Thus, the lack of tools and methods for assessing the performance of mycoherbicides in a field drug-crop control situation does not allow an informed choice about using mycoherbicides to control illicit-drug crops.

PERSISTENCE IN THE ENVIRONMENT

The persistence of the proposed *F. oxysporum* mycoherbicides in the environment is important to determine whether their population density would remain high enough and last long enough to infect the target crops and whether they could survive in the soil and organic matter at levels necessary to affect later plantings of the crop. Another consideration is whether the mycoherbicide strains pose risks to nontarget organisms after release. If such risks occur, the prolonged persistence of the strains would be a disadvantage rather than an advantage. The ability to persist in the environment is an important consideration in proposing the use of a mycoherbicide strain. Monitoring for persistence of registered mycoherbicides has been based on molecular characteristics of the fungus (Dauch et al. 2003; Ditmore et al. 2008).

In general, the efficacy and survival of fungal pathogens depends on environmental conditions, such as temperature, soil composition, moisture (dew period, relative humidity, and rainfall for foliar pathogens; soil moisture and water potential for root pathogens), and ultraviolet radiation. Mycoherbicides are typically formulated to enhance pathogen survival after application to ensure successful infection and establishment on the target plant and possibly to allow for the production of secondary inoculum to keep the disease going during the season or to provide inoculum for the next season. Longer-term survival would depend on favorable environmental conditions. Other factors that influence survival include geographic considerations, the movement of the pathogen outside the application area, the ability of the pathogen to survive on other plant hosts, and the nature and composition of the resident soil biota.

Geographic and Climatic Considerations

Only one study that provides data on the environmental conditions that affect the establishment of *F. oxysporum* f.sp. *cannabis* on inoculated plants was found (Tiourebaev et al. 2001). Infection of cannabis began to appear in field trials conducted in Kazakhstan in May and June, when temperatures were 20-30°C. Disease was highest during late August and September and then declined as "cooler conditions prevailed"; this suggests that the host plant continued to grow while disease development was slowed or arrested at cooler temperatures. However, no quantitative data on the pathogen population size were reported, so it is unknown whether the decreased disease progression was due to decreased numbers of the pathogen. No studies have specifically investigated the effects of different environmental conditions (such as soil composition, temperature, and moisture) on the persistence of *F. oxysporum* f.sp. *cannabis*, but any single strain may have a narrower set of climatic boundaries than cannabis, especially inasmuch as cannabis is grown in a wide variety of environmental conditions (see Chapter 3).

Three studies have evaluated the effects of environmental factors—such as soil type, moisture (soil matric potential and relative humidity), and temperature—on the proliferation and survival of *F. oxysporum* f.sp. *erythroxyli* (see Tables 4-9 and 4-10 for details). Fravel et al. (1996) found that *F. oxysporum* f.sp. *erythroxyli* (strain EN4-FT) can survive in different soil types, at temperatures of 10-35°C, and under stable moisture conditions (such as relative humidity of about 80-97%, regular misting, or irrigation). However, some of the soils tested appear to have had a "suppressive" effect on the pathogen's survival. Followup studies with autoclaved and nonautoclaved soils indicated that native microbial communities in the soils inhibit the survival of the pathogen.

Studies by Bailey et al. (1997, 1998) showed that under some field conditions *F. oxysporum* f.sp. *erythroxyli* (EN-4 strain) can persist for at least 6 or 7 months in the soil with a decline in density over that period. The three formulations tested performed similarly in enhancing soil colonization. Most of the colonization occurred in the top 1-5 cm of soil, and the fungus was shown to colonize coca roots. Moisture was controlled during the first 10-14 days after treatment by drip irrigation, presumably to provide favorable conditions for infection. Enhancement of the *F. oxysporum* f.sp. *erythroxyli* population in soil appeared to be greater when spores were applied to fields previously planted with coca.

Greenhouse and small field tests indicated that *F. oxysporum* f.sp. *erythroxyli* EN-4 can grow and survive at temperatures ranging from 10 to 35°C and that survival is poor below 10°C and above 40°C (Fravel et al. 1996; Bailey et al. 1997, 1998). Moreover, high soil moisture is needed for its initial establishment.

TABLE 4-9 Effects of Temperature, Soil Type, and Matric Potential on Proliferation and Survival of *Fusarium oxysporum* f.sp. *erythroxyli* (Strain EN4-FT) in the Laboratory[a]

Test Factors and Methods	Results
Matric potential	
-10, -100, and -500 kPa 10 prills/plate Soil types tested: Galestown gravelly loamy sand (GGLS) Hatbro loamy sand (HLS) Red clay subsoil (RC) Hawaiian clay (HCL) Soil samples taken after 1 wk Treatments replicated three times Experiment repeated three times	Matric potential of soils affected survival of *F. oxysporum* f.sp. *erythroxyli;* density in HCL soil greatest at -100 kPa, but density in GGLS soil greatest at -10 kPa; in all soils, survival significantly less at -500 kPa (drier soil) than at -100 or -10 kPa (moister soils) Population density of *F. oxysporum* f.sp. *erythroxyli* greatest in HCL soil and least in HLS soil
Constant temperature	
10, 18, 25, 32, and 40°C Soil types tested: GGLS, HLS, and RC Soil samples taken after 1, 5, 9, 13, and 17 wk 10 prills/petri dish (75 petri dishes/soil type) Treatments were replicated three times Experiment repeated once	*F. oxysporum* f.sp. *erythroxyli* survival greatest at 10-32°C; no significant differences in survival between soils tested Population density 10^3-10^5 CFU/g after 1 wk, depending on temperature, and declined to 0-10^4 after 17 wk
Fluctuating temperature	
Alternating 15C° and 25°C every 12 h Alternating 25°C and 35°C every 12 h Soil types tested: GGLS, HLS, and RC Soil samples taken after 1, 5, 9, 13, and 17 wk 10 prills/petri dish (75 petri dishes/soil type) Treatments replicated three times Experiment repeated once	*F. oxysporum* f.sp. *erythroxyli* survival did not differ between temperature regimes, but persistence was affected by time and soil type Initial population density was 10^4-10^6 CFU/g and declined over study; decline most rapid in RC and HLS soils, especially for 25-35°C regime, in which populations fell below detection levels after 17 wk

[a]For all tests, alginate prill formulation (10^5 CFU/prill) was used and 10 prills were added to 25 g of soil. Treatments replicated three times.
Source: Adapted from Fravel et al. 1996.

TABLE 4-10 Greenhouse and Field Studies of Effects of Environmental Factors on Survival of *Fusarium oxysporum* f.sp. *erythroxyli*

	Greenhouse	Field
Bailey et al. 1997		
Formulation and application	Rice alginate prill *F. oxysporum* f.sp. *erythroxyli* strain EN-4 (primarily chlamydospores) Application rate, 33 g/ha	Rice alginate prill *F. oxysporum* f.sp. *erythroxyli* strain EN-4 (primarily chlamydospores) Application rate, 33 g/ha
Coca species and plant age	*E. coca* seedlings	Experiment 1: *E. coca*, 15 mo Experiment 2: *E. coca*, 16 mo Experiment 3: *E. coca*, 7 mo
Environmental conditions	Four soil types tested: Galestown gravelly loamy sand Hatboro loamy sand Red clay subsoil Hawaiian clay 29°C, 14-h daylight; 22°C, 12-h night for 7 wk Soils watered once per week, misted daily	Kauai, Hawaii 17-21°C (average minimum), 23-27°C (average maximum), <80% to >97% relative humidity, drip irrigation for first 10 d Experiments 1 and 2: fields previously grown with coca for 7 yr or more Experiment 3: field not previously grown with coca
Soil sampling method	Samples taken 0, 1, 2, 4, and 7 wk after treatment EN-4 distinguished from background *Fusarium* on basis of colony morphology and distinct orange color	Experiments 1 and 2: single samples taken from each plot 0, 28, 56, and 229 d after treatment Experiment 3: single samples taken 0 and 200 d after treatment (technical problems prevented collection of data between these days) Samples taken from top 7.6 cm of soil and at 7.6-15.2 cm
Results	• EN-4 population was significantly greater than control, similarly in all four soil types • EN-4 maximum population of 4,265 CFU/g after 2 wk, declined to 1,060 CFU/g over next 5 wk	Experiment 1: • EN-4 population significantly greater in soil than control • Population highest in top 7.6 cm of soil (maximum, 534 CFU/g after 1 mo, declined to 95 CFU/g over next 6 mo)

(Continued)

TABLE 4-10 Continued

	Greenhouse	Field
	• EN-4 population highest in top 5.1 cm of soil • EN-4 in control samples averaged less than 4 CFU/g for entire study period	• At soil depths of 7.6-15.2 cm, EN-4 populations low (maximum, 22 CFU/g after 1 mo) • EN-4 in control plots below 10 CFU/g for entire study period Experiment 2: • EN-4 population highest in top 7.6 cm of soil (maximum, 9,015 CFU/g after 1 mo, declined to 114 CFU/g over next 6 mo; not detected after 7 mo) • At soil depths of 7.6-15.2 cm, EN-4 populations low (maximum, 36 CFU/g after 2 mo) • EN-4 in control plots below 10 CFUs/g for entire study period Experiment 3: Data collected only at 6 mo because of technical problems • EN-4 population 58 CFU/g in top 7.6 cm of soil • EN-4 not detected at 7.6-15.2 cm of soil
Replication	Three replications of each soil-treatment combination Experiment repeated	Experiment 1: five replications of each treatment Experiment 2: four replications of each treatment Experiment 3: five replications of each treatment
Bailey et al. 1998		
Formulation and application	Rice alginate prill, wheat-flour kaolin, and rice and wheat-flour mixture *F. oxysporum* f.sp. *erythroxyli* strain EN-4 Application rate, 33 g/ha	Rice alginate prill, wheat-flour kaolin, and rice and wheat-flour mixture *F. oxysporum* f.sp. *erythroxyli* strain EN-4 Application rate, 33 g/ha
Coca species and plant age	*E. coca* seedlings	Experiment 1: *E. coca*, 9 mo old Experiment 2: *E. coca*, 5 mo old Experiment 3: *E. coca*, 7 mo old

Environmental conditions	Galestown gravelly loamy sand 29°C 14-h daylight, 22°C 10-h night for 6 wk	Kauai, Hawaii Drip irrigation for first 14 d after application Experiment 1: field not previously grown with coca; temperature and moisture not specified Experiment 2: field previously grown with coca for 7 yr or more; 24°C (soil), 21°C (air), <80% humidity over first 10 d of treatment Experiment 2: field previously grown with coca for 7 yr or more; 23°C (soil), 20°C (air), >97% humidity over first 10 d of treatment
Soil sampling method	Two samples taken 1, 4, and 6 wk after treatment Three samples taken 2 and 6 wk after treatment	Experiment 1: sampling method not specified Experiment 2: samples taken 0, 10, 33, 60, and 232 d after treatment Experiment 3: samples taken at 0, 11, 28, 62, and 213 d after treatment Samples taken from top 1 cm of soil and at 7.6-15.2 cm
Results	• All formulations resulted in greater populations of EN-4 in soil than in untreated controls; rice alginate prill had lowest increase • EN-4 populations highest in top 1 cm of soil (mean, 5,587 CFU/g) • At depths of 5.1-10.2 cm, EN-4 populations low (mean, 20 CFU/g) • After 2-5 wk, mean EN-4 populations declined from 31,804 CFU/g to 5,587 CFU/g	Experiment 1: No data on EN-4 populations reported Experiment 2: • All three formulations resulted in greater populations of EN-4 in top 1 cm of soil than in control (mean maximum, 15,135 CFU/g,within 10 d, declined to 29 CFU/g at 232 d) • No significant difference in EN-4 populations between soil depths of 7.6-15.2 cm and controls and EN-4 not detected at end of study Experiment 3: • All three formulations resulted in greater populations of EN-4 in top 1 cm of soil and at 7.6-15.2 cm than in untreated controls • Maximum mean of 79,432 CFU/g in top 1 cm of soil was about five times greater than in Experiment 2 • EN-4 barely detectable at depths of 7.6-15.2 cm after 213 d
Replication	Four replications of each treatment Experiment was repeated	Experiment 1: four replications of each treatment Experiment 2: six replications of each treatment Experiment 3: six replications of each treatment

Transmission and Spread

The potential pathways by which spores and other propagules from a particular application site might move via physical transport mechanisms are illustrated in Chapter 2 (see Figure 2-1). In general, dispersal of the proposed mycoherbicides after application will depend on the production and natural dispersal of secondary inoculum. In the case of *F. oxysporum*, secondary spread was originally thought to be limited to root-to-root contact and distribution of infected plant tissues and seeds (Gambogi 1983; Gracia-Garza et al. 1999; Rekah et al. 2001). However, additional evidence indicates that under humid conditions, several formae speciales of *F. oxysporum* produce conidia in stroma (mass of hyphae) on the lower stems of infected plants (Rowe et al. 1977; Timmer 1982; Woudt et al. 1995; Gamliel et al. 1996; Vakalounakis 1996; Katan et al. 1997; Rekah et al. 1999, 2000, 2001; El-Hamalawi and Stanghellini 2005). These conidia can be dispersed by rain, wind, or insects (Gillespie and Menzies 1993; El-Hamalawi 2008). Inoculum could also be transferred from the application site by several vectors, including humans (transporting plant material or infested seeds), the hides or fur of mammals (such as pack animals), the droppings of birds, or the surface of insects. Long-distance transport via waterways is a potential route inasmuch as viable and infectious propagules of *F. oxysporum* have been found in rivers and seas (Palmero et al. 2009), although the distance over which such transport might occur is unknown. Finally, windstorms could carry inoculum over long distances to areas far outside the target areas (Maldonado-Ramirez et al. 2005). Thus, the potential exists for the proposed mycoherbicides to move out of the geographic areas where they are applied.

Only a few studies have examined the dispersal of *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli.* In a field study in Kazakhstan, consumption and dispersal by insects, lizards, rodents, and birds was reported for a seed-based inoculum of *F. oxysporum* f.sp. *cannabis* (Tiourebaev et al. 2001). In a field study in Hawaii, the applied inoculum of *F. oxysporum* f.sp. *erythroxyli* was foraged by ants and became clustered in their nests (Gracia-Garza et al. 1998).

In addition, many *Fusarium* species, including *F. oxysporum*, survive on and in seeds (Nelson et al. 1997; Garcia-Garza et al. 1999; Mbofung and Pryor 2007). Transmission by seeds and planting materials carries the risk of unintended spread beyond target areas in that farmers and traders could carry infected materials throughout a region or even into new areas, as has been proposed for the transport of *F. oxysporum* f.sp. *erythroxyli* that appears to have arrived in Hawaii from Peru (Fravel et al. 1996; Sands et al. 1997).

Other Host Plants

In general, *Fusarium* species are ubiquitous in the environment and can survive on host plants that may or may not be susceptible to them (Blok and

Bollen 1997). In a host-range study of *F. oxysporum* f.sp. *erythroxyli*, Sands et al. (1997) reported that the pathogen was isolated from the crown tissue of symptomless plants of 26 species grown in treated soil. Thus, this fungus could colonize plants other than coca. However, no studies have evaluated the long-term persistence of either *F. oxysporum* f.sp. *erythroxyli* or *F. oxysporum* f.sp. *cannabis* through observations on their survival in soil after the host plant dies or evaluated their potential to colonize later plantings of coca or cannabis.

The recent study of genetic variation at two loci among *F. oxysporum* individuals pathogenic on animals and plants (O'Donnell et al. 2009) showed that *F. oxysporum* f.sp. *erythroxyli* has a unique genotype at both loci but that *F. oxysporum* f.sp. *cannabis* shared one or both alleles with individuals that were isolated from other plants. Although it certainly is possible that either *Fusarium* mycoherbicide could attack nonhost plants, it also is true that the mycoherbicide strains would not be expected to behave differently from members of the two *Fusarium* species that are already present in the endemic areas.

Competition with Other Soil Microorganisms

Although it was demonstrated that *F.* oxyspsorum f.sp. *erythroxyli* can be suppressed in some soil types (Fravel et al. 1996), no studies are available on the interactions of *F. oxysporum* f.sp. *cannabis* or *F. oxysporum* f.sp. *erythroxyli* with particular soil microorganisms or other organisms. Studies of other *F. oxysporum* formae speciales and other fungi suggest that the presence of competitor or antagonistic microorganisms could reduce persistence. For example, springtails and mites feed on plant pathogenic fungi (Nakamura et al. 1992; Okabe 1993), and several bacteria and fungi can suppress the pathogens responsible for *Fusarium* wilt of melon (Suárez-Estrella et al. 2007), tomato (Larkin and Fravel 1999), and chickpea (Landa et al. 2004). In fact, such antagonists have been pursued as biological control agents against these wilt diseases. Thus, antagonistic microorganisms in the soil could theoretically lessen the likelihood that the proposed mycoherbicides would establish sufficiently high inoculum levels in the rhizosphere to cause wilt disease of cannabis or coca. In contrast, it is possible that the mycoherbicide strains could displace resident strains; but, again, no data are available to evaluate this possibility.

Conclusions

It is clear that *F. oxysporum* f.sp. *erythroxyli* has the ability to colonize and survive in soil and coca roots for several months, but there are insufficient data to predict the long-term persistence of *F. oxysporum* f.sp. *erythroxyli*, in that none of the studies lasted for more than 7 months. However, the natural presence of this fungus and the recurrent incidence of the coca wilt disease in the Huallaga Valley of Peru (see discussion later in this chapter) suggest that *F. oxysporum* f.sp. *erythroxyli* is capable of long-term persistence. Inasmuch as *F.*

oxysporum f.sp. *erythroxyli* could survive on plants other than coca and on decaying organic material, it is likely that the mycoherbicide strain would persist in the indigenous fungal populations at some level once it is introduced in coca fields. In general, the environmental factors that affect the persistence of *F. oxysporum* f.sp. *erythroxyli* may also affect *F. oxysporum* f.sp. *cannabis*, but too little information is available on the latter forma specialis to draw any conclusions beyond this generalization.

EFFECTS ON NONTARGET ORGANISMS

Microbial pesticides are regulated by the U.S. Environmental Protection Agency, which requires a variety of testing of the environmental fate and safety of pesticides before they are registered. Chapter 2 and Appendix B describe the types of testing required, including product analysis, pesticide residue analysis, toxicity testing, toxicity and pathogenicity testing on nontarget organisms, and assessment of environmental fate. The aforementioned studies have not been conducted with the goal of registering the tested strains of *F. oxysporum* f.sp. *cannabis* or *F. oxysporum* f.sp. *erythroxyli* as mycoherbicides, so this section reviews the existing data on them that are available in the open literature and are pertinent to understanding potential adverse effects on nontarget plants and organisms. Consideration is given to the issues specified in the committee's statement of task, including potential effects on licit crops, other soil fungi, animals, humans, biodiversity, and other relevant aspects of environmental health.

Effects on Nontarget Plants

Some research has been performed to evaluate the effects of *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* on native plant species, but the reported data are sparse. For example, Tiourebaev et al. (2000, 2001) tested *F. oxysporum* f.sp. *cannabis* on several plant species in Kazakhstan, including food crops and native plants (see Table 4-11). The first paper appeared in the proceedings of a conference and reported that none of 13 plants tested exhibited symptoms of *Fusarium* wilt but provided no experimental data to support the claim (Tiourebaev et al. 2000). The second paper reported that host-range tests were conducted under greenhouse conditions on five crops that were commonly cultivated in the test region of Kazakhstan (tomato, corn, wheat, bean, and potato) but contained no data or discussion of the results obtained pertaining to those crops (Tiourebaev et al. 2001); instead, the authors simply state that 12 nontarget plants "showed no evidence of infection by the pathogen" and seemed to imply that the results were the results of field tests.

The possible taxonomic and etiological relationships of *F. oxysporum* f.sp. *cannabis* to other wilt-causing *Fusarium* species that attack plants related to cannabis, such as the economically important hops (*Humulus lupulus*), were not

adequately addressed either by McCain and Noviello (1985) or by Tiourebaev et al. (2001). The claim by McCain and Noviello that hops "are not known to be susceptible to a [*Fusarium*] wilt disease anywhere in the world" is erroneous (McPartland and West 1999; Solarska 2001).

One host-range study of *F. oxysporum* f.sp. *erythroxyli* was identified. Sands et al. (1997) tested strains of this fungus from Hawaii and South America on 26 plant species (see Table 4-12) grown in growth chambers from seeds planted in pathogen-infested soil. Wilt symptoms were not observed on any of the native species. However, when crown tissues of the species grown in the infested soil were plated on a selective medium at the conclusion of the experiment, *F. oxysporum* was isolated from all species. Those results indicate that *F. oxysporum* f.sp. *erythroxyli* was not pathogenic to the tested hosts, but because it was present in the crowns the authors concluded that it could infect nontarget host tissues.

TABLE 4-11 Plants Reportedly Tested in Host-Range Studies of *Fusarium oxysporum* f.sp. *cannabis*

Tiourebaev et al. 2000	Tiourebaev et al. 2001
Agropyron spicatum (wheat grass)	Crops:
Cucumus sativus (cucumber)	*Lycopersicon esculentum* (tomato)
Daucus carota (wild carrot)	*Phaseolus vulgaris* (kidney bean)
Festuca arundinaceae (tall fescue; grass)	*Solanum tuberosum* (potato)
Gossypium hirsutum (cotton)	*Triticum aestivum* (wheat)
Hordeum vulgare (barley)	*Zea mays* (corn)
Lycopersicon esculentum (tomato)	
Melilotus indica (sweet clover; herb)	Native plants:
Phaseolus vulgaris (kidney bean)	*Agropyrum pectiniforme* (crested wheatgrass)
Pisum sativum (garden pea)	*Alhagi pseudalhagi* (camelthorn; legume)
Raphanus sativus (radish)	*Artemisia vulgaris* (common wormwood; herbaceous perennial)
Triticum aestivum (wheat)	*Atriplex alba* (lambsquarters goosefoot; shrub)
Zea mays (corn)	*Bromus inermis* (awnless brome; grass)
	Ceratocarpus arenarius (jiao gulo li; herb)
	Chenopodium sp. (common lamb's quarters; herbaceous annual)
	Dactylis glomerata (orchardgrass)
	Glycyrrhiza glabra (cultivated licorice; legume)
	Kochia prostrata (forage kochia; shrub)
	Stipa dasyphylla (grass)
	Tragopogon major (yellow salsify)

TABLE 4-12 Plants Reportedly Tested in Host-Range Studies of *Fusarium oxysporum* f.sp. *erythroxyli*

Abelmoschus esculentus (okra)	*Helianthus annuus* (sunflower)
Allium cepa (onion)	*Hordeum vulgare* (barley)
Arachis hypogaea (peanut)	*Lactuca sativa* (lettuce)
Beta vulgaris (sugar beet)	*Lycopersici esculentum* (tomato)
Capsicum annuum (cayenne pepper)	*Oryza sativa* (rice)
Carthamus tinctorius (safflower)	*Phaseolus vulgaris* (kidney bean)
Citrullus lanatus (watermelon)	*Pisum sativum* (garden pea)
Cucumis melo var. *cantalupensis* (cantaloupe)	*Raphanus sativus* (radish)
Cucumis sativus (cucumber)	*Sorghum bicolor* (sorghum)
Cucurbita moschata (pumpkin)	*Triticum aestivum* (wheat)
Daucus carota subsp. *sativus* (carrot)	*Vigna unguiculata* (cowpea)
Glycine max (soybean)	*Zea mays* (corn)
Gossypium barbadense (annual long-fiber cotton)	

Source: Adapted from Sands et al. 1997.

Overall, the data on the host range of *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* are insufficient to draw any conclusions. *Erythroxylum* is a large genus; it has as many as 250 species, of which about 200 have the same native habitat as the coca plants (Plowman 1980). Thus, it is important to determine whether *F. oxysporum* f.sp. *erythroxyli* could attack potential alternative hosts and whether any of them are of ecological significance, for example, endangered, threatened, or of particular value.

Conducting such tests and interpreting the data from them may be difficult because *F. oxysporum* is ubiquitous and "the host range tests required to prove specificity [of the *formae speciales*] would be very large and likely prohibitive" (Berner and Bruckart 2005, p. 227).

Effects on Legal Crop Production

The potential risk to legal production of cannabis and coca has not been given much attention. Commercial cultivation of hemp is not legal in the United States, but hemp is grown in Canada and many other countries (for example, several European countries, China, and Russia) for fiber, for linen production, and for seeds, which are a source of meal and oil used in food and other products (USDA 2000).

McCain and Noviello (1985) claimed that "because of the restricted host range, no special precautions are necessary for controlled use of the fungus," even though the industrial hemp cultivars tested developed 10-100% disease. They also claimed that cannabis cultivars or seed collections from Czechoslovakia, India, Iran, Mexico, Nepal, Pakistan, Poland, South Africa, Thailand, and

Turkey were "all highly susceptible to the disease and in general there were no survivors or escapes in [a] growth chamber study." However, no data were provided on the extent of cultivar differences in susceptibility. On the basis of the McCain and Noviello (1985) data, *F. oxysporum* f.sp. *cannabis* could pose a risk to commercial hemp grown for fiber.

Toxicity to Wildlife, Domestic Animals, and Humans

This subsection focuses on the potential toxicity to nontarget organisms. As discussed in Chapter 1, toxicity refers to the degree or extent of harm caused by a *chemical*. In the case of the proposed mycoherbicides, the greatest risk to animals and humans probably would come from the consumption of the pathogen in colonized plant material. Toxins might be produced in the materials used to deliver the inoculum. Tiourebaev et al. (2001) found that when *F. oxysporum* f.sp. *cannabis* was applied to wheat and oat seeds, insects, lizards, and rodents consumed the inoculum. There are no studies of mycotoxin production by *F. oxysporum* f.sp. *cannabis* or *F. oxysporum* f.sp. *erythroxyli.* As noted earlier in this chapter, *F. oxysporum* is a large and diverse species complex, and the genus *Fusarium* is even larger, so generalizations or assumptions about the production of mycotoxins by the proposed mycoherbicide strains cannot be made, even though there is a great deal of information about mycotoxins produced by members of the genus *Fusarium* (Marasas et al. 1984; Desjardins 2006).

Filamentous fungi produce a variety of metabolites. Primary metabolites are chemicals that are required for the survival of an organism, and secondary metabolites are organic compounds whose production by an organism is not required for growth, development, reproduction, or immediate survival. Biosynthesis of secondary metabolites often begins with components derived from primary metabolism. Nearly all fungi produce at least one secondary metabolite. Although the functions of most secondary metabolites are unknown, many have been shown to be biologically active. Many have antimicrobial activity and thus might play a role in competition with other microorganisms. Others are toxic to plants and function as pathogenicity factors.

Secondary metabolites that are toxic to animals and humans are called mycotoxins. Mycotoxins are grouped into classes on the basis of their chemical structure. All members of a class have a common core structure; each member has its own unique chemical modifications of the common core. The toxicity of members of a class is diverse; they can range from nontoxic to lethal. Sensitivity to mycotoxins also varies: some animals exhibit acute reactions to a particular mycotoxin and members of other species find it innocuous or require long exposures to produce any observable symptoms.

Some members of the genus *Fusarium* produce toxic secondary metabolites (Marasas et al. 1984; Desjardins 2006). Table 4-13 presents a summary of the classes of mycotoxins reportedly produced by *F. oxysporum*. Most research

TABLE 4-13 Classes of Mycotoxins Produced by *Fusarium oxysporum*

Mycotoxin Class	Source	Reference
Beauvericin	Maize culture	Logrieco et al. 1998
Fumonisins	Wheat culture	Seo et al. 1996
Fusaric acid	Maize culture	Bacon et al. 1996
Fusarin C	Defined medium	Cantalejo et al. 1999
Moniliformin	Rice culture	Abbas et al. 1990
Trichothecenes	Defined medium	Cantalejo et al. 1999
Wortmannin	Rice culture	Abbas and Mirocha 1988

on *Fusarium* mycotoxins has focused on the ones that contaminate important food and feed crops, such as corn, wheat, barley, and potato. Some highly toxic metabolites—such as trichothecenes, moniliformin, fumonisins, fusarin C, and zearalenone—have been found (Kim and Lee 1994; Cantalejo et al. 1999; Seo et al. 1999; Miller 2002; Rheeder et al. 2002).

Trichothecene toxins include T-2 toxin and diacetoxyscirpenol, which are on the U.S. select-agents list and pose severe threats to animal or plant health or products. T-2 and diacetoxyscirpenol are most commonly produced by *Fusarium* species other than *F. oxysporum*. The data on trichothecene production in the report by Cantalejo et al. (1999) are questionable because the identification of the one *F. oxysporum* isolate to produce trichothecene was based solely on morphologic characteristics of cultures that were not grown under the standard conditions (Leslie and Summerell 2006) used for the accurate identification of these fungi. Given that background, it is unlikely that any *F. oxysporum* strains proposed as mycoherbicides would mutate to produce T-2 or diacetoxyscirpenol, although it would be straightforward to test mycoherbicide strains for these molecules. Because the biosynthetic pathways for these toxins are known (e.g., Desjardins et al. 1993; Kimura et al. 2007), sequencing of appropriate portions of the genomes of the proposed mycoherbicide strains could be used to determine whether the genes encoding the enzymes in these pathways are present. If some of or all the necessary genes are missing, it is unlikely that the strains could mutate to produce either of the toxins.

Fumonisins are a large family of related compounds, a few of which are produced by some strains of *F. oxysporum*. The analogues most commonly associated with human health problems are those in the fumonisin B group. *F. oxysporum* strains are known to produce members of the fumonisin C series (Seo et al. 1996; Sewram et al. 2005), but these toxins are not produced at high concentrations even under optimal conditions. Some isolates have also been shown to produce small amounts of fumonisin B_1/B_2 in culture (Waskiewicz et al. 2010). Strains of *F. verticillioides* have been identified as responsible for the production of the fumonisin that caused neural-tube defects in newborns in the Rio Grande valley of Texas and in the Transkei region of South Africa (Ncayi-

yana 1986; Hendricks 1999; Gelineau-van Wase et al. 2009). A strain of *F. oxysporum* used as a bioherbicide of striga was grown in culture medium known to encourage mycotoxin production (Savard et al. 1997). Small amounts of fusaric acid were detected but no other mycotoxins.

Pathogenicity to Animals and Humans

There are reports in the literature of about 150 cases of *Fusarium* infections in immunocompetent people and 330 cases in immunocompromised people (references include Nelson et al. 1994; Guarro and Gene 1995; Baran 1997; Gianni et al. 1997; Romano et al. 1998, 2010; Sander et al. 1998; Musa et al. 2000; Bodey et al. 2002; Nucci and Anaissie 2002; Albisetti et al. 2004; Naiker and Odhav 2004; O'Donnell et al. 2004; Guilhermetti et al. 2007; Nucci and Anaissie 2007; Nucci et al. 2010). Among the cases reported in immunocompetent people, virtually all were nail infections (onychomycosis) with or without accompanying localized cutaneous infection (paronychia). Cases of keratitis were excluded from this review because *Fusarium solani* was reported as the etiologic agent in the overwhelming majority of cases. Increased *Fusarium* inoculum in the soil and in the environment could potentially increase the number of these types of cases in the population of the target areas. Although the infections lead to cosmetic disfigurement of affected nails, the infections are self-limited in immunocompetent hosts.

In immunocompromised hosts, fusariosis is a much more serious infection. Although many of the infections in this population begin as nail and superficial skin infections, the organism rapidly invades blood vessels and spreads hematogenously (Nucci and Anaissie 2002, 2007). These infections are difficult to diagnose and treat and lead to poor survival rates in infected patients. Although increased *Fusarium* in the environment could lead to increased infections in immunocompromised populations, the population at risk in developing nations would be estimated to be somewhat smaller than in developed countries, in part because of the much lower numbers of procedures performed in developing countries that involve treatments for suppressing the immune system, such as organ transplantation (Ota 2004). However, the committee is aware that the variability of conditions that contribute to immunosuppression in members of a population and the difficulty of estimation of the prevalence of such conditions clearly hinder long-term estimation of risk. A final caveat in interpreting these studies is that in many cases the species of *Fusarium* was not determined and in others the accuracy of the identification, which was almost always based only on structure, could be called into question. In addition, the phenotype of the potential mycoherbicide strains of *Fusarium* appears to be largely uncharacterized.

Although a few cases of infection with *Fusarium* have been reported in animals (for example, see Mayayo et al. 1999; Conkova et al. 2003; Evans et al. 2004; Marangon et al. 2009), most of the reports deal with cases of proven or presumed *Fusarium* toxicosis.

An additional factor that should be considered in an evaluation of potential effects of *Fusarium* on humans is the paucity of effective antifungal drugs available to treat systemic fusariosis (Azor et al. 2009). In a recent review, Lortholary et al. (2010) reported an overall rate of success of voriconazole treatment for systemic fusariosis of 47%, with a range of 0-64%, depending on the site of infection. Combination therapy with amphotericin B did not improve overall success. The median survival rate of patients infected with *F. oxysporum* was 112 days. That is perhaps not surprising, in light of the poor in vitro response of *F. oxysporum* isolates to antifungal drugs. Although the efficacy of voriconazole is low, it compares favorably with the administration of amphotericin B and its lipid derivatives (Pfaller et al. 2002). Voriconazole and the other mold-active azoles posaconazole and ravuconazole are likely to be the mainstays of therapy for systemic fusariosis for now (Stanzani et al. 2007).

MUTATION

The potential of fungi used as mycoherbicides to mutate is similar to that of other fungi in general. Fungal genetic variation can be affected by many processes, including nucleotide substitution (Kasuga et al. 2002), gene loss (Sharpton et al. 2009), gene gain by duplication (Sharpton et al. 2009), and gene gain by horizontal gene transfer (that is, not from parent to offspring) from closely related (Neafsey et al. 2010) or distantly related (Friesen et al. 2006) fungi. The gain of genetic material can be as small as a few genes or as large as complete chromosomes (Ma et al. 2010). Genetic variation can become established, or fixed, in fungal populations by natural selection or by chance. Natural selection leads to adaptation to changing environments, and the adaptation could include the ability to attack new cultivars of existing host species or new host species. Adaptation may occur in fungi that reproduce sexually or asexually, but genetic recombination speeds the process by enabling the rapid synthesis of numerous genotypes (Goddard et al. 2005). Recombination is accomplished by mating and meiosis but also could occur by nonsexual anastomosis and nonmeiotic recombination via the parasexual cycle (even though many fungi have mechanisms to limit nonsexual hyphal anastomosis to partners with almost identical genotypes). All those evolutionary processes apply to fungi used as mycoherbicides.

Strains of *Fusarium* have a reputation for being highly mutable in culture, especially if the strains are maintained for a long period on a medium high in simple sugars (Puhalla 1981; Leslie and Summerell 2006). Mass production of *Fusarium* would involve such long-term maintenance of the strains. Mutations that occur under laboratory conditions usually are of a loss-of-function nature, such as the inability to synthesize a toxin, the inability to produce one or more types of spores, abnormal hyphal structure, or loss of plant pathogenic capabilities. Such mutations are probably due to the inactivation of one or more functional genes that are already in the strain's genome. Under field conditions, mu-

tations would certainly occur, although there is no reason to think that they would be more likely in these fungi than in indigenous strains or any other fungi.

Although no sexual stage has been reported for *F. oxysporum*, it is not possible to rule out its existence. For example, there are two mating types in sexual *Fusarium*, *MAT1-1* and *MAT1-2*, and both are found in the strains of *F. oxysporum* that have been evaluated (Enya et al. 2008; Lievens et al. 2009). Therefore, it is possible that adaptation in *F. oxysporum* could be aided by recombination if an as yet undetected sexual stage is occurring.

It is possible that mutation could appear to be driven in *F. oxysporum* by selective conditions. For example, some conditions might favor a particular strain among the very large pre-existing populations of *F. oxysporum* in the soil. *F. oxysporum* is ubiquitous in soils where crops are grown, and its populations would harbor substantial genetic variation. As a consequence, the sudden appearance of such a strain could be attributed to mutation even though the strain had existed at low levels for an extended period. Alternatively, phenotypic changes could result from a mutagenic mechanism, such as the one responsible for the spontaneous generation of *nit* mutants, which are rendered insensitive to chlorate toxicity when the strains are cultured in the presence of high concentrations of chlorate (Klittich and Leslie 1988). The mechanism underlying this mutagenic process has not been characterized, but it appears to have a multigenic base (Klittich et al 1988). In both those cases, mutations appear to be directed by the selective conditions under which they occur.

Mutations could make mycoherbicide strains more virulent and thus make a host plant more susceptible to them. It is equally likely that the host plant would produce offspring that could be more resistant to the mycoherbicide. Changes in susceptibility and pathogenicity are expected as the host-pathogen system evolves. Selection of plants resistant to mycoherbicide strains should be relatively straightforward; the more virulent the mycoherbicide strain is and the fewer genetically susceptible plants escape it by chance, the more rapidly selection should occur.

Mutations could affect mycotoxin production, provided that a mycoherbicide has the genes for toxin production. Mutations also could alter the synthesized toxin by blocking its biosynthetic pathway; this would lead to the accumulation of an intermediate in the pathway rather than of the end product. No on toxin production in the proposed *F. oxysporum* mycoherbicides are available.

WHAT WE CAN LEARN FROM A NATURAL EPIDEMIC OF *FUSARIUM OXYSPORUM* F.SP. *ERYTHROXYLI* WILT OF COCA IN PERU

The natural occurrence of diseases is common in plant populations, and plants that are intensively grown as crops are particularly vulnerable to periodic devastating epidemics. Recent history tells us that coca populations are no ex-

ceptions. For example, an epidemic on coca in Peru in the late 1980s was linked to *F. oxysporum* f.sp. *erythroxyli* (Arévalo et al. 2000). That natural epidemic can be viewed to some extent as a large-scale field trial that is informative in considering several questions: Did the epidemic inflict sufficient damage to reduce crop production? Did it cause recurrent or permanent effects on coca in the affected areas? If the effects of this natural epidemic are an indication, how effective would it be if the same pathogen were applied as a mycoherbicide to curtail the production of illicit-drug crops?

A wilt of coca (*E. coca*) caused by *F. oxysporum* resulted in extensive losses to the coca crop in the Huallaga Valley, the main coca-producing region in Peru (Arévalo et al. 2000). Although definitive reports of the presence of the disease go back at least as far as 1932, its incidence increased sharply in the 1980s, at the time of the increase in coca production, the subsequent increase in use of agricultural chemicals, and the reduction in cultivation time (Nelson et al. 1997). The pathogen was reported as *F. oxysporum* f.sp. *erythroxyli* (Nelson et al. 1997; Arévalo et al. 2000) and one of its strains was found to be identical to a strain of the pathogen studied in Hawaii by Sands et al. (1997) and Nelson et al. (1997).

The most recent epidemic of *F. oxysporum* f.sp. *erythroxyli* wilt of coca in the Huallaga Valley of Peru began in 1987. It was estimated that 52% of the coca crops were affected during 1992-1994. To describe the impacts of the disease, Arévalo et al. (2000) quantified its incidence and development in 11 coca fields in five regions in the valley. The coca plants sampled were 14-93 months old. Overall, the coca leaf yields were reduced by 74%. Disease incidence (the proportion of sampled plants that were diseased) ranged from 54% of 32-month-old plants to 79% of 93-month-old plants. The level of disease, measured as the area under the disease-progress curve, ranged from 5.67 on 39-month-old plants to 28.2 on 93-month-old plants (that is, the older plants were more diseased). Thus, the Peruvian *F. oxysporum* f.sp. *erythroxyli* epidemics of the 1980s and 1990s were highly devastating to coca production in the Huallaga Valley.

No formal followup studies of the disease have been performed, but anecdotal observations suggest that the coca wilt disease persists and affects coca leaf production in some portions of the valley. In some areas, however, cultivation of coca has been moved to new fields to escape the disease (personal communication, E. Arévalo, Instituto de Cultivos Tropicales, November 16, 2010, to B. Bailey, USDA). Hence, the epidemic, even while inflicting severe losses, has not deterred coca production, which has continued even in the presence of sporadic disease outbreaks.

5

Crivellia papaveracea and *Brachycladium papaveris* as Candidate Biological Control Agents against Opium Poppy

Fungal diseases are a normal occurrence where opium poppy (*Papaver somniferum*) is grown repeatedly. Among the fungal pathogens that can cause serious damage to poppy plants are species of *Brachycladium*, *Crivellia, Fusarium, Peronospora,* and *Verticillium* (Scott 1877; Harrison and Schmitt 1967; Dolgovskaya et al. 1996; Podlipaev et al. 1996; Reznik et al. 1996; Finetto 2008). *Crivellia papaveracea* and *Brachycladium papaveris* (formerly known as *Pleospora papaveracea* and *Dendryphion penicillatum*, respectively) have received the most attention as parasites that might substantially limit licit poppy cultivation (Milatović 1975a b) and as potential mycoherbicides against illicit opium poppy crop (Del Serrone and Annesi 1990; O'Neill et al. 2000; Bailey et al. 2000b, 2004a, 2004b; UNODC 2002). Both fungi are distributed worldwide wherever poppy is grown (Milatović 1975a; Munro 1978; Sivanesan and Holliday 1982).

As noted in Chapter 1, a single fungus may have more than one name, and this can complicate interpretation of the scientific literature. Nowhere is that problem more evident than in the literature on *C. papaveracea* and *B. papaveris*. In this report, the publication by Inderbitzin et al. (2006) is used for the taxonomy of those fungi because it used DNA sequence data to identify strains, including many of the ones used in the studies cited in this chapter. Inderbitzin et al. (2006) describe how different authors have used the names in different ways. In the present report, where possible, we have translated the names used by the various authors into the currently accepted names, *C. papaveracea* and *B. papaveris*. Readers who consult the original literature may find Table 5-1 useful in attempting to equate the names used in that literature with the names used

here. The attributes of *C. papaveracea* and *B. papaveris* are listed in Table 5-2 with the two isolates studied by O'Neill et al. (2000) as reference strains. When it is unclear which of the two species was studied, we refer to both in this manner: *C. papaveracea/B. papaveris*.

C. papaveracea/B. papaveris was reported as a pathogen of poppy by Milatović (1975a,b) and Czyzewska and Zarzycka (1960). *C. papaveracea* and *B. papaveris* are seedborne pathogens that are widely distributed around the world wherever poppy is grown but are most commonly observed in Europe and Asia (Schmitt and Lipscomb 1975). Both can cause serious damage to poppy when conditions for disease are optimal (Krikorian and Ledbetter 1975; Schmitt and Lipscomb 1975). *C. papaveracea/B. papaveris* attacks plant roots and parts above the ground; disease symptoms include seedling damping-off, girdling of roots, and lesions on leaves, stems, and capsules (Meffert 1950; O'Neill et al. 2000).

TABLE 5-1 Fungi Used in Various *Papaver* Mycoherbicide Studies

Fungal Strain (Reference)	Identification Given in Reference	Identification by Inderbitzin et al. 2006
Strain 7359 (Meffert 1950)	*Dendryphion penicillatum*	*Crivellia papaveracea*
Strain 3 (Meffert 1950)	*Helminthosporium papaveris*	*Brachycladium papaveris*
Isolates used by Del Serrone and Annesi 1990	*Dendryphion* state of *P. papaveracea*	Strains not studied by Inderbitzin et al. 2006. Inferred to be a heterothallic fungus.
Strain Cf96 (Farr et al. 2000)	*Dendryphion penicillatum*	*Crivellia papaveracea*
Strain Pf96 (Farr et al. 2000)	*Pleospora papaveracea*	*Brachycladium papaveris*
Strain Cf96 (O'Neill et al. 2000)	*Dendryphion penicillatum*	*Crivellia papaveracea*
Strain B96 (O'Neill et al. 2000)	*Pleospora papaveracea*	*Brachycladium papaveris*
Strain Pf96 (Bailey et al. 2000a, 2000b)	*Pleospora papaveracea*	*Brachycladium papaveris*
Strain Cf96 (Bailey et al. 2000b)	*Dendryphion penicillatum*	*Crivellia papaveracea*
No strain number; inferred to be Pf96 (Bailey et al. 2004a)	*Pleospora papaveracea*	*Brachycladium papaveris* (if the isolate is indeed Pf96)
Strain Pf96 (Bailey et al. 2004b)	*Pleospora papaveracea*	*Brachycladium papaveris*
Strain C-6-3 (UNODC 2002)	*Pleospora papaveracea*	(Not studied by Inderbitzin et al. 2006)

TABLE 5-2 Attributes of the *Papaver* Mycoherbicides *Crivellia papaveracea* and *Brachycladium papaveris*

Attribute	*Crivellia papaveracea*	*Brachycladium papaveris*	Reference
Reference strain[a]	Cf96 *Dendryphion penicillatum*	Pf96 *Pleospora papaveracea*	O'Neill et al. 2000
Teleomorph (sexual form)	*Crivellia papaveracea*	None described[b]	Inderbitzin et al. 2006
Anamorph (asexual form)	*Brachycladium penicillatum*	*Brachycladium papaveris*	Inderbitzin et al. 2006
Sexual reproduction	Heterothallic (requires mating partner)	Homothallic (self-mating)	Inderbitzin et al. 2006
Macroconidiophores[c]	Produced	Not produced	Inderbitzin et al. 2006
Microsclerotia[d]	Present	Absent	Inderbitzin et al. 2006; Meffert 1950
Chlamydospores[e]	Not reported	Infrequent; intercalary (between apex and base)	Farr et al. 2000; Meffert 1950
Pseudothecia[f]	Present in field material	Present in field material; produced by laboratory cultures older than 30 days	O'Neill et al. 2000
Relative virulence	Less virulent	More virulent	Bailey et al. 2000b; O'Neill et al. 2000

[a]Strains used by O'Neill et al. 2000 and studied by Inderbitzin et al. 2006.
[b]The situation with *B. papaveris* is unusual because this fungus has a sexual, meiotic spore state (Farr et al. 2000), but Inderbitzin et al. (2006) were unable to locate a specimen with the sexual structures to serve as a type for a teleomorph name.
[c]Hyphae (vegetative threads) that bear cells that produce macroconidia or large asexual spores.
[d]Very small rounded mass of hyphae.
[e]Asexual thick-walled one-cell spores.
[f]Specialized structures that bear asci (which contain ascospores or sexual spores).

In 1986, *C. papaveracea/B. papaveris* was evaluated as a potential biological control agent against *Papaver rhoeas,* a major weed of wheat in Italy (Covarelli 1981; Pignatti 1982). Results of pathogenicity and host-range experiments indicated that *C. papaveracea/B. papaveris* infected *P. rhoeas* and reduced its biomass and that it did not infect wheat, maize, barley, sorghum, or oats (Del Serrone and Annesi 1990).

In 1991, researchers at the Institute of Genetics and Plant Experimental Biology in Tashkent, Uzbekistan, recovered a "highly virulent" isolate of *C. papaveracea/B. papaveris* from poppy plants (UNODC 2002). They reported

that the isolate caused 50-75% losses in licit and illicit poppy crops but did not specify under what environmental conditions the losses occurred. Symptoms of disease included damping-off of seedlings and leaf and stem lesions. Poppy capsules and seeds also were affected, and this resulted in smaller capsules and reduced seed production. The discovery of the isolate became the basis of a project on the development of *C. papaveracea/B. papaveris* as a biological control agent against poppy (discussed in more detail later in this chapter). It is important to note that, particularly for pathogens of the foliage, environmental conditions dramatically influence the amount of damage that a pathogen can cause. Losses can approach 100% under environmental conditions favorable to the fungus, but there might be no infection or damage in conditions unfavorable to the fungus.

In the late 1990s, research on the biological control of poppy (*P. somniferum*) was carried out at the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) laboratory in Beltsville, Maryland, with isolates of *C. papaveracea* and *B. papaveris* recovered from poppy plants that were grown in a field in Beltsville. *C. papaveracea* and *B. papaveris* were isolated from the same diseased poppy seeds, seedlings, foliage, capsules, and field stubble and from asymptomatic plants (O'Neill et al. 2000). The two fungi are morphologically distinct, and this makes it possible to distinguish them in culture. Greenhouse and field tests conducted by Bailey et al. (2000b) showed that *B. papaveris* caused more severe damage on poppy than *C. papaveracea*. When both fungi were applied in the field, *B. papaveris* was the more frequently recovered fungus from poppy seed capsules and was the only fungus isolated from the field in the following year (Bailey et al. 2000b). Other studies of *B. papaveris* focused on whether its efficacy could be enhanced with a phytotoxic protein, Nep1, isolated from *Fusarium oxysporum* (Bailey et al. 2000a) or the addition of other adjuvants (Bailey et al. 2004b) and on determining the best technique for mass production of its inoculum (Bailey et al. 2004a).

There have been no other publications on the evaluation or development of *C. papaveracea* or *B. papaveris* as mycoherbicides against opium poppy since the termination of the project in Uzbekistan in 2001 and the publication of the results of the studies conducted by the USDA ARS in 2000 and 2004.

EFFICACY AND IMPLEMENTATION

Three studies can be used as basis for assessing the efficacy of *C. papaveracea* or *B. papaveris* as a mycoherbicide agent against opium poppy. A study conducted in Italy was published in the proceedings of a conference (Del Serrone and Annesi 1990), a study conducted in the United States (Beltsville, Maryland) was published in a peer-reviewed journal (O'Neill et al. 2000), and a study conducted in Uzbekistan (UNODC 2002 report) that was sponsored by the UN International Drug Control Program under the auspices of the UN Office on Drugs and Crime (UNODC) and conducted in 1998–2002 at the Institute of

Genetics and Plant Experimental Biology. The goal of the latter study was to assess the potential of *C. papaveracea/B. papaveris* "as an effective, reliable, and environmentally safe biological control agent for opium poppy in realistic field conditions." The findings were presented in a report made available to the present committee; the report was reviewed by an independent expert and a group of technical experts enlisted by UNODC, but the results have not been published in peer-reviewed journals.

Experiments at the Istituto Sperimentale per la Patologia Vegetale, Rome, Italy

Del Serrone and Annesi (1990) conducted studies to evaluate the feasibility of controlling a species of poppy, *P. rhoeas,* with *C. papaveracea/B. papaveris* isolated from infected *P. rhoeas* plants in the field. *P. rhoeas* is an annual weed in cereal crops, especially wheat, in Italy. The committee reviewed this work because of the interest in *C. papaveracea/B. papaveris* as a mycoherbicide against opium poppy, although Del Serrone and Annesi did not propose to use it to control illicit poppy. Table 5-3 provides a summary of the experimental details.

According to the published report, disease symptoms were observed 3-4 days after inoculation as water-soaking of petiole tissues, followed by drying of the petioles, and finally the wilting and death of leaves. The greatest damage (with over 99% of the leaves infected) was observed in plants that had four to seven true leaves and had been sprayed with a suspension of 1.5×10^6 spores/mL followed by a 24-hour dew period at 25°C. Damage (in percentage of leaves infected) was less severe when inoculated plants were given 12 hours of dew at 23°C (59%) or 24 hours of dew at 15°C (72%). The requirement for 24 hours of dew to cause the most severe damage led the authors to conclude that it is "too long" to consider the use of this isolate as a mycoherbicide. They noted that it would be useful to conduct additional investigations to find "more adaptable isolates to be used under field conditions" (Del Serrone and Annesi 1990).

Experiments at the U.S. Department of Agriculture Agricultural Research Service Laboratory in Beltsville, Maryland

O'Neill et al. (2000) discovered that *P. somniferum* seedlings and mature plants produced from USDA plant introduction seed accessions grown in greenhouses and growth chambers in Beltsville, Maryland, were dying of an unknown destructive disease. The pathogen was identified as *C. papaveracea/B. papaveris*. O'Neill et al. conducted replicated experiments in growth chambers to determine the pathogenicity and comparative virulence of *B. papaveris* (isolate B96) and *C. papaveracea* (isolate Cf96) to poppy plants from three

accessions (White Cloud, Indian Grocery, and Venezuela). The details of the experiments are given in Table 5-4.

The results confirmed that both fungi were pathogenic to the three poppy accessions tested, but *B. papaveris* B96 was generally more virulent than *C. papaveracea* Cf96. Symptoms of infection included chlorosis, water-soaking of the stems and leaves, and tissue death. The average disease rating (on a scale of 0-9) on all test plants 7 days after inoculation (DAI) was 8.75-9.00 (93-100% foliage necrosis) with *B. papaveris,* whereas the same test plants inoculated with *C. papaveracea* had a rating of 2.75-7.50 (6-12% to 87-93% foliage necrosis) (see Table 5-3). When a similar experiment was conducted with 18-day-old seedlings of White Cloud and Indian Grocery, the seedlings were highly susceptible to both fungi, and necrotic lesions were observed 48 hours after inoculation. Mortality was 100% in seedlings inoculated with *P. papaveracea* and 97% in seedlings inoculated with *D. penicillatum* 5 DAI. The seedlings inoculated with *B. papaveris* were dead, and those inoculated with *C. papaveracea* exhibited 87-93% necrosis.

O'Neill et al. (2000) also determined the efficacy of *C. papaveracea* and *B. papaveris* in replicated experiments by inoculating poppy plants with different spore concentrations (10^5, 10^6, and 10^7 spores/mL) and then exposing them to different wetness periods by misting them for 0, 6, 12, 24, and 48 hours. The inoculated plants were killed within 9 days when the spore concentration was 10^6/mL and the wetness period was 24 hours or longer. For *B. papaveris* B96, at least 6 hours of wetness was required to attain 25-50% foliar necrosis 12 DAI. The efficacy of the spore inoculum increased when the inoculum was formulated with unrefined corn oil; White Cloud and Indian Grocery plants inoculated with *C. papaveracea* or *B. papaveris* spores with 30% oil and exposed to 6 hours of wetness exhibited 25-50% necrosis 3 DAI (O'Neill et al. 2000).

TABLE 5-3 Greenhouse Study by Del Serrone and Annesi (1990)

Factors	Details
Inoculum	*C. papaveracea/B. papaveris* Spore suspension containing 1.5×10^6 spores (conidia) per milliliter with 0.001% Teepol (detergent) sprayed onto test plants until runoff; control plants sprayed with water and Teepol only
Test plants	Poppy plants inoculated at four growth stages: cotyledon to three true leaves, four to seven true leaves, eight to 11 true leaves, and 12-16 true leaves
Environmental conditions	Inoculated and control plants exposed to different dew periods (6, 12, 18, and 24 h) and temperatures (15, 20, 25, 30°C)
Assessment method	Efficacy assessment was based on reduction in plant dry weight and percentage of infected leaves Experiment was performed twice

TABLE 5-4 Growth-Chamber Studies by O'Neill et al. (2000)

Factors	Details
Inoculum	Single-conidia isolates of *C. papaveracea* and *B. papaveris* obtained from diseased poppy foliage and capsules of plants growing in a field and growth chambers in Beltsville, Maryland
Test plants	Poppy plants from Indian Grocery (IG), VEN (from Venezuela, used in opium production), and White Cloud (WC) accessions
Treatments	In one experiment (two trials), 8-wk IG, VEN, and WC plants sprayed with *B. papaveris* isolate B96 and *C. papaveracea* isolate Cf96 spore suspensions in water (containing 4×10^4 spores/mL) and provided with 100% relative humidity (RH) for 24 h; plants then moved to a growth chamber with 40% RH, 28°C/22°C day/night temperature, and 11-h photoperiod
	In another experiment (two trials), 18-d-old seedlings of IG and WC sprayed with spore suspensions of isolates B96 and Cf96 in water (containing 3×10^4 spores/mL) and provided 100% RH for 24 h
	Different spore concentrations (10^5, 10^6, and 10^7 spores/mL) of isolates and different wetness periods (misting for 0, 6, 12, 24, and 48 h) also tested in growth-chamber experiments
Assessment method	Efficacy assessed on disease-severity rating scale (based on visual estimation of percentage of foliage blight) of 0-9 where 0 = 0-3%, 1 = 3-6%, 2 = 6-12%, 3 = 12-25%, 4 = 25-50%, 5 = 50-75%, 6 = 75-87%, 7 = 87-93%, 8 = 93-96%, and 9 = 96-100% necrosis

Experiments in Uzbekistan and Tajikistan

Researchers at the Institute of Genetics and Plant Experimental Biology conducted experiments in 2000-2001 to test the efficacy of fungi that they had isolated from diseased poppy plants in Uzbekistan. Their report refers to the pathogens as *P. papaveracea* (now referred to as *C. papaveracea*) and *D. penicillatum* (now referred to as *B. papaveris*) (UNODC 2002). Details of the field experiments are given in Table 5-5.

In the trials in Uzbekistan, several formulations were tested, but they were not described in the report. In 2000 and 2001, application of conidia in formulation "19" resulted in reductions of 50% and 52% in poppy capsule numbers, respectively, 45% and 63% in capsule weight, and 78% and 81% in seed weight per capsule. Application of formulation "1" had similar results in the Uzbekistan trials. In the 2001 trial in Tajikistan, formulations "24," "25," and "27" caused the most damage: a 60% reduction in plant height, 60% in capsule numbers, and 90% in plant weight. Formulations "19" and "1," which caused the most damage to poppies in Uzbekistan, were not as efficacious in the Tajikistan trial. The researchers thought that the higher ultraviolet-radiation levels at the Tajikistan site, which was 2,500 m above sea level, reduced the efficacy of *C. papaveracea/B. papaveris* formulations "19" and "1."

TABLE 5-5 Field Trials in Uzbekistan and Tajikistan (2000-2001)

Factors	Details
Inoculum	*C. papaveracea/B. papaveris* isolated from diseased opium poppy (*Papaver somniferum*) and wild poppies (*P. arrhenium*, *P. pavonium*, *P. rhoeas*, and *P. refracta*) in Uzbekistan and Tajikistan
	Isolate referred to as C-6-3 used in field experiments because it had "the greatest virulence and highest growth rate"
Location and time of inoculation	Trials conducted in Uzbekistan and Tajikistan field sites
	Inoculations in autumn, at "budding phase" of poppy plants
Treatments	Inoculum suspension containing 1×10^6 conidia/mL sprayed at 500 L/ha (5×10^{11} conidia/ha); treatments consisted of spores in different formulations identified by numerical designations but of undescribed compositions; control treatment consisted only of formulation without spores
Assessment method	Efficacy of fungus assessed on basis of reduction in poppy capsule numbers, capsule weight, and weight of seeds per capsule in Uzbekistan trials; in Tajikistan trials, efficacy assessed on basis of reduction in plant height, capsule numbers, and plant weight
	Final yield data not obtained, because "it was too dangerous to keep the trial long enough for this"

According to the Uzbek researchers, inoculation of poppy plants at the rosette stage resulted in leaf infections followed by stem infections and finally plant death within 48 hours of inoculation. Infection at a more mature, capsule-forming stage resulted only in capsule discoloration and stunting. The researchers further reported that under natural field conditions, *C. papaveracea* or *B. papaveris* can cause disease in plants at the "budding phase" (capsule formation) and that although inoculation at this stage did not cause plant death, the capsule was so affected that it was commercially unusable; capsules of infected plants were small, hard, and blackened and contained seeds with lower viability. Laboratory analysis for alkaloids showed reduced concentrations of morphine, codeine, thebaine, narcotine, and papaverine in plants that were inoculated with fungal formulations "1" and "19" (UNODC 2002). The authors recommended application of the fungus to poppy plants at the rosette stage (before flowering) to destroy the crop (UNODC 2002).

Del Serrone and Annesi (1990) demonstrated that younger plants are more susceptible to damage and that the fungus requires 24 hours of dew to cause severe damage. O'Neill et al. (2000) also showed that young plants are more susceptible to damage and that *C. papaveracea* or *B. papaveris* can cause severe damage or death relatively quickly if the inoculated plants are exposed to a long wetness period, that is, at least 24 hours. However, in a study by Bailey et al. (2000b), field-grown poppy plants from the accession White Cloud inoculated at the rosette stage did not become severely infected until the plants began to flower and form capsules. Bailey et al. postulated that the susceptibility of younger plants in greenhouse experiments was due to the optimal conditions in

the greenhouse, which may not always occur in the field. An important caveat here is that the different research groups worked with different isolates of *C. papaveracea* or *B. papaveris* (see Table 5-1).

Results from greenhouse and field studies provide evidence that *C. papaveracea* and *B. papaveris* can cause disease on poppy, but the extent of damage or yield loss will depend on several factors, including the virulence of the isolate or strain used, the inoculum formulation, the application rate, the plant growth stage, the poppy cultivar (genotype), and the environmental conditions in the field during and immediately after inoculation.

Mechanisms of Pathogenicity

Pathogenicity is the ability to cause disease; disease may result from a pathogen's infecting, colonizing, and disrupting the normal cellular functions of a plant. *C. papaveracea* and *B. papaveris* infect aerial parts of the plant, initially causing chlorotic spots on the leaf, chlorosis on leaf margins, and water-soaking on the leaf and stem, which may be followed by withering and drying of the leaf and the development of stem lesions (Milatović 1975b; Del Serrone and Annesi 1990; Bailey et al. 2000b; O'Neill et al. 2000). Infected leaves change from green to gray-green and enter senescence prematurely (Miltović 1975b). Both *C. papaveracea* and *B. papaveris* form appressoria, specialized suction-cup-like structures that some fungi require to penetrate plant epidermal cells and cause infection, on poppy leaves, but they can also penetrate the leaf through open stomata (Bailey et al. 2000b). The disease can attack any part of the opium poppy plant—leaf, stem, capsule, root, or seed—at any stage of development (Milatović 1975a). Although diseased seedlings and immature plants might be killed (Del Serrone and Annesi 1990; Bailey et al. 2000b; O'Neill et al. 2000), premature drying of the plant at any of the later stages of development (flowering, capsule formation, or capsule maturity) can greatly reduce the number, size, and weight of the capsules (Bailey et al. 2000b) and the amount of opium that could be harvested from the still-green capsules (UNODC 2010d). *C. papaveracea* produces sexual and asexual spores on infected poppy tissues (Milatović 1975b; O'Neill et al. 2000); these spores may disperse the pathogen throughout the field and region. Being a seedborne pathogen (Milatović 1975b), *C. papaveracea* also could be spread through the use of infected seed for planting.

Little is known about the mechanisms underlying the pathogenicity of *C. papaveracea* or *B. papaveris*. As noted in Chapter 4, filamentous fungi produce a variety of secondary metabolites (organic compounds produced by an organism that are not required for its immediate survival). Although the function of most secondary metabolites is unknown, many have been shown to have biological activity, including phytotoxicity. The UNODC (2002) report listed several metabolites produced by *C. papaveracea* that were identified in methanol extracts of fungal cultures and that were found to be phytotoxic in a

detached-leaf assay. Phytotoxicity was measured semiquantitatively (+, ++, +++) over a 48-hour period. Phytotoxins identified included 1, 2-benzene dicarboxylic acid; 1, 2-benzene dicarboxylic acid, dipropyl ester; 1, 2-benzene dicarboxylic acid, bis (ethylhexyl) ester; and two derivatives of tetracosahexaene (squalene). These metabolites have no clear role in disease development (UNODC 2002). Some experiments combining a culture filtrate with the fungus were performed to determine whether fungal efficacy was enhanced by toxic metabolites; the results showed little enhancement of the efficacy of *C. papaveracea* (UNODC 2002). The role of the metabolites in the pathogenicity of *C. papaveracea*, if any, remains unknown.

INOCULUM PRODUCTION AND DELIVERY

Facility, Equipment, and Technology for Large-Scale Manufacture

A general discussion of the fermentation methods used for large-scale production of commercial mycoherbicides is provided in Chapter 4 (see section "Inoculum Production and Delivery"). *C. papaveracea/B. papaveris* can be grown to produce infective propagules on a variety of solid substrates and liquid media (Table 5-6). On the basis of studies reviewed in Table 5-6 and the general literature on microbial pesticides, it appears feasible to mass-produce these fungi. The available data on the proposed mycoherbicides provide useful leads but are exploratory; any large-scale attempt at production would be more efficient if it begins with basic studies of fungal growth and fermentation, preferably in collaboration with an industrial partner. Such studies are needed to determine the choice of fermentation method (liquid, solid, or biphasic), the type of propagule (mycelium, conidium, chlamydospores, microsclerotia, pseudothecia, or ascospores), the formulation (liquid concentrate, dust, pellet, food-grain-based, or seed-based), the intended delivery method (aerial or ground-based), and the expected efficacy, shelf-life, and handling qualities of the product.

To estimate the amounts of the mycoherbicide product that might be required for a hypothetical program to control illicit opium poppy worldwide, the committee made calculations on the basis of published data on the amounts of inoculum used in field trials (Table 5-7). However, as noted in Chapter 4, the published data are only a guide; the actual amounts cannot be determined without testing the finished mycoherbicide product under conditions that simulate operational programs. Typically, this phase of mycoherbicide research and development is done by an industrial producer in collaboration with the mycoherbicide researchers. Therefore, the actual amounts of opium-poppy mycoherbicide required may be higher or lower than the amounts projected in the table. The estimates in Table 5-7 project that large volumes of water would be necessary to apply the opium-poppy inoculum, and this could be an important limiting factor in developing it as a mycoherbicide.

TABLE 5-6 Methods Used for Production of *C. papaveracea*/*B. papaveris* Inoculum for Experimental Trials

Reference	Method	Inoculum Produced
Del Serrone and Anessi 1990	Inoculum produced by culturing *C. papaveracea/ B. papaveris* on malt extract agar (30% maltose) for 9 days at 25°C with 12-h light and dark periods	Spores produced (type not mentioned; presumed to be conidia) used for inoculation
Bailey et al. 2000b; O'Neill et al. 2000	*C. papaveracea/B. papaveris* cultured on V8 agar medium for 7-10 days at 23°C with 16-h photoperiod	Conidia from agar plate cultures used in laboratory, greenhouse, and field experiments
Bailey et al. 20004b	*C. papaveracea* and *B. papaveris* cultured on V8 agar medium for 6-10 days at 25°C in the dark	Conidia from agar plate cultures used in laboratory, greenhouse, and field experiments
Bailey et al. 2004a	*C. papaveracea/B. papaveris* cultured on agar with molasses, wheat bran, pectin, rice flour, dextrin, cornstarch, soy flour, corncobs, or cottonseed meal, each with brewer's yeast	Greatest amount of radial growth occurred on molasses-brewer's yeast, soy fiber-brewer's yeast, and wheat bran-brewer's yeast agar media; greatest number of conidia produced on soy fiber-brewer's yeast agar medium; only chlamydospores produced on cornstarch-brewer's yeast and dextrin-brewer's yeast agar media
Bailey et al. 2004a	Liquid media (in 100-mL shake flasks) containing dextrin, cornstarch, wheat bran, or soy fiber, each supplemented with brewer's yeast	1×10^6 colony forming units per milliliter produced on all four substrates after 5 days of growth; chlamydospores (6×10^5/mL) produced only in media containing cornstarch-brewer's yeast and dextrin-brewer's yeast; conidia (10^4/mL) produced only in media containing soy fiber-brewer's yeast and wheat bran-brewer's yeast
Bailey et al. 2004a	Bench-top fermentation (2.5-L commercial bench-top fermentor) with dextrin (30.0 g) and brewer's yeast (15.0 g) mixture as medium; medium maintained at 25°C with 200-rpm agitation, fermentation period was 7 days	Biomass produced on bench-top fermentor consisted of nonmelanized mycelial fragments and chlamydospores
UNODC 2002	10-mL conidial suspension of *C. papaveracea* added to 250 mL of liquid medium in flask; inoculated liquid medium (not identified in report) incubated on rotary shaker (70 rpm) at room temperature for 3-4 days, after which mycelia were recovered and spread out on muslin cloth that had been stretched over metal frame; mycelia on muslin incubated at 100% relative humidity for 48 h; spores then collected with vacuum harvester and stored as dry powder	Dry spores stored in sealed or nonsealed containers or mineral oil; tests conducted over a 5-year period indicated minimal reduction in spore viability and virulence; number of propagules produced not discussed in report

TABLE 5-7 Estimated Amounts of the Proposed *C. papaveracea*/*B. papaveris* Needed for a Single Application against Illicit Opium-Poppy Crops Worldwide

Reference	Amount of Inoculum and Volume of Carrier (Water) Used in Field Trials[a]	Amount of Mycoherbicide (No. Spores) and Volume of Water Needed *for Each Application* over Worldwide Area[b,c,d]	Amount of Mycoherbicide (No. Spores) and Volume of Water Needed per Hectare *for Each Application*
Bailey et al. 2000b	2×10^5 conidia/mL suspended in Tween 20 or Tween 20 plus corn oil, applied at 3 mL/Plant or 180-360 L/ha[e]	6.5×10^{15} to 13×10^{15} (6.5-13 quadrillion) spores and 33-66 million liters of water	3.6×10^{10} to 7.2×10^{10} spores and 182-364 L of water
Bailey et al. 2000a[e]	5×10^5 conidia/mL suspended in water or aqueous adjuvant, applied at 5×10^{10} conidia/ha in 1,290 L of water (with or without adjuvants)	1.2×10^{17} (1.2 sextillion) spores and 234 million liters of water	6×10^{11} spores and 1,290 L of water
Bailey et al. 2004b[e]	1×10^6/mL conidia applied at 8.7×10^{11} conidia/ha in 866 L/ha	1.6×10^{17} (1.6 sextillion) spores and 157 million liters of water	88×10^{10} spores and 866 L of water
	1×10^6/mL conidia applied at 3.3×10^{12} conidia/ha in 3,300 L/ha	6×10^{17} (6 sextillion) spores and 599 million liters of water	331×10^{10} spores and 3,303 L of water
	2×10^6/mL conidia applied at 9.9×10^{12} conidia/ha in 4,950 L/ha	1.8×10^{18} (1.8 quintillion) spores and 898 million liters of water	992×10^{10} spores and 4,951 L of water
UNODC 2002	5 g/0.3 m^2 of inoculum composed of fungus-colonized millet seeds; proportion of fungus to millet seed not specified	90×10^{15} (90 quadrillion) spores and 91 million liters of water	50×10^{10} spores and 502 L of water

[a]Amounts of inoculum used in field experiments were not aimed at defining the minimum inoculum quantity needed for effectiveness; this remains to be determined with the actual mycoherbicide product.

[b]Based on most recent UNODC estimate of total area under opium-poppy cultivation worldwide is 181,373 ha (UNODC 2010a). These calculations are provided on the basis of a potential worldwide target area to indicate the industrial production capacity that might be needed. The committee regards simultaneous worldwide application or even a worldwide application within a growing season as logistically unrealistic.

[c]A typical opium-poppy field has 60,000-120,000 plants/ha (see Chapter 3).

[d]Without knowing the weight of each spore, it is not possible to estimate the required amount in metric tons.

[e]Descriptions of plot size are incomplete. Estimation is based on the committee's interpretation of the available details.

Adjuvants and Formulation

For a general discussion of adjuvants and formulations, see the section "Adjuvants and Formulation in Chapter 4. The use of adjuvants to improve the efficacy of *C. papaveracea* and *B. papaveris* against opium poppy was studied by Bailey et al. (2000b, 2004b) and by O'Neill et al. (2000). Among the adjuvants tested, the most effective were Tween 20 (polyoxyethylene [20] sorbitan monolaurate) and unrefined corn oil. The details and results of the experiments are summarized in Table 5-8.

O'Neill et al. (2000) showed that the addition of unrefined corn oil to *C. papaveracea* and *B. papaveris* inoculum increased the severity of disease caused by these two fungi in White Cloud and Indian Grocery poppy plants. They noted that the formulation of *C. papaveracea* spores with corn oil and the provision of a 6-hour wetness period rendered *C. papaveracea* "almost as virulent" as *B. papaveris* on the tested poppy cultivars.

Researchers in the Institute of Genetics and Plant Experimental Biology also tested various formulations of *C. papaveracea*/*B. papaveris* under field conditions, but they identified the formulations only by number and did not provide any information on their composition (UNODC 2002).

The use of a low concentration of Tween 20 as an adjuvant might be cost-effective, but the environmental effects of its use might require documentation for registration purposes. The use of unrefined corn oil at 10-30% by volume of the spray mixture is impractical in light of the large spray volumes required (Table 5-6).

TABLE 5-8 Effect of Adjuvants on the Efficacy of *C. papaveracea/B. papaveris* in Greenhouse and Field Experiments

Reference	Adjuvant Tested	Efficacy
Bailey et al. 2000b	0.001% Tween 20 in 10% (unrefined) corn oil	In greenhouse experiments, application of spores (10^6/mL) mixed with 0.001% Tween 20 in 10% corn oil resulted in 100% infection by *B. papaveris* and nearly 100% infection by *C. papaveracea*; *B. papaveris* with 0.001% Tween 20 in 10% corn oil caused 57.7% mortality in poppy seedlings; *C. papaveracea* with 0.001% Tween 20 in 10% corn oil caused 34.7% mortality in poppy seedlings; and 17% of plants sprayed with spores of either pathogen with 0.0001% Tween 20 in water did not exhibit any disease symptoms
O'Neill et al. 2000	30% (unrefined) corn oil	In greenhouse tests, poppy plants inoculated with *C. papaveracea* and *B. papaveris* spores mixed with 30% corn oil and provided with a 6-hour wetness period had 25-50% foliar necrosis at 3 DAI
Bailey et al. 2004b	1% Tween 20	In field experiments, *B. papaveris* spores mixed with 1% Tween 20 caused 68% and 56% necrosis and 22% and 27% reduction in capsule weight per plot within 2 weeks of application in trials 1 and 2, respectively

Delivery

On-ground application of a mycoherbicide against opium poppy is possible, but from a tactical standpoint, aerial application may be the only feasible delivery method over inaccessible areas. An aerial pathogen such as *C. papaveracea/B. papaveris* is likely to be most effective when sprayed on the plants (that is, aerial application). However, all the studies that the present committee reviewed have examined only land-based spray application of *C. papaveracea/B. papaveris* directed at the plants. Of the five papers that the committee reviewed (Bailey et al. 2000a,b, 2004b; O'Neill et al. 2000; UNODC 2002), two identified the tool used to apply the inoculum (Bailey et al. 2000a, 2004b), namely, a Binks spray gun model #15 delivering a spore suspension at 15 lb/in^2. Presumably, the other studies used some type of hand-held or backpack sprayer.

Aerial spraying of a liquid formulation of *C. papaveracea/B. papaveris* is unlikely to be practical, because of the large quantities of water that would be required if the methods used by the researchers were implemented on a large scale (see Table 5-6). Producing the large number of spores required might not constitute a problem, provided that a suitable fermentation method is found, but the availability of water and the ability to transport and apply the required quantities in the field would pose major challenges.

Assessment of Performance

The opium-poppy crop is grown in open fields under full sun, so it should be possible to assess the performance of the *C. papaveracea/B. papaveris* mycoherbicide with aerial imagery. Although the technology for aerial imagery is available, it has to be adapted and tested for use in measuring mycoherbicide efficacy. As an alternative, on-ground assessment of crop damage combined with interviews with growers could be used; however, this approach requires the ability to ensure personnel security and access to targeted regions.

The assessment methods used by the researchers were not developed for use in operational drug-crop control programs. For example, in the field trials conducted in Uzbekistan, the efficacy of *C. papaveracea/B. papaveris* was assessed on the basis of reductions in poppy capsule numbers, capsule weight, and weight of seeds per capsule. In the Tajikistan trials, efficacy assessment was based on reductions in plant height, capsule numbers, and plant weight. Final yield data were not obtained in these studies, because it was too "dangerous" to continue the trial (UNODC 2002). O'Neill et al. (2000) assessed the efficacy of *C. papaveracea* and *B. papaveris* in growth-chamber studies with a disease-severity rating scale (see Table 5-4). Del Serrone and Annesi (1990) assessed the efficacy of *C. papaveracea/B. papaveris* on the basis of plant dry-weight reduction and percentage of infected leaves.

PERSISTENCE IN THE ENVIRONMENT

The persistence of *C. papaveracea/B. papaveris* in the environment is an important factor in determining whether the applied fungal strain(s) could potentially affect later plantings of the crop. If the mycoherbicide poses risks to nontarget organisms after release, its prolonged persistence would be a disadvantage rather than an advantage. Thus, as noted in Chapter 4, an understanding of the ability to persist in the environment is an important consideration in proposing the use of a mycoherbicide.

Geographic and Climatic Considerations

Only a few studies provide quantitative information on the survival of *C. papaveracea/B. papaveris* (Del Serrone and Annesi 1990; Bailey et al. 2000b; UNODC 2002). Del Serrone and Annesi (1990) found that moisture on the plant surface for sufficiently long durations (length of time depending on the temperature) at favorable temperatures (10-30°C) are required for spore germination and later penetration of host tissue. All the studies were done at 100% humidity or on wet leaflets.

Bailey et al. (2000b) performed a detached-leaf assay with *C. papaveracea* and *B. papaveris* to assess the effect of temperature on their survival and growth on poppy leaf surfaces. Survival and growth were measured in terms of conidial germination, germ-tube growth, and formation of appressoria at various temperatures. Germ-tube growth and appressorium formation were favored at higher temperatures (16-29°C), whereas conidial germination was similar throughout the range of temperatures tested (7-29°C). *B. papaveris* formed more appressoria than *C. papaveracea* regardless of temperature and required fewer hours of moisture.

The long-term survival of *C. papaveracea* and *B. papaveris* also was assessed as part of field tests of their infectivity (discussed above in the section "Efficacy and Implementation"). After tests during the first season were completed, the remaining poppy plant residues in the field were chopped and left on the soil surface over the winter. In the spring, opium poppies were planted in the fields, and plants that had symptoms of disease were tested for the presence of the fungi. Only *B. papaveris* was isolated from diseased poppy. The investigators suggested that their results could be due to this pathogen's ability to produce ascospores in the spring (Bailey et al. 2000b).

In the studies in Uzbekistan (UNODC 2002), laboratory and field tests indicated that conidia of *C. papaveracea/B. papaveris* required the presence of the host plant to survive for longer than 2-3 months. In the presence of host tissue, the pathogen remained viable for up to 15 months when applied to the top 5 cm of soil and for up to 6 months when applied at a depth of 15 cm. In the absence of the host plant, persistence was reduced to 3 months at all depths

during the winter and to 2 months at all depths during the summer. When plant debris was buried in the soil, viable fungi were detected for up to 10 months. However, the UNODC report provided no description of the testing protocols or analytical methods that were used.

As previously stated, such physical factors as unfavorable temperature, moisture, and solar radiation and such biological factors as antagonistic microorganisms in the soil could influence survival of *C. papaveracea* and *B. papaveris*, but there are no specific data on the effects of these factors on the two pathogens. Inasmuch as *C. papaveracea* and *B. papaveris* already are present in most areas where poppies are grown, the environmental conditions and the life cycle of the plants seem to be such that at least some level of persistence would be achieved.

Transmission and Spread

The potential pathways by which spores and vegetative propagules from a particular application site might move into environmental media are illustrated in Chapter 2 (see Figure 2-1). In general, dispersal of the proposed mycoherbicides after application would depend on the production and natural dispersal of secondary inoculum. Experience with other plant pathogens indicates that short-distance spread of ascospores is facilitated by rain and wind, whereas dispersal of conidia occurs primarily by wind (Li and Kendrick 1995; Hildebrand 2002). Long-distance transport and dispersal by wind and water are possible for infected seeds, infected plant tissues, infested dead plant material, and conidia (Meffert 1950; UNODC 2002). It is also possible for farmers and traders to carry infected or infested materials throughout a region or even into new areas.

Other Considerations

The occurrence and susceptibility of the host plant appears to be a major factor in determining the population size of many pathogens, including *C. papaveracea* and *B. papaveris*. The pathogens are somewhat host-specific, being capable of infecting *P. somniferum* and other species of *Papaver* (Del Serrone and Annesi 1990; UNODC 2002). The pathogens might survive on related hosts or colonize unrelated hosts that are not necessarily susceptible to them, as has been shown with formae speciales of *Fusarium oxysporum* (see Chapter 4), but there are no data on the presence of *C. papaveracea* and *B. papaveris* on or in tissues of nontarget plants. These fungi are widely prevalent across the range where opium poppy is grown (Schmitt and Lipscomb 1975).

No studies are available on the interactions of *C. papaveracea* or *B. papaveris* with soil microorganisms or other organisms. In general, as noted in Chapter 4, the presence of competitor or antagonistic microorganisms could reduce the persistence *C. papaveracea* or *B. papaveris*. For example, insects and

soil organisms can feed on or suppress plant-pathogenic fungi (Nakamura et al. 1992; Okabe 1993; Suárez-Estrella et al. 2007). Thus, antagonistic microorganisms in the soil could theoretically reduce the likelihood that a *C. papaveracea/B. papaveris* mycoherbicide would establish populations that are large enough to cause recurrent disease in opium poppy. It is possible that the introduced strains of *C. papaveracea* or *B. papaveris* could displace the resident strains; but there are no data available to determine the probability of such displacement or its consequences.

Overall, the ecological requirements for the spread and survival of *C. papaveracea* and *B. papaveris* cannot be adequately described on the basis of the studies conducted so far. In general, it appears that the pathogens could persist in soil for at least two growing periods in the presence of host tissue. The fact that they are found almost everywhere that opium poppy is grown indicates that the strains distributed are likely to persist at some level once introduced into a site. Although no data are available specifically on the potential dispersal of the fungi from the site of application, it is reasonable to assume that they would spread through infected or infested seeds, plant tissues, or plant debris and by wind and rain.

EFFECTS ON NONTARGET ORGANISMS

Microbial pesticides are regulated by the U.S. Environmental Protection Agency (EPA), which requires a variety of tests on the environmental fate and safety of pesticides before they are registered. Chapter 2 and Appendix B describe the types of testing required—including product analysis, pesticide-residue analysis, toxicity testing, and toxicity and pathogenicity testing of nontarget organisms—and the assessment of environmental fate. The aforementioned studies have not been systematically performed for the EPA registration of *C. papaveracea/B. papaveris.* This section reviews the data available on *C. papaveracea/B. papaveris* in the open literature that are pertinent to an understanding of their potential adverse effects on nontarget plants, animals, and microorganisms.

Effects on Nontarget Plants or Microorganisms

In the UNODC (2002) study, the host specificity of *C. papaveracea/B. papaveris* was tested against 239 species of plants of economic, medicinal, and ornamental importance, including trees and shrubs, and 52 wild species of plants belonging to 24 families. Some nontarget *Papaver* species were reported to be susceptible, but none of the other nontarget plants was susceptible. The report provides no names of the plant species and families tested, including the names or numbers of the species in *Papaveraceae*, and no details of the testing and assessment methods. Hence, the information available cannot be used to assess the risks posed to nontarget relatives of poppy by *C. papaveracea/B. papaveris*.

Del Serrone and Annesi (1990) tested 14- to 20-day-old plants of "several varieties" of cereal crops and three *Papaver* species by spraying them with "the optimum" suspension of mitospores (conidia) of *C. papaveracea/B. papaveris* and growing the plants under "the best conditions for disease development." "Poppy" plants were included as a control. None of the cereal-crop varieties tested developed disease by 14 DAI, and the fungus was never reisolated from them. *Papaver dubium* and *P. nudicaule,* a wild and a cultivated species, respectively, developed a hypersensitive (resistant) reaction. A few small, black, round spots appeared on the surface of the leaves. Some 30% of the *P. somniferum* plants had died at 5 DAI. The fungus was not recovered from *P. dubium*, *P. nudicale*, and *P. somniferum*, so there was no evidence that the fungus caused the disease.

Effects on Legal Crop Production

The potential risk to legal production of poppy has not been given any attention in the literature reviewed by the committee. Licit poppy crops may be cultivated for seed, oil, ornamental uses, and pharmaceutical purposes to extract narcotics. It is possible that *C. papaveracea/B. papaveris* would be present in fields used for legal production. If legal poppy production occurs in or near illicit-opium-producing regions, the inundative release of *C. papaveracea/B. papaveris* as a mycoherbicide to control the illicit plants could similarly enhance the development of disease in the legal crop owing to drift during application or by secondary inoculum produced on infected plant tissues.

Toxicity to Wildlife, Domestic Animals, and Humans

Although the UNODC (2002) report described secondary metabolites of *C. papaveracea/B. papaveris* that are phytotoxic, none of the metabolites was tested for mycotoxigenic activity. Two secondary metabolites were identified as derivatives of tetracosahexaene (squalene). Why the authors described the squalene derivatives as "fumonisin-like" is not clear. Squalene and fumonisins are not derived from the same biosynthetic pathway, so there is no rationale, on the basis of the spectral analyses (mass, infrared, and ultraviolet spectrometry), to conclude that the derivatives are fumonisin-like, and the conclusion that *C. papaveracea* produces fumonisin mycotoxins is not well supported. The committee found no publications other than the UNODC report on biologically active metabolites, including mycotoxins, produced by *C. papaveracea/B. papaveris*.

Pathogenicity in Animals and Humans

No reports of human or animal infection with *Pleospora*, *Crivellia*, or

Brachycladium were found. A single report on the toxicity of culture extracts of *Pleospora* for cell lines was reviewed (Ge et al. 2005), but it was not helpful in evaluating the potential risks of infection of humans or animals. If those fungi are shown to be thermotolerant (that is, able to grow efficiently at human body temperature, 37°C), there would be a theoretical risk that increasing their amounts in the environment might lead to infection in immunocompromised humans and animals. However, on the basis of the absence of any case reports, the likelihood appears quite low.

MUTATION

The potential for mycoherbicides to mutate is similar to that of fungi in general, as described in Chapter 4. The diversity of fungal genotypes also is affected by sexual recombination within species. Many fungi that have not been observed to reproduce sexually may do so cryptically, judging from population-genetics evidence (Taylor et al. 2000). In some cases, population-genetics evidence on sex has led to confirmation by laboratory mating (O'Gorman et al. 2009). New genetic variation can become established in fungal populations by natural selection or by chance. Adaptation to new environments, for example, to new plant hosts or to new cultivars of crop plants can be accelerated by outbreeding and recombination due to sexual reproduction (Goddard et al. 2005; Zhan et al. 2007; Sommerhalder et al. 2010). All those processes could affect *Crivellia* or *Brachycladium* species.

However, there is little basic genetic information on *C. papaveracea* or *B. papaveris*, so only a generalization about the potential for mutation can be made. There is no reason to expect that the mutation rate of these fungi would be different from that of other filamentous fungi or that they would be more or less susceptible to gene gain, gene duplication, or horizontal gene transfer. *C. papaveracea* outbreeds by sexual reproduction. *B. papaveris* reproduces sexually but is homothallic (self-mating) and need not produce recombined progeny (Farr et al. 2000). Thus, adaptation involving virulence or host range could be accelerated by genetic recombination in the case of *C. papaveracea* but not necessarily in the case of *B. papaveris*.

Mutation could play a role in determining the toxicity (with respect to secondary metabolites produced) of a mycoherbicide to the extent that mutation results in changes in the amount of toxin produced or the environmental conditions under which the toxins are produced. Concerns about mutation-related changes in the toxicity of *C. papaveracea* or *B. papaveris* are all but impossible to assess because very little research has been performed. As noted earlier, the available data are insufficient to determine what secondary metabolites are produced by *C. papaveracea* or *B. papaveris*, let alone in what quantities or how production would be affected by mutations.

A PRESUMPTIVE DISEASE EPIDEMIC IN OPIUM POPPY IN AFGHANISTAN

According to UNODC, diseases of opium poppy are a normal occurrence in Afghanistan. Farmers report various degrees of damage to their crops in practically all years and regions since UNODC began systematic yield surveys (UNODC 2010d). In the spring of 2010, a fungal disease was speculated to be the possible cause of an opium-poppy blight in Afghanistan. The poppy plants exhibited wilting and other disease symptoms that appeared to be consistent with a fungal infection. Tests of diseased tissues identified two *Fusarium* species, but they were probably secondary colonizers of the decaying tissue rather than the cause of the disease. *C. papaveracea/B. papaveris*, which has been linked with past diseases of opium poppy in Afghanistan, was not detected (personal communication, Justice Tetty, UNODC, November 19, 2010), but it was noted that the tissue samples examined were of poor quality (personal communication, Eric Boa, CABI, November 25, 2010).

The UN Afghanistan Opium Survey of 2010 reported that opium production was 48% lower than in 2009 (UNODC 2010d) although the overall area under poppy cultivation remained the same. Disease was considered a major contributor to the reduction in opium yield, but farmers also reported losses due to frost, drought, and pests, such as aphids, other insects, and worms. Poppy capsules were fewer and smaller than in previous years. It is important to note that diseases in major growing areas affected opium poppy plants at the late stage of plant development. The diseased plants were described as exhibiting wilt symptoms with yellowing of leaves, drooping, and finally desiccating completely, all of which are indicative of a collar (stem-root interface) or upper root rot. Those symptoms are consistent with the ones observed previously in the region in connection with fungal infections (UNODC 2010d) but are inconsistent with the typical symptoms of infection by *C. papaveracea/B. papaveris*. The southern region was the most affected: about 42% of the area under opium cultivation was damaged. The western region was also affected by diseases but to a much smaller degree. In the west, a combination of factors, including frost, played a role, according to farmer reports (UNODC 2010d).

On the basis of the foregoing account, the cause of the reduction in opium production in 2010 in Afghanistan is unknown. Diseases, drought, frost, and pests might have contributed to it. Adverse weather conditions (such as frost and drought) might have predisposed the 2010 crop in different parts of Afghanistan to diseases. Without conclusive evidence based on positive identification of the pathogen, *C. papaveracea/B. papaveris* could not be implicated in the 2010 Afghan poppy blight epidemic. Therefore, it is not possible to gain any insight from this epidemic to guide the use of *C. papaveracea/B. papaveris* as a mycoherbicide.

6

Answers to Charge Questions

On the basis of the reviews presented in Chapters 2-5, the committee concluded that the available studies of *Fusarium oxysporum* f.sp. *cannabis*, *F. oxysporum* f.sp. *erythroxyli*, and *Crivellia papaveracea* or *Brachycladium papaveris* are preliminary and exploratory. Thus, in answering the specific charge questions, the committee attempted to capture what conclusions could be drawn from the limited dataset and what might be expected on the basis of what is known about fungal pathogens and mycoherbicides in general.

EFFECTIVENESS

- **Are the drug crops (cannabis, coca, and opium poppy) known to be susceptible to the proposed mycoherbicides?**
    - The committee found documented evidence from greenhouse and small field studies that cannabis (*Cannabis sativa*), coca (*Erythroxylum coca* and *E. novogranatense*), and opium poppy (*Papaver somniferum*) are susceptible to *F. oxysporum* f.sp. *cannabis*, *F. oxysporum* f.sp. *erythroxyli*, and *C. papaveracea/B. papa-veris*, respecttively. However, the few studies available involved controlled applications to the target plants and often used artificial environmental conditions that favored infection. Thus, the committee judged that the information is insufficient to conclude that the proposed mycoherbicides would be efficacious for the control of illicit-drug crops under field conditions.

- **Have the mechanisms of action of the proposed mycoherbicides' toxicity to illicit-drug crops been established?**
    - The types of diseases produced by the proposed mycoherbicide strains are wilt diseases in cannabis and coca and a blight of the aerial parts of opium poppy. Some members of the fungal species to which the proposed mycoherbicide strains belong can produce tox-

ins involved in disease development under laboratory conditions, but no reliable data are available on the proposed mycoherbicide strains. The molecular mechanisms underlying the host-pathogen interactions are unknown, and the nature of disease progression and fungal spread after disease onset, which are critical determinants of mycoherbicide efficacy, are not documented.

- **Are the proposed mycoherbicides host-plant-specific?**
  - The proposed mycoherbicide strains can cause disease in their target plants, including those grown legally and those which occur naturally near the illicit crops. However, the few host-range studies conducted with nonrelated species were of little value because they only report that the mycoherbicide strains did not cause disease in some native plants and crop species and do not provide experimental details (or in some cases even the names of the plants). Furthermore, none of the available studies used a standard, systematic process to select the most relevant plants to test in host-range studies. For example, of about 200 species of *Erythroxylum* native to South America, only two were tested for susceptibility to the coca mycoherbicide. Thus, the data are insufficient to conclude that the proposed mycoherbicides would not pose a risk to other plants or crops.

- **What quantities of mycoherbicides would be needed to eradicate illicit drug crops?**
  - Workable mycoherbicide formulations, delivery methods, and application strategies need to be developed in partnership with an industrial producer before this question can be answered. The committee made estimates for a single application per hectare solely on the basis of published laboratory production methods, which may or may not be realistic. Its estimates indicate that tens to hundreds of kilograms of dry formulations per hectare or hundreds to thousands of liters of liquid-spray formulation (containing billions to trillions of spores) per hectare would be required for a single application of the mycoherbicides.

- **How would the method of delivery affect the effectiveness of the mycoherbicides in eradicating the drug crops?**
  - For all three mycoherbicides, on-ground application would be the most precise and uniform. However, it is not feasible, because the growers of illicit drug crops would be uncooperative and possibly hostile. Aerial application is probably the most practical approach, but it would need to be from altitudes greater than those normally used for agricultural applications.

- *F. oxysporum* f.sp. *cannabis* and *F. oxysporum* f.sp. *erythroxyli* are soilborne, root-infecting pathogens; for greatest efficacy, they should be delivered to the soil. Aerial application of dry formulations, such as prills and pellets, could reduce the efficacy of such soilborne pathogens because the formulations would be subject to scattering by wind, which would lead to nonuniform, discontinuous placement of the inoculum over the target area and reduce the size of the plant-pathogen interface.
- *C. papaveracea/B. papaveris* is a pathogen that attacks primarily the aerial parts of opium poppy; for greatest efficacy, it should be sprayed on the foliage of the plants. All tests of this pathogen have used a wet application. Aerial application of liquid formulations would be subject to wind-driven drift, which would lead to irregular deposition on the target area. The large amount of water needed for aerial delivery of the mycoherbicide could be an even more important limiting factor. Low-volume and ultra-low-volume applications have not been tried with the proposed mycoherbicides.

FEASIBILITY OF LARGE-SCALE MANUFACTURE AND DELIVERY

- **What sort of facility would be required for the large-scale Industrial manufacture of the proposed mycoherbicides?**
  - An entity with industrial-scale liquid or solid-state fermentation and formulation capabilities and capacity to produce sufficient tonnage of the finished products per year would be needed for large-scale manufacture of mycoherbicides. The experience gained in industrial production of registered mycoherbicides could be adapted to produce the materials needed.

- **What sort of equipment and technology would be required for the delivery of the proposed mycoherbicides on a large scale?**
  - Delivery of the mycoherbicides is expected to pose a challenge. The most likely scenario is aerial application of the proposed mycoherbicides from an aircraft capable of delivering a dry or liquid formulation.
  - On the basis of published data, the *Fusarium* mycoherbicides would be best delivered as dry formulations and the *Crivellia/Brachycladium* mycoherbicide as a spray. However, the latter appears impractical because of the amount of water required for spraying and the possible inability to apply from low altitudes.

- **What is the overall technical feasibility of the large-scale industrial manufacture of the proposed mycoherbicides?**
  - Technology for large-scale industrial production of commercial my-

coherbicides against weeds exists. It probably could be adapted for production of the proposed mycoherbicides. However, there are no data on whether the proposed strains could be mass-produced on a large scale.

- **What consideration would need to be made for large-scale delivery of the proposed mycoherbicides?**
  - In addition to the equipment and technology required for large-scale manufacture and application, consideration should be given to the potential impediments to implementing the use of the mycoherbicides. The impediments include the need for international approval and cooperation with the countries in which the mycoherbicides are to be used and the uncooperative and possibly hostile growers who might try to prevent or counteract the effects of mycoherbicide application.

- **What types of field trials of the proposed agents are needed? For example, is testing in tropical or arid environments required?**
  - More epidemiological data on the proposed mycoherbicides from experiments with consistent protocols are needed before extensive field tests can be conducted. In general, the field-test conditions should mimic the climatic and environmental conditions under which the illicit-drug crops are grown, and the mycoherbicides should be applied in a manner that simulates the intended delivery method.
  - The published data shed some light on the environmental conditions that favor the mycoherbicides' performance. This information is useful for guiding further research. There would have to be several on-site trials over a few years and in different areas where the drug crops are grown to identify field conditions that are conducive to the efficacious performance of the mycoherbicide and to establish the best timing for application. This phase of research should be done in cooperation with industry.
  - As part of any future research program, ecoclimatic data on major drug-producing regions should be gathered to select test sites and regions where the mycoherbicides could be tested and used successfully. Climate-matching computer programs could help identify geographic regions where the climatic conditions would be conducive for the mycoherbicides to cause substantial damage on their target crops.

PERSISTENCE IN THE ENVIRONMENT

- **How long are the mycoherbicides likely to persist in the environment after application?**

- Persistence of the mycoherbicide fungi would depend on environmental conditions at the application site and whether the type of fungal inoculum (asexual or sexual spores, microsclerotia, pseudothecia, or mycelia) applied would be able to survive environmental stress. Simply determining the abiotic conditions that favor or reduce persistence under controlled environments is insufficient because persistence would also depend on interactions with other living organisms and on environmental conditions that might not be replicable in controlled settings.

- **Do the mycoherbicides have geographic or climatic boundaries?**
  - *F. oxysporum* f.sp. *cannabis, F. oxysporum* f.sp. *erythroxyli,* and *C. papaveracea/B. papaveris* occur naturally in the native geographic ranges of their target crops. Particular strains probably have geographic and climatic boundaries that would limit their survival and infectivity, but the strains that were tested were evaluated under particular environmental conditions, so it is not possible to define those boundaries.

- **What combination of environmental conditions (such as temperature, depth in soil, and pH) would favor persistence after application?**
  - Moisture (from dew, high relative humidity, or rainfall) for several hours over several days with favorable temperatures would probably be required for the pathogens to become established on the target crops.
  - Information on moisture, temperature, and other requirements for pathogen survival are based on data on a few strains collected from very small areas relative to the entire geographic and climatic ranges of the target drug crops. The available data are not sufficient to conclude that the strains studied would be effective in all areas where the illicit crops are grown.
  - Studies have not examined the survival of various types of fungal propagules (spores, mycelia, or other structures) either in general or derived from the particular strains studied for development as mycoherbicides.

- **What conditions would shorten the persistence of the proposed mycoherbicides in the environment?**
  - In general, a variety of factors could shorten the persistence of fungi, such as moisture conditions (too much or too little moisture), extreme temperatures (too high or too low), ultraviolet radiation, soil depth and compaction, and the presence of antagonistic organisms, including other microorganisms, plants, and microfauna.

- **Could persistence of the mycoherbicides in the environment be controlled?**
  - *Shortening persistence*: The persistence of mycoherbicides in the environment could be shortened by the application of chemicals, such as fungicides or soil fumigants. Such a control strategy would be effective for reducing mycoherbicide agents only in small areas and would be impractical or impossible for large areas. Controlling the mycoherbicides in this manner carries substantial risk of harming the environment or other organisms in the treated area.
  - *Prolonging persistence*: The available data support the hypothesis that increased inoculum levels could be maintained for a few months after application but do not support the hypothesis that the mycoherbicide strains could persist indefinitely at higher population densities than those of the indigenous strains of the same fungi.

TOXICITY TO NONTARGET ORGANISMS

- **Would the proposed mycoherbicides harm licit crops or kill other soil fungi?**
  - Licit crops of cannabis, coca, and poppies could be adversely affected if they were exposed to the proposed mycoherbicides. Such exposures could occur through accidental drift of aerially applied mycoherbicides; through transport by human, insect, or rodent carriers; through seed transmission; or through long-distance aerial transport of spores. The available data are insufficient to determine whether any of the proposed mycoherbicides would harm other types of licit crops, native plants, or soil microbiota.

- **Would the proposed mycoherbicides threaten biodiversity or pose other risks to the environment?**
  - There are few data on risks posed by the application of the proposed mycoherbicides. To the extent that a large volume of fungal inoculum would be introduced by the application of the mycoherbicides, the biodiversity and ecology of the target regions could be affected. The risk of disease in related, nontarget species of *Cannabis*, *Erythroxylum*, and *Papaver* and to native plants caused by the mycoherbicide strains and the consequent indirect effects on microorganisms and fauna are unknown.

- **What is the nature of the health risks to animals and humans posed by the use of the proposed mycoherbicides?**
  - There are no reliable data on the health risks posed by *F. oxysporum* f.sp. *cannabis*, *F. oxysporum* f.sp. *erythroxyli*, and *C. papaveracea/B. papaveris* to animals or humans. Some strains of *F. ox-*

ysporum can infect humans or produce low levels of mycotoxins under laboratory conditions, but the variation within this species complex and the relative rarity of human infections and known mycotoxin-producing strains means that other strains in the species complex cannot serve as surrogates for the proposed mycoherbicide strains.

- There are no data on the types or quantities of toxins or allergens produced, if any, by the proposed mycoherbicide fungi. Without such data, risks to human and animal health cannot be evaluated.

- **What would be the range of transmission of the proposed mycoherbicides and what factors would influence their spread?**
  - There is potential for the spread of the mycoherbicides beyond the site of application by a variety of means, including dispersal by wind, rain, insects, animals, and humans and spread with seed or soil. Therefore, neither the range nor the speed of transmission of the proposed mycoherbicides can be predicted.

- **Once released, would the pathogens uncontrollable?**
  - Control or containment of the mycoherbicide strains after they are released would be all but impossible. The fungal strains are living organisms that interact with and adapt to their environment. Their ability to survive, propagate, and disperse beyond the target area would depend on environmental factors that can be neither predicted nor controlled. The persistence of indigenous strains of the fungi across the native range of their hosts is consistent with the conclusion that introduced mycoherbicide strains are unlikely to be contained or eradicated once they are released.

POTENTIAL FOR MUTATION AND RESULTING TOXICITY TO TARGET AND NONTARGET ORGANISMS

- **What would be the potential of the pathogens to mutate?**
  - The potential of mycoherbicide fungi to mutate would be similar to that of fungi in general. The genomes of fungi could change by nucleotide substitution, the gain of genetic material either from closely related species (by introgression) or from distantly related fungi (by horizontal gene transfer), the duplication of genetic material, and the loss of genetic material. New genetic variation could become established in fungal populations by natural selection or by chance. Natural selection results in adaptation to changing environments, including adaptation to new cultivars of the target crops or to new host species (nontarget crops). Adaptation might occur in fungi that reproduce sexually or asexually. Sexual reproduction allows new

genotypes to be produced more quickly through recombination than does mutation alone, as would occur in asexually reproducing organisms.

- One place where the mycoherbicides would be expected to adapt would be during the fermentation necessary to produce large amounts of inoculum. In such a case, adaptation to the fermentation conditions might alter the virulence of the mycoherbicides. It seems likely that the alteration would be to lower virulence rather than to raise it. Thus, as in any industrial fermentation, quality control of both the product at the end of fermentation and the inoculum that is used to start the fermentation process would be essential.
- *F. oxysporum* reproduces clonally; no sexual stage is reported. However, the distribution of mating type alleles and genotypic diversity in soil populations of *F. oxysporum* are consistent with sexual recombination in field populations of this fungus. *C. papaveracea* reproduces clonally and by sexual recombination. *B. papaveris* reproduces clonally and, although it also reproduces sexually, is self-fertile; the relative proportions of inbreeding and outbreeding are unknown.

- **Are there environmental or other conditions that would drive mutations in the mycoherbicides?**
  - Potential mutagenic changes in the proposed mycoherbicide strains should be the same as in any fungus. There is no reason to expect the proposed mycoherbicides to be more or less susceptible to mutation due to environmental or other conditions than indigenous strains.

- **Does the potential for mutation pose additional risks to nontarget organisms (including other plants, fungi, animals, and humans) or the environment?**
  - It is not possible to predict what types of mutations that might adversely affect nontarget organisms would occur.

- **How might mutations affect the susceptibility of the target crops to the mycoherbicides?**
  - Planting resistant crop plants selects for new fungal genotypes that can overcome the host plant's resistance. Selection for crop varieties that are resistant to existing fungal genotypes follows, and the cycle repeats. Selection for resistant drug crop varieties would reduce the effectiveness of the mycoherbicides and would require continued development of new mycoherbicide strains that could attack the newly developed target crop genotypes.

- Mutation of the proposed mycoherbicide strains could reduce, increase, or have no effect on the efficacy of the mycoherbicides on a target crop. It is not possible to predict what type of mutations might occur, how the pathogen or host plant might be affected, or whether the mutations would be favored by natural selection.

- **How might mutations affect the toxicity of mycoherbicides generally?**
  - Given that *F. oxysporum* and *C. papaveracea/B. papaveris* are already present where the illicit-drug crops are grown, there is no reason to expect that toxins, or mutations that affect toxins, found in the mycoherbicides would differ from those already present in indigenous strains.

RESEARCH AND DEVELOPMENT NEEDS

- **Could the effectiveness of the mycoherbicides in eradicating illicit-drug crops be improved through research and development?**
  - Only a few strains of the proposed mycoherbicides considered in this report have been investigated. Before improvements can be considered, more research is needed to obtain and identify the most efficacious strains available (preferably from plants common to the target regions), the most susceptible stage in the life cycle of the target crop, and the climatic and environmental conditions required for disease development.
  - Once those conditions are met, there are ways to improve the effectiveness of the mycoherbicides, such as the use of adjuvants and the development of formulation and delivery methods to facilitate and maximize infection.

- **What types of expertise would be most relevant for improving the effectiveness and safety of the proposed mycoherbicides?**
  - At a minimum, a team with expertise in the growing practices and conditions under which the drug crops are grown; in plant ecology; in plant disease epidemiology; in plant pathology; in fungal genetics; in fermentation, formulation, and application technology; and in nontarget risk assessment is needed to develop the proposed strains as mycoherbicides, to assess their effectiveness and safety, and to gather data for Environmental Protection Agency (EPA) registration.
  - Partnerships between industry and science are needed to guide technical development and to assess the feasibility of producing the proposed mycoherbicides.

- **What types of research and technology would improve the production and delivery of the mycoherbicides?**
    - Research is needed to determine whether the proposed mycoherbicide strains could be produced on a large scale. Research is also needed to guide product formulation, storability, and delivery. The technology to support production and delivery could be identified or developed as needed by an industrial producer.

- **What type of testing would be needed before mycoherbicides could be safely and effectively used to eradicate illicit-drug crops (for example, mode-of-action studies)?**
    - A full complement of research documenting the efficacy, specificity, and mode of action of the proposed mycoherbicide products is needed.
    - For safety assessments, at a minimum, EPA's toxicology, nontarget-organism, and environmental-fate data requirements for microbial pesticides should be met.
    - Testing in countries where the mycoherbicides would be used should be done, particularly to address local biodiversity and nontarget-risk considerations.

- **What would be required under U.S. federal and state laws to test and approve a mycoherbicide of this type, and what guidelines of the International Organisation for Biological and Integrated Control of Noxious Animals and Plants[1] would apply?**
    - The committee did not feel comfortable in developing a checklist of testing requirements that must be met, especially with regard to state and international rules, which differ by state and country. The experience from testing registered mycoherbicides suggests that the requirements become part of an evolving process as dictated by the experimental findings with regard to individual organism, host, and conditions. The committee therefore took a more general approach of reviewing and identifying the types of data required for registration in the United States and the special considerations for domestic and international uses of the proposed mycoherbicides.

[1]The International Organisation for Biological and Integrated Control of Noxious Animals and Plants is a professional society that promotes the use of biological control. While the organization and some of its members have been involved in the development of certain international standards for testing of pesticides and guidelines for transport and release of biological control agents, the IOBC does not have its own set of requirements. The committee interpreted this question to be a more general one about what international guidelines are relevant to mycoherbicides, and reviewed pertinent international treaties and guidance from the United Nations.

- For a microbial pesticide to be registered by EPA for use in the United States, a variety of tests and reports are required, for example, on product analysis, pesticide residues, toxicology, effects on nontarget organisms, and environmental fate of the pesticide. Such testing is required for each *strain* of a mycoherbicide to be registered. An environmental assessment or an environmental impact statement would probably be required for each mycoherbicide and perhaps for each strain.
- At the international level, applicable guidelines and potential limitations for testing, approval, and application of mycoherbicides against illicit crops fall under the International Plant Protection Convention, the International Standards for Phytosanitary Measures, the Biological Weapons Convention of 1972, and legal requirements in the country where the mycoherbicides would be used. Collectively, the guidelines are intended to prevent the spread and introduction of plant pests by requiring their safe export, import, shipment, and release. Some of the requirements involve documentation for the transport of agents, conducting risk assessments, and implementing risk-management strategies.

References

Abbas, H.K., and C.J. Mirocha. 1988. Isolation and purification of a hemorrhagic factor (wortmannin) from *Fusarium oxysporum* (N17B). Appl. Environ. Microbiol. 54(5):1268-1274.

Abbas, H.K., C.J. Mirocha, R.F. Vesonder, and R. Gunther. 1990. Acute toxic effects of an isolate of moniliformin-producing *Fusarium oxysporum* and purified moniliformin on rats. Arch. Environ. Contam. Toxicol. 19(3):433-436.

Abel, E.L. 1980. Marihuana: The First 12,000 Years. New York: Plenum Press.

Abruzzese, R. 1989. Coca-leaf production in the countries of the Andean subregion. Bull. Narc. 41(1-2):95-98.

Acock, M.C., J. Lydon, E. Johnson, and R.D. Collins. 1996. Effects of temperature and light levels on leaf yield and cocaine content in two *Erythroxylum* species. Ann. Bot. 78(1):49-53.

Adams, E.B., and R.F. Line. 1984. Biology of *Puccinia chondrillina* in Washington. Phytopathology 74(6):742-745.

Adams, T.C., Jr., and L.A. Jones. 1973. Long chain hydrocarbons of *Cannabis* and its smoke. J. Agr. Food Chem. 21(6):1129-1131.

Albisetti, M., R.P. Lauener, T. Gungor, G. Schar, F.K. Niggli, and D. Nadal. 2004. Disseminated *Fusarium oxysporum* infection in hemophagocytic lymphohistiocytosis. Infection 32(6):364-366.

ARC (Agricultural Research Council). 2010. Plant Species Arranged According to Growth Habit and Common Name: Black Wattle: *Acacia mearnsil (Fabaceae).*Agricultural Research Council, Pretoria, South Africa [online]. Available: http://www.arc.agric.za/home.asp?PID=1041&ToolID=63&ItemID=2986 [accessed June 28, 2010].

Arévalo, G.E., C.L. Zuniga, and H. Cabezas. 1994. Coca plant wilt and its ecological implications in Alto Huallaga [in Spanish]. P. 17 in Resúmenes del XIII Congreso Peruano de Fitopatologia, 18-23 Setiembre, Tingo Maria, Peru.

Arévalo, E., O. Cabezas, and L. Zuniga. 2000. Progress of coca *(Erythroxylum coca)* wilt caused by *Fusarium oxysporum* f.sp. *erythroxyl* in coca growing areas of Peru [in Spanish]. Fitopatol. 35(1):10-11.

Armstrong, G.M., and J.K. Armstrong. 1981. *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. Pp 391-399 in *Fusarium*: Diseases, Biology and Taxonomy, P.E. Nelson, T.A. Toussoun, and J.K. Cook, eds. University Park, PA: Pennsylvania State University Press.

Assigbetse, K.B., D. Fernandez, M.P. Dubois, and J.P. Gieger. 1994. Differentiation of *Fusarium oxysporum* f. sp. *vasinfectum* races on cotton by random amplified polymorphic DNA (RAPD) analysis. Phytopathology 84(6):622-626.

Auld, B.A., and L. Morin. 1995. Constraints in the development of bioherbicides. Weed Technol. 9(3):638-652.

Azor, M., J. Cano, J. Gene, and J. Guarro. 2009. High genetic diversity and poor in vitro response to antifungals of clinical strains of *Fusarium oxysporum*. J. Antimicrob. Chemother. 63(6):1152-1155.

Baayen, R.P., K. O'Donnell, P.J.M. Bonants, E. Cigelnik, P.N. Laurens, M. Kroon, E.J.A. Roebroeck, and C. Waalwijk. 2000. Gene genealogies and AFLP analyses in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic *formae speciales* causing wilt and rot disease. Phytopathology 90(8):891-900.

Bacon, C.W., J.K. Porter, W.P. Norred, and J.F. Leslie. 1996. Production of fusaric acid by *Fusarium* species. Appl. Environ. Microbiol. 62(11):4039-4043.

Bailey, B.A., K.P. Hebbar, M. Strem, L.C. Darlington, and R.D. Lumsden. 1997. An alginate prill formulation of *Fusarium oxysporum* Schlechtend:Fr. f.sp. *erythroxyli* for biocontrol of *Erythroxylum coca* var. *coca.* Biocontrol Sci. Technol. 7(3):423-435.

Bailey, B.A., K.P. Hebbar, M. Strem, R.D. Lumsden, L.C. Darlington, W.J. Connick, Jr., and D.J. Daigle. 1998. Formulations of *Fusarium oxysporum* f.sp. *erythroxyli* for biocontrol of *Erythroxylum coca* var. *coca.* Weed Sci. 46(6):682-689.

Bailey, B.A., P.C. Apel-Birkhold, O.O. Akingbe, J.L. Ryan, N.R. O'Neill, and J. Anderson. 2000a. Nep1 protein from *Fusarium oxysporum* enhances biological control of opium poppy by *Pleospora papaveracea*. Phytopathology 90(8):812-818.

Bailey, B.A., P.C. Apel-Birkhold, N.R. O'Neill, J. Plaskowitz, S. Alavi, J.C. Jennings, and J.D. Anderson. 2000b. Evaluation of infection processes and resulting disease caused by *Dendryphion penicillatum* and *Pleospora papaveracea* on *Papaver somniferum*. Phytopathology 90(7):699-709.

Bailey, B.A., K.P. Hebbar, R.D. Lumsden, N.R. O'Neill, and J.A. Lewis. 2004a. Production of *Pleospora papaveracea* biomass in liquid culture and its infectivity on opium poppy (*Papaver somniferum*). Weed Sci. 52(1):91-97.

Bailey, B.A., N.R. O'Neill, and J.D. Anderson. 2004b. Influence of adjuvants on disease development by *Pleospora papaveracea* on opium poppy (*Papaver somniferum*). Weed Sci. 52(3):424-432.

Baran, R. 1997. Uncommon clinical patterns of *Fusarium* nail infection: Report of three cases. Br. J. Dermatol. 136(3):424-427.

Bailey, K.L., and S. Falk. Turning research on microbial bioherbicides into commercial products—A *Phoma* story, Pest. Technol. (*in press*).

Baudoin, A.B., R.G. Abad, L.T. Kok, and W.L. Bruckart. 1993. Field evaluation of *Puccinia carduorum* for biological control of musk thistle. Biol. Control 3(1):53-60.

Bennett, A. 1873. An experimental inquiry into the physiological action of theine, caffeine, guaranine, cocaine, and theobromine. Edinb. Med. J. 19(1):323-341.

Berkoff, J. 2007. The opium economy: A possible approach to reform. World Econ. 8(4):9-45.

Berner, D.K., and W.L. Bruckart. 2005. A decision tree for evaluation of exotic plant pathogens for classical biological control of introduced invasive weeds. Biol. Control 34(2):222-232.

Bhattacharji, R. 2007. India's Experiences in Licensing Poppy Cultivation for the Production of Essential Medicines: Lessons for Afghanistan. Poppy for Medicine Project, The International Council on Security and Development [online]. Available: http://www.poppyformedi*133*cine.net/modules/support/documents/india_case_study [accessed on Dec. 15, 2010].

Bioline International. 1995. News: Philom Bios Drops BioMal. Agbiotech. Bull. 3(8) [online]. Available: http://www.bioline.org.br/request?nl95026 [accessed June 24, 2010].

Blok, W.J., and G.J. Bollen. 1997. Host specificity and vegetative compatibility of Dutch isolates of *Fusarium oxysporum* f .sp. *asparagi*. Can. J. Bot. 75(3):383-393.

Bodey, G.P., M. Boktour, S. Mays, M. Duvic, D. Kontoyiannis, R. Hachem, and I. Raad. 2002. Skin lesions associated with *Fusarium* infection. J. Am. Acad. Dermatol. 47(5):659-666.

Bosca, I., and M. Karus. 1998. The Cultivation of Hemp: Botany, Varieties, Cultivation and Harvesting. Sebastopol, CA: Hemptech.

Bourdôt, G.W., D.J. Saville, G.A. Hurrell, I.C. Harvey, and M.D. De Jong. 2000. Risk analysis of *Sclerotinia sclerotiorum* for biological control of *Cirsium arvense* in pasture: Sclerotium survival. Biocontrol Sci. Technol. 10:411-425.

Bowers, R.C. 1982. Commercialization of microbial biological control agents. Pp. 157-173 in Biological Control of Weeds with Plant Pathogens, R. Charudattan, and H.L. Walker, eds. New York: Wiley.

Boyette, C.D., and H.L. Walker. 1985. Factors influencing biocontrol of velvetleaf (*Abutilon theophrasti*) and prickly sida (*Sida spinosa*) with *Fusarium lateritium*. Weed Sci. 33(2): 209-211.

Brook, G. 2008. National Industrial Hemp Strategy. Prepared for Manitoba Agriculture Food and Rural Initiative Agriculture and Agri-Food Canada. Ottawa: The Agricola Group [online]. Available: http://www.hemptrade.ca/docs/National_Industrial_Hemp_Strategy_Final_Complete2.pdf [accessed Mar. 28, 2011].

Burdon, J.J., R.H. Groves, and J.M. Cullen. 1981. The impact of biological control on the distribution and abundance of *Chondrilla juncea* in south-eastern Australia. J. Appl. Ecol. 18(3):957-966.

Burgess, L.W. 1981. General ecology of the Fusaria. Pp. 225-235 in *Fusarium*: Diseases, Biology and Taxonomy, P.E. Nelson, T.A. Toussoun, and J.K. Cook, eds. University Park, PA: Pennsylvania State University Press.

Cantalejo, M.J., P. Toronel, L. Amate, J.M. Carrasco, and E. Hernandez. 1999. Detection of fusarin C and trichothecenes in *Fusarium* strains from Spain. J. Basic Microbiol. 39(3):143-153.

Carolan, J.C., I.L.I. Hook, M. Chase, J.W. Kadereit, and T.R. Hodkinson. 2006. Phylogenetics of Papaver and related genera based on DNA sequences from ITS nuclear ribosomal DNA and plastid trnL intron and trnL-F intergenic spacers. Ann. Bot. 98(1):141-155.

Charudattan, R. 1990. Assessment of efficacy of mycoherbicide candidates. Pp. 455-464 in Proceedings of the VII International Symposium on Biological Control of Weeds, 6-11 March 1988, Rome, Italy, E.S. Delfosse, ed. Rome: Instituto Sperimentale per la Patologia Vegetale (MAF).

Charudattan, R. 1991. The mycoherbicide approach with plant pathogens. Pp. 24-57 in Microbial Control of Weeds, D.O. TeBeest, ed. New York: Chapman and Hall.

Charudattan, R. 2005. Ecological, practical, and political inputs into selection of weed targets: What makes a good biological control target? Biol. Control 35(3):183-196.

Chouvy, P.A. 2009. Opium: Uncovering the Politics of the Poppy. London: L.B. Tauris.

Churchill, B.W. 1982. Mass production of microorganisms for biological control. Pp. 139-156 in Biological Control of Weeds with Plant Pathogens, R. Charudattan, and H.L. Walker, eds. New York: Wiley.

Conkova, E., A. Laciakova, G. Kovac, and H. Seidel. 2003. Fusarial toxins and their role in animal diseases. Vet. J. 165(3):214-220.

Connick, W.J., Jr., D.J. Daigle, A.B. Pepperman, K.P. Hebbar, R.D. Lumsden, T.W. Anderson, and D.C. Sands. 1998. Preparation of stable, granular formulations containing *Fusarium oxysporum* pathogenic to narcotic plants. Biol. Control 13(2):79-84.

Cotler, J. 1996. Coca Sociedad y Estado en el Peru. PNUD (Lima, 1996).

Covarelli, G. 1981. Il diserbo delle colture erbacee: Frumento, orzo, avena. I'Italia Agricola 118(3):121-133.

Cullen, J.M. 1978. Evaluating the success of the programme for the biological control of *Chondrilla juncea* L. Pp. 117-121 in Proceedings of the IV International Symposium on Biological Control of Weeds, August 30-September 2, 1976, Gainesville, FL, T.E. Freeman, ed. Gainesville: University of Florida, Center for Environmental Programs.

Cullen, J.M. 1985. Bringing the cost benefit analysis of biological control of *Chondrilla-juncea* up to date. Pp. 145-152 in Proceedings of the VI International Symposium on Biological Control of Weeds, 19-25 August 1984, Vancouver, Canada, E.S. Delfosse, ed. Ottawa: Agriculture Canada.

Cullen, J.M., and R.H. Groves. 1977. The population biology of *Chondrilla juncea* L. in Australia. Proc. Ecol. Soc. Aust. 10:121-134.

Cullen, J.M., P.F. Kable, and M. Katt. 1973. Epidemic spread of a rust imported for biological control. Nature 244(5416):462-464.

Cura, J.J., W. Heiger-Bernays, T.S. Bridges, and D.W. Moore. 1999. Ecological and Human Health Risk Assessment Guidance for Aquatic Environments. Technical Report DOER-4. Prepared for the U.S. Army Corps of Engineers, Engineer Research, and Development Center, Vicksburg, MS [online]. Available: http://el.erdc.usace.army.mil/elpubs/pdf/trdoer4.pdf [accessed Jan. 31, 2011].

Czyzewska, S., and H. Zarzycka. 1960. Niektóre dane z biologii grzyba *Helminthosporium papaveris* Hennings, stadium doskonale *Pleospora papaveracea* (Wint.) de Not. Acta Agrobot. 10(1):41-51.

Daniel, J.T., G.E. Templeton, R.J. Smith, and W.T. Fox. 1973. Biological control of northern jointvetch in rice with an endemic fungal disease. Weed Sci. 21(4):303-307.

Dar, G.H.H., T. Rashid, A.R. Naqshi, A.A Khuroo and A.H. Malik. 2010. Two new species of *Papaver L.* (Papaveraceae) from Kashmir Himalaya, India. Pak. J. Bot. 42:57-62

Darlington, L. 1996. History of *Erythroxylum* and notes on diseases and pests at Kauai field site. P. 43 in Proceedings of the 1st International Workshop on *Fusarium* Biocontrol, October 28-31, 1996, Beltsville, MD.

Dauch, A.L., A.K. Watson, and S.H. Jabaji-Hare. 2003. Detection of the biocontrol agent *Colletotrichum coccodes* (183088) from the target weed velvetleaf and from soil by the strain-specific PCR markers. J. Microbiol, Methods 55:51-64.

De Jong, M.D., P.C. Scheepens, and J.C. Zadoks. 1990. Risk analysis for biological control: A Dutch case study in biocontrol of *Prunus serotina* by the fungus *Chondrostereum purpureum*. Plant Dis. 73(3):189-194.

De Jong, M.D., D.E. Aylor, and G.W. Bourdôt. 1999. A methodology for risk analysis of plurivorous fungi in biological weed control: *Sclerotinia sclerotiorum* as a model. BioControl 43:397-419.

De Jong, M.D., G.W. Bourdôt, G.A. Hurrell, D.J. Saville, H.J. Erbrink, and J.C. Zadoks. 2002. Risk analysis for biological weed control—simulating dispersal of *Sclerotinia sclerotiorum* (Lib.) de Bary ascospores from a pasture after biological control of *Cirsium arvense* (L.) Scop. Aerobiologia 18:211-222.

de Zeeuw, R.A., T.M. Malingre, and F.W. Merkus. 1972. Tetrahydrocannabinolic acid, an important component in the evaluation of *Cannabis* products. J. Pharm. Pharmacol. 24(1):1-6.

de Zeeuw, R.A., J. Wijsbeek, and T.M. Malingre. 1973. Interference of alkanes in the gas chromatographic analysis of *Cannabis* products. J. Pharm. Pharmacol. 25(1):21-26.

DEA (U.S. Drug Enforcement Administration). 1993. Coca Cultivation and Cocaine Processing. [online]. Available: http://www.druglibrary.org/schaffer/govpubs/coccp.htm [accessed Dec. 13, 2010].

DeGrandchamp, R.L., and M.G. Barron. 2005. PCB Analysis and Risk Assessment at Naval Installations, Part C: Ecological Risk Assessment for PCB Sites: A Guide for Determining the Risk of PCB Exposure to Ecological Receptors. Prepared for the Navy Environmental Health Center, Portsmouth, VA. December 2005.

Del Serrone, P., and T. Annesi. 1990. Pathogenicity and host-specificity of *Pleospora papaveracea*, a candidate for biological control of poppy (*Papaver rhoeas*). Pp. 465-469 in Proceedings of the VII International Symposium on Biological Control of Weeds, 6-11 March 1988, Rome, Italy, E.S. Delfosse, ed. Rome: Instituto Sperimentale per la Patologia Vegetale (MAF).

Desjardins, A.E., T.M Hohn, and S.P. McCormick. 1993. Trichothecene biosynthesis in *Fusarium* species: Chemistry, genetics, and significance. Microbiol. Rev. 57(3): 595-604.

Desjardins, A.E. 2006. *Fusarium* Mycotoxins: Chemistry, Genetics and Biology. St. Paul, MN: APS Press. 260 pp.

Di Pietro, A., M.P. Madrid, Z. Caracuel, J. Delgado-Jarana, and M.I.G. Roncero. 2003. *Fusarium oxysporum*: Exploring the molecular arsenal of a vascular wilt fungus. Mol. Plant Pathol. 4(5):315-325.

Ditmore, M., J.W. Moore, and D.O. TeBeest. 2008. Interactions of two selected field isolates of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* on *Aeschynomene virginica.* Biol. Control 47(3):298-308.

DOJ (U.S. Department of Justice). 2005. National Drug Threat Assessment 2005. Document No. 2005-Q0317-003. National Drug Intelligence Center, U.S. Department of Justice, Johnstown, PA. February 2005 [online]. Available: http://www.justice.gov/ndic/pubs11/12620/12620p.pdf [accessed Mar. 29, 2011].

DOJ (U.S. Department of Justice). 2010. National Drug Threat Assessment 2010. National Drug Intelligence Center, U.S. Department of Justice, Washington, DC. February 2010 [online]. Available: http://www.justice.gov/ndic/pubs38/38661/index.htm [accessed June 8, 2010].

Dolgovskaya, M.Yu, S.A. Podlipaev, S.Ya Reznik, M.G. Volkovich, M. McCarthy, and D. Sands. 1996. Screening of fungal pathogens for the control of *Papaver somniferum* in the former Soviet Union. P. 543 in Proceedings of the IX International Symposium on Biological Control of Weeds, January 19-26, Stellenbosch, South Africa, V.C. Moran, and J.H. Hoffman, eds. Cape Town: University of Cape Town.

Duke, J. 1983. Handbook of Energy Crops. NewCROP [online]. Available: http://www.hort.purdue.edu/newcrop/duke_energy/dukeindex.html [accessed Dec. 12, 2010].

EC (European Commission). 2009. Flax and Hemp, AGRI C5. Agriculture and Rural Development [online]. Available: http://www.eiha.org/attach/553/09-02_C1_Flax_hemp_presentation_26_February_2009_circa.pdf [accessed Mar. 28, 2011].

Eccleston, C.H. 2008. NEPA and Environmental Planning: Tools, Techniques, and Approaches for Practitioners. Boca Raton, FL: CRC Press.

Edel, V., C. Steinberg, N. Gautheron, G. Recorbet, and C. Alabouvette. 2001. Genetic diversity of *Fusarium oxysporum* populations isolated from different soils in France. FEMS Microbiol. Ecol. 36(1):61-71.

El Comercio. 1995. Hongo que destruye plantaciones de coca en Columbia no procede de Perú. El Comercio, A15. March 20, 1995.

El-Hamalawi, Z.A. 2008. Attraction, acquisition, retention and spatiotemporal distribution of soilborne plant pathogenic fungi by shore flies. Ann. Appl. Biol. 152(2): 169-177.

El-Hamalawi, Z.A., and M.E. Stanghellini. 2005. Disease development on lisianthus following aerial transmission of *Fusarium avenaceum* by adult shore flies, fungus gnats, and moth flies. Plant Dis. 89(6):619-623.

Elzein, A., and J. Kroschel. 2004. Influence of agricultural by-products in liquid culture on chlamydospore production by the potential mycoherbicide *Fusarium oxysporum* Foxy 2. Biocontrol Sci. Technol. 14(8):823-836.

Emge, R.G., J. Stanley Melching, and C.H. Kingsolver. 1981. Epidemiology of *Puccinia chondrillina*, a rust pathogen for the biological control of rush skeleton weed in the United States. Phytopathology 71(8):839-843.

ENACO (Empresa Nacional de la Coca). 2002. Oficio No. 028-2002-ENACO S.A.PD Lima (as cited in Thoumi 2005).

Enya, J., M. Togawa, T. Takeuchi, S. Yoshida, S. Tsushima, T. Arie, and T. Sakai. 2008. Biological and phylogenetic characterization of *Fusarium oxysporum* complex, which causes yellows on *Brassica spp.*, and proposal of *F. oxysporum* f. sp *rapae*, a novel forma specialis pathogenic on *B. rapa* in Japan. Phytopathology 98(4):475-483.

EPA (U.S. Environmental Protection Agency). 1995. Site Conceptual Models for BRAs. Model Site Conceptual Model for RI/FS Baseline Risk Assessments of Human and Ecological Health. Region 8 Superfund Technical Guidance SOP 9RA-05. U.S. Environmental Protection Agency Region 8, Denver, CO. May 1995 [online]. Available: http://www.epa.gov/region8/r8risk/pdf/r8_ra05-scms.pdf [accessed Apr. 7, 2011].

EPA (U.S. Environmental Protection Agency). 1997. *Colletotrichum gloeosporioides* f.sp. *aeschynomene,* R.E.D. Facts. EPA-738-F-96-026. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. April 1997 [online]. Available: http://www.epa.gov/oppsrrd1/REDs/factsheets/4103fact.pdf [accessed June 28, 2010].

EPA (U.S. Environmental Protection Agency). 2002. *Puccinia thlaspeos* Strain Woad (Dyer's Wood Rust) (006489) Fact Sheet, June 2002 [online]. Available: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_006489.htm [accessed June 28, 2010].

EPA (U.S. Environmental Protection Agency). 2004a. *Chondrostereum purpureum* Stain PFC 2139 (081308) Fact Sheet, October 2004 [online]. Available: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_081308.htm [accessed June 28, 2010].

EPA (U.S. Environmental Protection Agency). 2004b. Biopesticide Registration Action Document: *Chondrostereum purpureum* Stain PFC 2139 (PC Code 081308). Office of Pesticides Programs, U.S. Environmental Protection Agency. September 20, 2004 [online]. Available: http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/brad_081308.pdf [accessed July 7, 2010].

EPA (U.S. Environmental Protection Agency). 2005a. Contaminated Sediment Remediation Guidance for Hazardous Waste Sites. EPA-540-R-05-012. OSWER 9355.0-

85. U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, December 2005 [online]. Available: http://www.epa.gov/superfund/health/conmedia/sediment/pdfs/guidance.pdf [accessed Jan. 31, 2011].

EPA (U.S. Environmental Protection Agency). 2005b. *Alternaria destruens* Strain 059 (028301) Fact Sheet, April 5, 2005 [online]. Available: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_028301.htm [accessed June 28, 2010].

EPA (U.S. Environmental Protection Agency). 2005c. *Chondrostereum purpureum* Stain HQ1 (081309) Fact Sheet, March 2005 [online]. Available: http://www.epa.gov/oppbppd1/biopesticides/ingredients/factsheets/factsheet_081309.htm [accessed June 28, 2010].

EPA (U.S. Environmental Protection Agency). 2005d. *Chondrostereum purpureum* Stain HQ1 (081309) Federal Register Notices: Conditional Approval of Active Ingredient, June 3, 2005 [online]. Available: http://www.epa.gov/pesticides/biopesticides/ingredients/fr_notices/frnotices_081309.htm [accessed June 30, 2010].

EPA (U.S. Environmental Protection Agency). 2006a. *Phytophthora palmivora* MWV. R.E.D. Facts. Office of Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. March 27, 2006 [online]. Available: http://www.epa.gov/opp00001/biopesticides/ingredients/factsheets/redfact_111301.pdf [accessed June 30, 2010].

EPA (U.S. Environmental Protection Agency). 2006b. Reregistration Eligibility Decision: *Phytophthora palmivora* MWV PC Code 111301. Office of Pesticide Programs, U.S. Environmental Protection Agency. February 15, 2006 [online]. Available: http://www.epa.gov/pesticides/biopesticides/ingredients/tech_docs/red_111301.pdf.

Evans, J., D. Levesque, A. de Lahunta, and H.E. Jensen. 2004. Intracranial fusariosis: A novel cause of fungal meningoencephalitis in a dog. Vet. Pathol. 41(5):510-514.

FAO (Food and Agriculture Organization). 2006a. Guidelines for Export, Shipment, Import and Release of Biological Control Agents and Other Beneficial Organisms (2005 Edition). International Standards for Phytosanitary Measures, ISPM No. 3. Secretariat of the International Plant Protection Convention, Food and Agriculture Organization [online]. Available: https://www.ippc.int/file_uploaded/1146657660135_ISPM3.pdf [accessed Feb. 28, 2011].

FAO (Food and Agriculture Organization). 2006b. Guidelines for Pest Risk Analysis (1995). International Standards for Phytosanitary Measures, ISPM No. 2. Secretariat of the International Plant Protection Convention, Food and Agriculture Organization [online]. Available: http://www.acfs.go.th/sps/downloads/34208_ISPM_2_E.pdf [accessed Feb. 28, 2011].

FAO (Food and Agriculture Organization). 2006c. Pest Risk Analysis for Quarantine Pests Including Analysis of Environmental Risks and Living Modified Organisms (2004). International Standards for Phytosanitary Measures, ISPM No. 11. Secretariat of the International Plant Protection Convention, Food and Agriculture Organization [online]. Available: https://www.ippc.int/file_uploaded/1146658377367_ISPM11.pdf [accessed Feb.28, 2011].

FAOSTAT (Food and Agriculture Organization Statistical Database). 2009. Production Crops by Agricultural Item: Poppy Seed [online]. Available: http://faostat.fao.org/site/567/default.aspx#ancor [accessed Mar. 14, 2011].

Farr, D.F., N.R. O'Neill, and P.B. van Berkum. 2000. Morphological and molecular studies on *Dendryphion penicillatum* and *Pleospora papaveracea*, pathogens of *Papaver somniferum*. Mycologia 92(1):145-153.

Fetterman, P.S., E.S. Keith, C.W. Waller, O. Guerrero, N.J. Doorenbos, and M.W. Quimby. 1971. Mississippi-grown *Cannabis sativa* L.: Preliminary observation on chemical definition of phenotype and variations in tetrahydrocannabinol content versus age, sex, and plant part. J. Pharm. Sci. 60(8):1246-1249.

Finetto, G. 2008. Opium poppy: Societal blessing and curse. Chronica Horticulturae 48(3):18-23.

Fjeldså, J., M.D. Álvarez, J.M. Lazcano, and B. Leon. 2005. Illicit crops and armed conflict as constraints on biodiversity conservation in the Andes region. Ambio 34(3):205-211.

Fravel, D.R., S.K. Stosz, and R.P. Larkin. 1996. Effect of temperature, soil type and matric potential on proliferation and survival of *Fusarium oxysporum* f. sp. *erythroxyli* from *Eythroxylum coca*. Phytopathology 86(3):236-240.

Friesen, T.L., E.H. Stukenbrock, Z. Liu, S. Meinhardt, H. Ling, J.D. Faris, J.B. Rasmussen, P.S. Solomon, B.A. McDonald, and R.P. Oliver. 2006. Emergence of a new disease as a result of interspecific virulence gene transfer. Nat. Genet. 38(8):953-956.

Fullaway, D.T. 1954. Biological control of cactus in Hawaii. J. Econ. Entomol. 47(4): 696-700.

Gambogi, P. 1983. Seed transmission of *Fusarium oxysporum*: Epidemiology and control. Seed Sci. Technol. 11:815-827.

Gamliel, A., T. Katan, H. Yunis, and J. Katan. 1996. *Fusarium* wilt and crown rot of sweeet basil: Involvement of soilborne and airborne inoculum. Phytopathology 86(1):56-62.

Garibaldi, A., J. Katan, and M.L. Gullino, eds. *Fusarium* Wilts of Greenhouse Vegetables and Ornamental Crops. St. Paul, MN: APS Press (*in press*).

Ge, H.M., Y.C. Song, C.Y. Shan, Y.H. Ye, and R.X. Tan. 2005. New and cytotoxic anthraquinones from *Pleospora* sp. IFB-E006, an endophytic fungus in *Imperata cylindrica*. Planta Med. 71(11):1063-1065.

Gelineau-van Wase, J., K.A. Voss, V.L. Stevens, M.C. Speer, and R.T. Riley. 2009. Maternal fumonisin exposure as a risk factor for neural tube defects. Adv. Food Nutr. Res. 56:145-181.

Gellert, M., I. Novak, M. Szell, and K. Szendrei. 1974. Glycosidic Components of *Cannabis sativa* L. I. Flavonoids. UN Document ST/SOA/SER.S/50. September 20, 1974.

Ghajar, F., P. Holford, E. Cother, and A. Beattie. 2006. Effects of ultraviolet radiation, simulated or as natural sunlight, on conidium germination and appressorium formation by fungi with potential as mycoherbicides. Biocon. Sci. Technol. 16(5):451-469.

Gianni, C., A. Cerri, and C. Crosti. 1997. Unusual clinical features of fingernail infection by *Fusarium oxysporum*. Mycoses 40(11-12):455-459.

Gillespie, D.R., and J.G. Menzies. 1993. Fungus gnats vector *Fusarium oxysporum* f.sp. *radicislycopersici.* Ann. Appl. Biol. 123(3):539-544.

Goddard, M.R., H.C.J. Godfray, and A. Burt. 2005. Sex increases the efficacy of natural selection in experimental yeast populations. Nature 434(7033):636-640.

Gootenberg, P. 2008. Andean Cocaine: The Making of a Global Drug. Chapel Hill: University of North Carolina Press.

Graham, G.L., G. Peng, K.L. Bailey, and F.A. Holm. 2007. Effect of plant stage, *Colletotrichum truncatum* dose, and use of herbicide on control of *Matricaria perforata*. BioControl 52(4):573-589.

Gracia-Garza, J.A., D.R. Fravel, B.A. Bailey, and P.K. Hebbar. 1998. Dispersal of formulations of *Fusarium oxysporum* f. sp. *erythroxyli* and *F. oxysporum* f. sp. *melonis* by ants. Phytopathology 88(3):185-189.

Gracia-Garza, J.A., D.R. Fravel, A.J. Nelson, K.S. Elias, B.A. Bailey, E. Arévalo Gardini, and L.C. Darlington. 1999. Potential for dispersal of *Fusarium oxysporum* f. sp. *erythroxyli* by infested seed. Plant Dis. 83(5):451-455.

Greaves, M.P., J.A. Bailey, and J.A. Hargreaves. 1989. Mycoherbicides: Opportunities for genetic manipulation. Pestic. Sci. 26(1):93-101.

Grey-Wilson, C. 2000. Poppies: A Guide to the Poppy Family in the Wild and in Cultivation. Portland, OR: Timber Press.

Grlic, L., and A. Andrec. 1961. The content of acid fraction in *Cannabis* resin of various age and provenance. Experientia 17:325-326.

Guarro, J., and J. Gene. 1995. Opportunistic fusarial infections in humans. Eur. J. Clin. Microbiol. Infect. Dis. 14(9):741-754.

Guilhermetti, E., G. Takahachi, C.S. Shinobu, and T.I. Svidzinski. 2007. *Fusarium* spp. as agents of onychomycosis in immunocompetent hosts. Int. J. Dermatol. 46(8):822-826.

Hajek, A.E. 2004. Natural Enemies: An Introduction to Biological Control. Cambridge, UK: Cambridge University Press. 378 pp.

Hanus, I. 1975a. The present state of knowledge in the chemistry of substances of *Cannabis sativa* L. III. Terpenoid substances. Acta Univ. Palacki. Olomuc. Fac. Med. 73:233-239.

Hanus, I. 1975b. The present state of knowledge in the chemistry of substances of *Cannabis sativa* L. IV. Nitrogen containing compounds. Acta Univ. Palacki. Olomuc. Fac. Med. 73:241-244.

Harrison, W.C., and C.G. Schmitt. 1967. Diseases of the Opium Poppy (*Papaver somniferum*): A Check List. Miscellaneous Publication 27. AD385869. U.S. Department of the Army, Fort Detrick, MD.

Heale, J.B., J.E. Isaac, and D. Chandler. 1989. Prospects for strain improvement in entomopathogenic fungi. Pestic. Sci. 26(1):79-92.

Health Canada. 2010. Medical Use of Marihuana. Health Canada [online]. Available: http://www.hc-sc.gc.ca/dhp-mps/marihuana/index-eng.php [accessed on Feb.17, 2011].

Health Canada. 2011a. Pesticides Public Registry: Chontrol Paste, Registration No. 27823. Pest Management Regulatory Agency, Health Canada [online]. Available: http://pr-rp.hc-sc.gc.ca/pi-ip/index-eng.php [accessed Feb. 28, 2011].

Health Canada. 2011b. Pesticides Public Registry: Myco-Tech Paste. Registration No. 27020. Pest Management Regulatory Agency, Health Canada [online]. Available: http://pr-rp.hc-sc.gc.ca/pi-ip/index-eng.php [accessed Feb. 28, 2011].

Health Canada. 2011c. Pesticides Public Registry: Sarritor Granular Biological Herbicide (Commercial), Registration No. 28545. Pest Management Regulatory Agency, Health Canada [online]. Available: http://pr-rp.hc-sc.gc.ca/pi-ip/index-eng.php [accessed Feb. 28, 2011].

Hebbar, K.P., R.D. Lumsden, S.M. Poch, and J.A. Lewis. 1997. Liquid fermentation to produce biomass of mycoherbicidal strains of *Fusarium oxysporum*. Appl. Microbiol. Biotechnol. 48(6):714-719.

Hebbar, K.P., B.A. Bailey, S.M. Poch, J.A. Lewis, and R.D. Lumsden. 1999. An improved granular formulation for a mycoherbicidal strain of *Fusarium oxysporum*. Weed Sci. 47(4):473-478.

Hendricks, H., T.M. Malingre, S. Batterman, and R. Bos. 1975. Mono- and sesquiterpene hydrocarbons of the essential oil of *Cannabis sativa*. Phytochemistry 14(3):814-815.

Hendricks, K. 1999. Fumonisins and neural tube defects in South Texas. Epidemiology 10(2):198-200.

Hildebrand, D.C., and A.H. McCain. 1978. The use of various substrates for large-scale production of *Fusarium oxysporum* f.sp. *cannabis* inoculum. Phytopathology 68(7):1099-1101.

Hildebrand, P.D. 2002. Dispersal of plant pathogens. Pp. 193-196 in Encyclopedia of Pest Management, D. Pimentel, ed. New York: Marcel Dekker.

Holcomb, G.E. 1982. Constraints on disease development. Pp. 67-71 in Biological Control of Weeds with Plant Pathogens, R. Charudattan, and H.L. Walker, eds. New York: Wiley.

Hood, L.V.S., M.E. Dames, and G.T. Barry. 1973. Headspace volatiles of marijuana. Nature 242(5397):402-403.

Inderbitzin, P., R.A. Shoemaker, N.R. O'Neill, B.G. Turgeon, and M.L. Berbee. 2006. Systematics and mating systems of two fungal pathogens of opium poppy: The heterothallic *Crivellia papaveracea* with a *Brachycladium penicillatum* asexual state and a homothallic species with a *Brachycladium papaveris* asexual state. Can. J. Bot. 84(8):1304-1326.

Inman, R.E. 1971. A preliminary evaluation of *Rumex* rust as biological control agent for curly dock. Phytopathology 61(1):102-107.

Jarvis, W.R., and R.A. Shoemaker. 1978. Taxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68(12):1679-1680.

Johnson, E.L. 1996. Alkaloid content in *Erythroxylum coca* tissue during reproductive. development. Phytochemistry 42(1):35-38.

Johnson, E.L., J.A. Saunders, S. Mischke, C.S. Helling, and S.D. Emche. 2003. Identification of *Erythroxylum* taxa by AFLP DNA analysis. Phytochemistry 64(1):187-197.

Johnson, K.B. 1994. Dose-response relationships and inundative biological control. Phytopathology 84(8):780-784.

Johnson, R. 2010. Hemp as an Agricultural Commodity. CRS Report for Congress RL32725. Congressional Research Service. December 22, 2010 [online]. Available: http://www.fas.org/sgp/crs/misc/RL32725.pdf [accessed Mar. 13, 2011].

Kapoor, L.D. 1995. Opium Poppy: Botany, Chemistry, and Pharmacology. New York: Haworth Press.

Karch, S.B. 1998. A Brief History of Cocaine. Boca Raton: CRC Press.

Kasuga, T., T.J. White, and J.W. Taylor. 2002. Estimation of nucleotide substitution rates in *Eurotiomycete* fungi. Mol. Biol. Evol. 19(12):2318-2324.

Katan, T., E. Shlevin, and J. Katan. 1997. Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. Phytopathology 87(7):712-719.

Kim, J.C., and Y.W. Lee. 1994. Sambutoxin, a new mycotoxin produced by toxic *Fusarium* isolates obtained from rotted potato tubers. Appl. Environ. Microbiol. 60(12): 4380-4386.

Kimura, M., and K. Okamoto. 1970. Distribution of tetrahydrocannabinolic acid in fresh wild *Cannabis*. Experientia 26(8):819-820.

Kimura, M., T. Tokai, N. Takahashi-Ando, S. Ohsato, and M. Fujimura. 2007. Molecular and genetic studies of *Fusarium* trichothecene biosynthesis: Pathways, genes, and evolution. Biosci. Biotechnol. Biochem. 71(9):2105-2123.

Kistler, H.C. 1997. Genetic diversity in the plant-pathogenic fungus *Fusarium oxysporum*. Phytopathology 87(4):474-479.

Klittich, C.J.R., and J.F. Leslie. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). Genetics 118(3):417-423.

Klittich, C.J.R., J.C. Correll, and J.F. Leslie. 1988. Inheritance of sectoring frequency in *Fusarium moniliforme* (*Gibberella fujikuroi*). Exp. Mycol. 12(3):289-294.

Krikorian, A.D., and M.C. Ledbetter. 1975. Some observations on the cultivation of opium poppy (*Papaver somniferum* L.) for its latex. Bot. Rev. 41(1):30-103.

Landa, B.B., J.A. Navas-Cortés, and R.M. Jiménez-Díaz. 2004. Integrated management of *Fusarium* wilt of chickpea with sowing date, host resistance, and biological control. Phytopathology 94(9):946-960.

Larkin, R.P., and D.R. Fravel. 1999. Mechanisms of action and dose-response relationships governing biological control of *Fusarium* wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 89(12):1152-1161.

Lee, G.A. 1986. Integrated control of rush skeletonweed (*Chondrilla juncea*) in the Western U.S. Weed Sci. 34(Suppl.1):2-6.

Leslie, J.F., and B.A. Summerell. 2006. The *Fusarium* Laboratory Manual. Ames, IA: Blackwell Publishing.

Levy, A., and J. Milo. 1998. Genetics and breeding of *Papaver somniferum*. Pp. 93-103 in Poppy: The Genus *Papaver,* J. Bernath, ed. Amsterdam: Harwood Academic Publishers.

Lievens, B., P. van Baarlen, C. Verreth, S. van Kerckhove, M. Rep, and B. Thomma. 2009. Evolutionary relationships between *Fusarium oxysporum* f.sp *lycopersici* and *F. oxysporum* f. sp *radicis-lycopersici* isolates inferred from mating type, elongation factor-1 alpha and exopolygalacturonase sequences. Mycol. Res. 113(Pt. 10):1181-1191.

Logrieco, A., A. Moretti, G. Castella, M. Kostecki, P. Golinski, A. Ritieni, and J. Chelkowski. 1998. Beauvericin production by *Fusarium* species. Appl. Environ. Microbiol. 64(8):3084-3088.

Lortholary, O., G. Obenga, P. Biswas, D. Caillot, E. Chachaty, A.L. Bienvenu, M. Cornet, J. Greene, R. Herbrecht, C. Lacroix, F. Grenouillet, I. Raad, K. Sitbon, and P. Troke. 2010. International retrospective analysis of 73 cases of invasive fusariosis treated with voriconazole. Antimicrob. Agents Chemother. 54(10):4446-4450.

Lotz, L.A.P., R.M.W. Groeneveld, B. Habekotte, and H. Van Oene. 1991. Reduction of growth and reproduction of *Cyperus esculentus* by specific crops. Weed Res. 31(3):153-160.

Luo, Y., and D.O. TeBeest. 1997. Infection components of wild-type and mutant strains of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* on northern jointvetch. Plant Dis. 81(4):404-409.

Luo,Y., and D.O. TeBeest. 1998. Behavior of a wild-type and two mutant strains of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* on northern jointvetch in the field. Plant Dis. 82(4):374-379.

Luo, Y., and D.O. TeBeest. 1999. Effect of temperature and dew period on infection of northern jointvetch by wild-type and mutant strains of *Colletotrichum gloeosporioides* f.sp. *aeschymenene*. Biol. Control 14(1):1-6.

Ma, L.J., H.C. van der Does, K.A. Borkovich, J.J. Coleman, M.J. Daboussi, A. Di Pietro, M. Dufresne, M. Freitag, M. Grabherr, B. Henrissat, P.M. Houterman, S. Kang, W.B. Shim, C. Woloshuk, X. Xie, J.R. Xu, J. Antoniw, S.E. Baker, B.H. Bluhm, A. Breakspear, D.W. Brown, R.A.E. Butchko, S. Chapman, R. Coulson, P.M. Coutinho, E.G.J. Danchin, A. Diener, L.R. Gale, D.M. Gardiner, S. Goff, K.E.

Hammond-Kosack, K. Hilburn, A. Hua-Van, W. Jonkers, K. Kazan, C.D. Kodira, M. Koehrsen, L. Kumar, Y.H. Lee, L. Li, J.M. Manners, D. Miranda-Saavedra, M. Mukherjee, G. Park, J. Park, S.Y. Park, R.H. Proctor, A. Regev, M.C. Ruiz-Roldan, D. Sain, S. Sakthikumar, S. Sykes, D.C. Schwartz, B.G. Turgeon, I. Wapinksi, O. Yoder, S. Young, Q. Zeng, S. Zhou, J. Galagan, C.A. Cuomo, H.C. Kistler, and M. Rep. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464(7287):367-373.

Makowski, R.M.D. 1993. Effect of inoculum concentration, temperature, dew period, and plant growth stage on disease of round-leaved mallow and velvetleaf by *Colletotrichum gloeosporioides* f. sp. *malvae*. Phytopathology 83(11):1229-1234.

Maldonado-Ramirez, S.L., D.G. Schmale, E.J. Shields, and G.C. Bergstrom. 2005. The relative abundance of viable spores of *Gibberalla zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of *Fusarium* head blight. Agr. Forest Meteorol. 132(1-2):20-27.

Mansfield, D. 2001. An Analysis of Licit Opium Poppy Cultivation: India and Turkey. April 2001 [online]. Available: http://www.geopium.org/Mansfield2001AnalysisLicitOpiumPoppyCultivation.pdf [accessed Dec. 13, 2010].

Marangon, A.V., T.I. Svidzinski, T.P. Salci, R. Meurer, M. da Cruz Fernandes, and L. Hernandes. 2009. Metabolic extract of *Fusarium oxysporum* induces histopathologic alterations and apoptosis in the skin of Wistar rats. Int. J. Dermatol. 48(7):697-703.

Marasas, W. F. O., P.E. Nelson, and T.A. Toussoun. 1984. Toxigenic *Fusarium* Species: Identity and Mycotoxicology. University Park, PA: Pennsylvania State University Press.

Masoud, A.N., and N.J. Doorenbos. 1973. Mississippi-grown *Cannabis sativa* L. III. Cannabinoid and cannabinoic acid content. J. Pharm. Sci. 62(2):313-315.

Mayayo, E., I. Pujol, and J. Guarro. 1999. Experimental pathogenicity of four opportunist *Fusarium* species in a murine model. J. Med. Microbiol. 48(4):363-366.

Mbofung, G.C., and B.M. Pryor. 2007. Potential for dispersal of *Fusarium oxysporum* f.sp. *lactucae* by infested lettuce seed. Phytopathology 97(7 suppl.):S72.

McCain, A.H., and C. Noviello. 1985. Biological control of *Cannabis sativa*. Pp. 635-642 in Proceedings of the VI International Symposium on Biological Control of Weeds, 19-25 August 1984, Vancouver, Canada, E.S. Delfosse, ed. Ottawa: Agriculture Canada.

McCoy, E.D., and J.H. Frank. 2010. How should the risk associated with the introduction of biological control agents be estimated? Agr. Forest Entomol. 12(1):1-8.

McPartland, J.M. 1992. The *Cannabis* pathogen project: Report of the second five-year plan. Mycological Society of America Newsletter 43(1):43.

McPartland, J.M. 1996. A review of *Cannabis* diseases. J. Int. Hemp Assoc. 3(1):19-23 [online]. Available: http://www.hempfood.com/Iha/iha03111.html [accessed Mar. 24, 2010].

McPartland, J.M., and K.W. Hillig. 2004. *Cannabis* clinic *Fusarium* wilt. J. Ind. Hemp 9(2):67-77.

McPartland, J.M., and D.P. West. 1999. Killing *Cannabis* with mycoherbicides. J. Int. Hemp Assoc. 6(1):1-8 [online]. Available: http://www.gametec.com/hemp/mcpartland/mycoherbicides.html [accessed Jan. 22, 2011].

McRae, C.F., and B.A. Auld. 1988. The influence of environmental factors on anthracnose of *Xanthium spinosum*. Phytopathology 78(9):1182-1186.

Meffert, M.E. 1950. Biology and morphology of the pathogen of the parasitic leaf blight of the poppy [in German]. Z. Parasitenkd. 14(5):442-498.

Merlin, M.D. 1984. On the Trail of the Ancient Opium Poppy. Cranbury, NJ: Associated University Presses.

Michielse, C.B., and M. Rep. 2009. Pathogen profile update: *Fusarium oxysporum*. Mol. Plant Pathol. 10(3):311-324.

Milatović, I. 1975a. A Very Dangerous Poppy Disease—Pleospora (*Pleospora calvescens* (Fr.) Tul.). Belgrade, Yugoslavia: Nolit [reprinted from Biljna Proizvodnja 2, 1949].

Milatović, I. 1975b. Study of the Fungus *Pleospora calvescens* (Fr.) Tulasne on Poppy. Belgrade, Yugoslavia: Nolit [reprinted from Poljoprivredna Znanstvena Smotra 13, 1952].

Miller, J.D. 2002. Aspects of the ecology of *Fusarium* toxins in cereals. Adv. Exp. Med. Biol. 504:19-27.

Mobarak, Z., D. Bieniek, and F. Korte. 1974a. Studies on non-cannabinoids of hashish. Isolation and identification of some hydrocarbons. Chemosphere 3(1):5-8.

Mobarak, Z., D. Bieniek, and F. Korte. 1974b. Studies on non-cannabinoids of hashish. II. An approach to correlate the geographical origin of *Cannabis* with hydrocarbon content by chromatographic analysis. Chemosphere 3(6):265-270.

Morin, L.A., A.K. Watson, and R.D. Reeleder. 1990. Effect of dew, inoculum density, and spray additives on infection of field bindweed by *Phomopsis convolvulus*. Can. J. Plant Pathol. 12(1):48-56.

Morris, M.J. 1997. Impact of the gall-forming fungus *Uromycladium tepperianum* on the invasive tree *Acacia saligna* in South Africa. Biol. Control 10(2):75-82.

Morris, M.J., A.R. Wood, and A. den Breeyen. 1999. Plant pathogens and biological control of weeds in South Africa: A review of projects and progress during the last decade. Pp. 129-137 in Biological Control of Weeds in South Africa (1990–1998), T. Olckers, and M.P. Hill, eds. African Entomology Memoir No.1. Hatfield, South Africa: Entomological Society of South Africa.

Munro, D. 1978. *Dendryphion penicillatum* (Corda) Fr. on opium poppy. Aust. Plant Pathol. Soc. Newsl. 7(1):8.

Musa, M.O., A. Al Eisa, M. Halim, E. Sahovic, M. Gyger, N. Chaudhri, F. Al Mohareb, P. Seth, M. Aslam, and M. Aljurf. 2000. The spectrum of *Fusarium* infection in immunocompromised patients with haematological malignancies and in non-immunocompromised patients: A single institution experience over 10 years. Br. J. Haematol. 108(3):544-548.

Naiker, S., and B. Odhav. 2004. Mycotic keratitis: profile of *Fusarium* species and their mycotoxins. Mycoses 47(1-2):50-56.

Nakamura, Y., I. Matsuzaki, and J. Itakura. 1992. Effect of grazing by *Sinella curviseta* (Collembola) on *Fusarium oxysporum* f.sp. *cucumerinum* causing cucumber disease. Pedobiologia 36:168-171.

Ncayiyana, D.J. 1986. Neural tube defects among rural blacks in a Transkei district. A preliminary report and analysis. S. Afr. Med. J. 69(10):618-620.

Neafsey, D.E., B.M. Barker, T.J. Sharpton, J.E. Stajich, D.J. Park, E. Whiston, C.Y. Hung, C. McMahan, J. White, S. Sykes, D. Heiman, S. Young, Q. Zeng, A. Abouelleil, L. Aftuck, D. Bessette, A. Brown, M. FitzGerald, A. Lui, J.P. Macdonald, M. Priest, M.J. Orbach, J.N. Galgiani, T.N. Kirkland, G.T. Cole, B.W. Birren, M.R. Henn, J.W. Taylor, and S.D. Rounsley. 2010. Population genomic sequencing of *Coccidioides* fungi reveals recent hybridization and transposon control. Genome Res. 20(7):938-946.

Nelson, A.J., K.S. Elias, G.E. Arévalo, L.C. Darlington, and B.A. Bailey. 1997. Genetic characterization by RAPD analysis of isolates of *Fusarium oxysporum* f. sp.

erythroxyli associated with an emerging epidemic in Peru. Phytopathology 87(12):1220-1225.

Nelson, P.E., T.A. Toussoun, and J.K. Cook. 1981. *Fusarium*: Diseases, Biology and Taxonomy. University Park, PA: Pennsylvania State University Press.

Nelson, P.E., M.C. Dignani, and E.J. Anaissie. 1994. Taxonomy, biology, and clinical aspects of *Fusarium* species. Clin. Microbiol. Rev. 7(4):479-504.

Novak, M., C.A. Salemink, and I. Khan. 1984. Biological activity of the alkaloids of *Erythroxylum coca* and *Erythroxylum novogranatense.* J. Ethnopharmacol. 10(3):261-274.

Nowak, B., and C. Eusebi. 2010. Methods for Delivering Mycoherbicides and Increasing System Effectiveness. Presentation at the Third Meeting on the Feasibility of Mycoherbicides in Eradicating Illicit Drug Crops, September 15, 2010, Washington, DC.

NRC (National Research Council). 1994. Science and Judgment in Risk Assessment. Washington, DC: National Academy Press.

Nucci, M., and E. Anaissie. 2002. Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: Implications for diagnosis and management. Clin. Infect. Dis. 35(8):909-920.

Nucci, M., and E. Anaissie. 2007. *Fusarium* infections in immunocompromised patients. Clin. Microbiol. Rev. 20(4):695-704.

Nucci, M., F. Queiroz-Telles, A.M. Tobón, A. Restrepo, and A.L. Colombo. 2010. Epidemiology of opportunistic fungal infections in Latin America. Clin. Infect. Dis. 51(5):561-570.

O'Brien, J.M., G.B. Kyser, D.M. Woods, and J.M. DiTomaso. 2010. Effects of the rust *Puccinia jaceae* var. *solstitialis* on *Centaurea solstitialis* (yellow starthistle) growth and competition. Biol. Control 52(2):174-181.

O'Donnell, K., H.C. Kistler, E. Cigelnik, E., and R.C. Ploetz. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA 95(5):2044-2049.

O'Donnell, K., D.A. Sutton, M.G. Rinaldi, K.C. Magnon, P.A. Cox, S.G. Revankar, S. Sanche, D.M. Geiser, J.H. Juba, J.A. van Burik, A. Padhye, E.J. Anaissie, A. Francesconi, T.J. Walsh, and J.S. Robinson. 2004. Genetic diversity of human pathogenic members of the *Fusarium oxysporum* complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analyses: Evidence for the recent dispersion of a geographically widespread clonal lineage and nosocomial origin. J. Clin. Microbiol. 42(11):5109-5120.

Oehrens, E. 1977. Biological control of the blackberry through the introduction of rust, *Phragmidium violaceum*, in Chile. FAO Plant Protect. B. 25(1):26-28.

O'Gorman, C.M., H.T. Fuller, and P.S. Dyer. 2009. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. Nature 457(7228):471-474.

Okabe, K. 1993. Population growth and dispersal behavior of *Histiogaster* sp. (*Acari, Acaridae*) on several economically important fungi. Appl. Entomol. Zool. 28(1):11-18.

O'Neill, N.R., J. Jennings, B.A. Bailey, and D.F. Farr. 2000. *Dendryphion penicillatum* and *Pleospora papaveraceae*, destructive seedborne pathogens and potential mycoherbicides for *Papaver somniferum*. Phytopathology 90(7):691-698.

OTA (Office of Technology Assessment). 1990. Beneath the Bottom Line: Agricultural Approaches to Reduce Agrichemical Contamination of Groundwater. U.S. Congress Office of Technology Assessment OTA-F-418. Washington, DC: U.S. Gov-

ernment Printing Office. November 1990 [online]. Available: http://www.fas.org/ota/reports/9006.pdf [accessed Mar. 28, 2011].

OTA (Office of Technology Assessment). 1993. Alternative Coca Reduction Strategies in the Andean Region. U.S. Congress Office of Technology Assessment OTA-F-556. Washington, DC: Government Printing Office. July 1993 [online]. Available: http://govinfo.library.unt.edu/ota/Ota_1/DATA/1993/9307.PDF [accessed March 7, 2011].

Ota, K. 2004. Current status of organ transplants in Asian countries. Transplant Proc. 36(9):2535-2538.

Palmero, D., C. Iglesias, M. de Cara, T. Lomas, M. Santos, and J.C. Tello. 2009. Species of *Fusarium* isolated from river and sea water of southeastern Spain and pathogenicity on four plant species. Plant Dis. 93(4):377-385.

Paris, M., F. Boucher, and L. Cosson. 1975. The constituents of *Cannabis sativa* polen. Econ. Bot. 29(3):245-253.

Pearson, D.E., and R.M. Calloway. 2005. Indirect nontarget effects of host-specific biological control agents: Implications for biological control. Biol. Control 35(3):288-298.

Peng, G., K.N. Byer, and K.L. Bailey. 2004. *Pyricularia setariae*: A potential bioherbicide agent for control of green foxtail (*Setaria viridis*). Weed Sci. 52(1):105-114.

Pfaller, M.A., S.A. Messer, R.J. Hollis, and R.N. Jones. 2002. Antifungal activities of posaconazole, ravuconazole, and and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: Report from SENTRY antimicrobial surveillance program, 2000. Antimicrob. Agents Chemother. 46(4):1032-1037.

Phatak, S.C., D.R. Sumner, H.D. Wells, D.K. Bell, and N.C. Glaze. 1983. Biological control of yellow nutsedge with the indigenous rust fungus *Puccinia canaliculata.* Science 219(4591):1446-1447.

Pignatti, S. 1982. Papaver L. Pp. 354-355 in Flora d'Italia, Vol. 1. Bologna, Italy: Edagricole.

Plowman, T. 1979. Botanical perspectives on coca. J. Psychedelic Drugs 11(1-2):103-117.

Plowman, T. 1980. Botanical perspectives on coca. Pp. 90-105 in Cocaine 1980: Proceedings of the Interamerican Seminar on Coca and Cocaine, F.R. Jeri, ed. Lima, Peru: Pacific Press.

Plowman, T., and N. Hensold. 2004. Names, types, and distribution of neotropical species of *Erythroxylum* (*Erythroxylaceae*). Brittonia 56(1):1–53.

Plowman, T., and L. Rivier. 1983. Cocaine and cinnamoylcocaine content of *Erythroxylum* species. Ann. Bot. 51(5):641-659.

Podlipaev, S.A., S. Ya. Reznik, M. Yu Dolgovskaya, M.G. Volkovitsh, and D. Sands. 1996. *Fusarium* strains isolated from *Papaver* spp. in the former Soviet Union. P. 11 in Abstracts of First International *Fusarium* Biocontrol Workshop, October 28-31, 1996, College Park, MD.

Puhalla, J.E. 1981. Genetic considerations of the genus *Fusarium*. Pp. 291-305 in *Fusarium*: Diseases, Biology, and Taxonomy, P.E. Nelson, T.A. Toussoun, and R.J. Cook, eds. University Park, PA: The Pennsylvania State University Press.

Rekah, Y., D. Shtienberg, and J. Katan. 1999. Spatial distribution and temporal development of *Fusarium* crown and root rot of tomato and pathogen dissemination in field soil. Phytopathology 89(9):831-839.

Rekah, Y., D. Shtienberg, and J. Katan. 2000. Disease development following infection of tomato and basil foliage by airborne conidia of the soilborne pathogens *Fusa-*

rium oxysporum f. sp. *radicis-lycopersici* and *F. oxysporum* f.sp. *basilici.* Phytopathology 90(12):1322-1329.

Rekah, Y., D. Shtienberg, and J. Katan. 2001. Population dynamics of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in relation to the onset of *Fusarium* crown and root rot of tomato. Eur. J. Plant Pathol. 107(4):367-375.

Reznik, S.Ya., M.G. Volkovitsh, S.A. Podlipaev, M. Yu. Dolgovskaya, and D. Sands. 1996. Biocontrol of *Papaver somniferum* and *Cannabis sativa* in Russia with *Fusarium*: Field evaluation. P. 45 in Abstracts of First Internernational *Fusarium* Biocontrol Workshop, October 28-31, 1996, College Park, MD.

Rheeder, J.P., W.F.O. Marasas, and H.F. Vismer. 2002. Production of fumonisin analogs by *Fusarium* species. Appl. Environ. Microbiol. 68(5):2101-2105.

Ridings, W.H. 1986. Biological control of stranglervine in citrus: A researcher's point of view. Weed Sci. 34(Suppl.1):31-32.

Roman, C.G., H. Ahn-Redding, and R.J. Simon. 2005. Illicit Drug Policies, Trafficking, and Use the World Over. Lanham, MD: Lexington Books.

Romano, C., C. Miracco, and E.M. Difonzo. 1998. Skin and nail infections due to *Fusarium oxysporum* in Tuscany, Italy. Mycoses 41(9-10):433-437.

Romano, C., P. Caposciutti, A. Ghilardi, C. Miracco, and M. Fimiani. 2010. A case of primary localized cutaneous infection due to *Fusarium oxysporum*. Mycopathologia 170(1):39-46.

Rosskopf, E.N., C.B. Yandoc, R. Charudattan, and J.T. DeValerio. 2005. Influence of epidemiological factors on the bioherbicidal efficacy of *Phomopsis amaranthicola* on *Amaranthus hybridus.* Plant Dis. 89(12):1295-1300.

Rottman, A. 1998. *Erythroxylum*: The coca plant. Ethnobotanical Leaflets 2(Winter): Article 6 [online]. Available: http://www.ethnoleaflets.com/leaflets/coca.htm [accessed March 3, 2011].

Rowe, R.C., J.D. Farley, and D.L. Coplin. 1977. Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. Phytopathology 67(12):1513-1517.

Sander, A., U. Beyer, and R. Amberg. 1998. Systemic *Fusarium oxysporum* infection in an immunocompetent patient with an adult respiratory distress syndrome (ARDS) and extracorporal membrane oxygenation (ECMO). Mycoses 41(3-4):109-111.

Sanders, P. 2010. Poppies could yield 100M. The Examiner, P. 27, December 30, 2010 [online]. Available: http://www.examiner.com.au/news/local/news/business/poppies-could-yield-100m/2036223.aspx [accessed Mar. 28, 2011].

Sands, D.C., E.J. Ford, R.V. Miller, B.K. Sally, M.K. McCarthy, T.W. Anderson, M.B. Weaver, C.T. Morgan, A.L. Pilgeram, and L.C. Darlington. 1997. Characterization of vascular wilt of *Erythroxylum coca* caused by *Fusarium oxysporum* f. sp. *erythroxyli forma specialis* nova. Plant Dis. 81(5):501-504.

Sands, D.C., K.S. Tiourebaev, A.L. Pilgeram, and T.W. Anderson. 2002. Carrier Methodology for Aerial Dispersal and Soil Penetration of Bioactive Agents. U.S. Patent No. 6,403,530 B1, June 11, 2002.

Savard, M.E., J.D. Miller, M. Ciotola, and A.K. Watson. 1997. Secondary metabolites produced by a strain of *Fusarium oxysporum* used for *Striga* control in West Africa. Biocontrol Sci. Technol. 7(1):61-64.

Schiff, P.L., Jr. 2002. Opium and its alkaloids. Am. J. Pharm. Educ. 66(2):186-194.

Schmitt, C.G., and B. Lipscomb. 1975. Pathogens of Selected Members of the *Papaveracea*—An Annotated Bibliography. Agricultural Research Service, U.S. Department of Agriculture, Beltsville, MD.

Schwarzenbach, K.A. 2008. Monitoring Soil Fungal Community Structures and Specific Fungal Biocontrol Strains for Ecological Effect and Fate Studies Used for Risk Assessment. Ph.D. Dissertation, Swiss Federal Institute of Technology, Zurich [online]. Available: http://e-collection.ethbib.ethz.ch/eserv/eth:30773/eth-30773-02.pdf [accessed Feb. 25, 2011].

Scott, J. 1877. Manual of Opium Husbandry. Bengal Secretariat Press, Calcutta, India.

Seo, J.A., L.C. Kim, and Y.W. Lee. 1996. Isolation and characterization of two new type C fumonisins produced by *Fusarium oxysporum*. J. Nat. Prod. 59(11):1003-1005.

Seo, J.A., J.C. Kim, and Y.W. Lee. 1999. N-Acetyl derivatives of type C fumonisins produced by *Fusarium oxysporum*. J. Nat. Prod. 62(2):355-357.

Sewram, V., N. Mshicileli, G.S. Shepard, H.F. Vismer, J.P. Rheeder, Y.W. Lee, J.F. Leslie, and W.F.O. Marasas. 2005. Production of fumonisin B and C analogues by several *Fusarium* species. J. Agric. Food Chem. 53(12):4861-4866.

Seymour, C.L., and R. Veldtman. 2010. Ecological role of control agent, and not just host-specificity, determine risks of biological control. Aust. Ecol. 35(6):704-711.

Sharpton, T.J., J.E. Stajich, s.D. Rounsley, M.J. Gardner, J.R. Wortman, V.S. Jordar, R. Maiti, C.D. Kodira, D.E. Neafsey, Q. Zeng, C.Y. Hung, C. McMahan, A. Muszewska, M. Grynberg, M.A. Mandel, E.M. Kellner, B.M. Barker, J.N. Galgiani, M.J. Orbach, T.N. Kirkland, G.T. Cole, M.R. Henn, B.W. Birren, and J.W. Taylor. 2009. Comparative genomic analyses of the human fungal pathogens *Coccidioides* and their relatives. Genome Res. 19(10):1722-1731.

Sivanesan, A., and P. Holliday. 1982. *Pleospora papaveracea*. IMI Descriptions of Fungi and Bacteria, 73, Sheet 730. CABI, Egham, Surrey, UK.

Small, E., and H.D. Beckstead. 1973. Common cannabinoid phenotypes in 350 stocks of *Cannabis*. Lloydia 36(2):144-165.

Solarska, E. 2001. The effect of *Fusarium sambucinum* infection on hops. Pp. 44-47 in Proceedings of the Scientific Commission, International Hop Growers` Convention, 5-7 August 2001, Canterbury, Kent, England, E. Seigner, ed. Bayerische Landesanstalt für Bodenkultur and Pflanzenbau, Hüll [online]. Available: http://www.lfltest.bayern.de/ipz/hopfen/10585/sc01-proceedings.pdf [accessed Jan. 22, 2011].

Sommerhalder, R.J., B.A. McDonald, F. Mascher, and J.S. Zhan. 2010. Sexual recombinants make a significant contribution to epidemics caused by the wheat pathogen *Phaeosphaeria nodorum*. Phytopathology 100(9):855-862.

Stanzani, M., F. Tumietto, N. Vianelli, and M. Baccarani. 2007. Update on the treatment of disseminated fusariosis: Focus on voriconazole. Ther. Clin. Risk Manag. 3(6):1165-1173.

Stevenson, S. 1991. Peru farmers blame U.S. for coca-killing fungus. Miami Herald, June 2:19A.

Stewart-Wade, S.M., and G.J. Boland. 2004. Selected cultural and environmental parameters influence disease severity of dandelion caused by the potential bioherbicidal fungi, *Phoma herbarum* and *Phoma exigua*. Biocontrol Sci. Technol. 14(6):561-569.

Stojanović, S., M. Starović, and D. Matijević. 1999. Factors affecting conidial germination of *Colletotrichum gloeosporioides* isolated from *Polystigma rubrum* subsp. *rubrum* stromata. Acta Phytopathol. Hun. 34(1-2):63-74.

Stowell, L.J. 1991. Submerged fermentation of biological herbicides. Pp. 225-261 in Microbial Control of Weeds, D.O. TeBeest, ed. New York: Chapman and Hall.

Suarez-Estrella, F., M.C. Vargas-Garcıa, M.J. Lopez, C. Capel, and J. Moreno. 2007. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. Crop Prot. 26(1):46-53.

Summerell, B.A., J.F. Leslie, D. Backhouse, W.L. Bryden, and L.W. Burgess, eds. 2001. *Fusarium:* Paul E. Nelson Memorial Symposium. St. Paul, MN: APS Press.

Supkoff, D.M., D.B. Joley, and J.J. Marois. 1988. Effect of introduced biological control organisms on the density of *Chondrilla juncea* in California. J. Appl. Ecol. 25(3):1089-1095.

Sylvan Bio. 2009. Agricultural Products: Sarritor. Sylvan, Inc. [online]. Available: http://www.sylvaninc.com/bioBiologicalControlAgents.html [accessed Aug. 17, 2010].

Taylor, J.W., D. Jacobson, and M.C. Fisher. 1999. The evolution of asexual fungi: Reproduction, speciation and classification. Annu. Rev. Phytopathol. 37:197-246.

TeBeest, D.O. 1982. Survival of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* in rice irrigation water and soil. Plant Dis. 66(6):469-472.

TeBeest, D.O. 1985. Techniques for testing and evaluating plant pathogens for weed control. J. Agric. Entomol. 2(1):123-129.

TeBeest, D.O. 1988. Additions to the host range of *Colletotrichum gloeosporioides* f.sp. *aeschynomene*. Plant Dis. 72(1):16-18.

TeBeest, D.O. 1991. Ecology and epidemiology of fungal plant pathogens studied as biological control agents of weeds. Pp. 97-114 in Microbial Control of Weeds, D.O. TeBeest, ed. New York: Chapman and Hall.

TeBeest, D.O., and J.M. Brumley. 1978. Survival of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* within seed of *Aeschynomene virginica*. Plant Dis. Rep. 62:675-678.

TeBeest, D.O., and G.E. Templeton. 1985. Mycoherbicides: Progress in the biological control of weeds. Plant Dis. 69 (1):6-10.

TeBeest, D.O., G.E. Templeton, and R.J. Smith, Jr. 1978. Histopathology of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* on northern jointvetch. Phytopathology 68(9): 1271-1275.

Thoumi, F.E. 2005. A modest proposal to clarify the status of coca in the United Nations conventions. Crime, Law Social Ch. 42(4-5):297-307.

Timmer, L.W. 1982. Host range and host colonization, temperature effects and dispersal of *Fusarium oxysporum* f.sp. *citri*. Phytopathology 72(6):698-702.

Tiourebaev, K.S., S. Nelson, N.K. Zidack, G.T. Kaleyva, A.L. Pilgeram, T.W. Anderson, and D.C. Sands. 2000. Amino acid excretion enhances virulence of bioherbicides. Pp. 295-299 in Proceedings of the X International Symposium on Biological Control of Weeds, July 4-14, 1999, Bozeman, MT, N.R. Spencer, ed. Bozeman, MT: Montana State University [online]. Available: http://www.invasive.org/publications/xsymposium/proceed/04pg295.pdf [accessed Apr. 6, 2011].

Tiourebaev, K.S., G.V. Semenchenko, M. Dolgovskaya, M.K. McCarthy, T.W. Anderson, L.D. Carsten, A.L. Pilgeram, and D.C. Sands. 2001. Biological control of infestations of ditchweed (*Cannabis sativa*) with *Fusarium oxysporum* f. sp. *cannabis* in Kazakhstan. Biocontrol Sci. Technol. 11(4):535-540.

Turner, C.E., K.W. Hadley, P.S. Fetterman, N.J. Doorenbos, M.W. Quimby, and C. Waller. 1973. Constituents of *Cannabis sativa* L. IV: Stability of cannabinoids in stored plant material. J. Pharm. Sci. 62(10):1601-1605.

Turner, C.E., M.A. Elsohly, and E.G. Boeren. 1980. Constituents of *Cannabis sativa* L. XVII. A review of the natural constituents. J. Nat. Prod. 43(2):169-234.

UNODC (United Nations Office on Drugs and Crime). 2000. World Drug Report 2000. New York: United Nations Publications [online]. Available: http://www.unodc.org/pdf/world_drug_report_2000/report_2001-01-22_1.pdf [accessed Mar. 28, 2011].

UNODC (United Nations Office on Drugs and Crime). 2002. Proceedings of Regional Technical Expert Review Meeting: Research and Development of an Environmentally Safe and Reliable Biological Control Agent for Opium Poppy, 25-27 November 2002, Tashkent, Uzbekistan, M.P. Greaves, ed. UNODC Regional Office for Central Asia.

UNODC (United Nations Office on Drugs and Crime). 2006. World Drug Report 2006. New York: U.N. Publications [online]. Available: http://www.unodc.org/unodc/en/data-and-analysis/WDR-2006.html [accessed Mar. 28, 2011].

UNODC (United Nations Office on Drugs and Crime). 2008. Coca Cultivation in the Andean Region: A Survey of Bolivia, Columbia and Peru. June 2008 [online]. Available: http://www.unodc.org/documents/crop-monitoring/Andean_report_2008.pdf [accessed Mar. 23, 2011].

UNODC (United Nations Office on Drugs and Crime). 2009. World Drug Report 2009. Vienna, Austria: United Nations Office on Drugs and Crime [online]. Available: http://www.unodc.org/unodc/en/data-and-analysis/WDR-2009.html [accessed July 27, 2010].

UNODC (United Nations Office on Drugs and Crime). 2010a. World Drug Report 2010. Vienna, Austria: United Nations Office on Drugs and Crime [online]. Available: http://www.unodc.org/unodc/en/data-and-analysis/WDR-2010.html [accessed July 27, 2010].

UNODC (United Nations Office on Drugs and Crime). 2010b. South-East Asia Opium Survey 2010. United Nations Office on Drugs and Crime [online]. Available: http://www.unodc.org/documents/crop-monitoring/sea/SEA_report_2010_withcover_small.pdf [accessed March 3, 2011].

UNODC (United Nations Office on Drugs and Crime). 2010c. Afghanistan *Cannabis* Survey 2009. United Nations Office on Drugs and Crime [online]. Available: http://www.unodc.org/documents/crop-monitoring/Afghanistan/Afghanistan_Cannabis_Survey_2009.pdf [accessed March 3, 2011].

UNODC (United Nations Office on Drugs and Crime). 2010d. Afghanistan Opium Survey 2010: Summary Findings. United Nations Office on Drugs and Crime. September 2010 [online]. Available: http://www.unodc.org/unodc/en/drugs/afghan-opium-survey-2010.html [accessed Nov. 29, 2010].

USDA (U.S. Department of Agriculture). 2000. Industrial Hemp in the United States: Status and Market Potential, Agricultural Economic Report No. AGES-ERSAGES001 [online]. Available: http://www.ers.usda.gov/Publications/AGES001e/ [accessed March 7, 2011].

U.S. State Department. 2010. International Narcotics Strategy Report, Vol. 1. Drug and Chemical Control. Bureau for International Narcotics and Law Enforcement Affairs, U.S. Department of State. March 2010 [online]. Available: http://www.wilsoncenter.org/news/docs/INCSR%20Mexico%20chapter%20March%202010.pdf [accessed March 3, 2011].

Vakalounakis, D.J. 1996. Root and stem rot of cucumber caused by *Fusarium oxysporum* f.sp. *radicis-cucumerinum* f. sp. nov. Plant Dis. 80(3):313-316.

Valent. 2010. Agriculture Products. Valent BioSciences Corporation [online]. Available: http://www.valent.com/agriculture/products/ [accessed July 23, 2010].

van Lenteren, J.C., D. Babendreier, F. Bigler, G. Burgio, H.M.T. Hokkanen, S. Kuske, A.J.M. Loomans, I. Menzler-Hokkanen, P.C.J. van Rijn, M.B. Thomas, M.G. Tommasini, and Q.Q. Zeng. 2003. Environmental risk assessment of exotic natural enemies used in inundative biological control. BioControl 48(1):3-38.

Veselovskaya, M.A. 1976. The Poppy, Its Classification and Importance as an Oliferous Crop. New Dehli, India: Amerind Publishing.

Waites, M.J., N.L. Morgan, J.S. Rockey, and G. Higton. 2001. Industrial Microbiology: An Introduction. Oxford, UK: Blackwell Science. 293 pp.

Waskiewicz, A., P. Golinski, Z. Karolewski, L. Irzykowska, J. Bocianowski, M. Kostecki, and Z. Weber. 2010. Formation of fumonisins and other secondary metabolites by *Fusarium oxysporum* and F. *proliferatum*: A comparative study. Food Addit. Contam. Part A. Chem. Anal. Control Expo. Risk Assess. 27(5):608-615.

Watson, A.K. 1991. The classical approach with plant pathogens. Pp. 3-23 in Microbial Control of Weeds, D.O. TeBeest, ed. New York: Chapman and Hall.

Watson, A.K., and L.A. Wymore. 1990. Identifying limiting factors in the biocontrol of weeds. Pp. 305-316 in New Directions in Biological Control: Alternatives for Suppressing Agricultural Pests and Diseases, R.R. Baker, and P.E. Dunn, eds. New York: A.R. Liss.

Watson, A.K., J. Gressel, D. Sands, S. Hallett, M. Vurro, and F. Beed. 2007. *Fusarium oxysporum* f.sp. *striga*, athletes foot or Achilles heel? Pp. 213-222 in Novel Biotechnologies for Biocontrol Agent Enhancement and Management, M. Vurro, and J. Gressel, eds. Dordrecht, the Netherlands: Springer.

Weaver, M.A., M.E. Lyn, C.D. Boyette, and R.E. Hoagland. 2007. Bioherbicides for weed control. Pp. 93-110 in Non-Chemical Weed Management: Principles, Concepts and Technology, M.K. Upadhyaya, and R.E. Blackshaw, eds. Cambridge, MA: CABI [online]. Available: http://www.ars.usda.gov/SP2UserFiles/Place/64022000/Publications/Weaver/Weaveretal07WMChap793-110.pdf [accessed Apr. 6, 2011].

Weidemann, G.J., and D.O. TeBeest. 1990. Biology of host range testing for biocontrol of weeds. Weed Technol. 4(3):465-470.

Weidemann, G.J., D.O. TeBeest, and R.D. Cartwright. 1988. Host specificity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* and *C. truncatum* in the leguminosae. Phytopathology 78(7):986-990.

Weston, V.C.M. 1999. The commercial realization of biological herbicides. Pp. 281-289 in Proceedings of the Brighton Crop Protection Conference-Weeds, Vol. 1. Farnham, U.K.: British Crop Protection Council.

Willis, A.J., and J. Memmott. 2005. The potential for indirect effects between a weed, one of its biocontrol agents and native herbivores: A food web approach. Biol. Control 35(3):299-306.

Wilson, C.L. 1965. Consideration of use of persimmon wilt as silvercide for weed persimmon. Plant Dis. Rep. 49:780-791.

Wilson, C.L. 1969. Use of plant pathogens in weed control. Annu. Rev. Phytopathol. 7(1):411-434.

Wood, A.R., and M.J. Morris. 2007. Impact of the gall-forming rust fungus *Uromycladium tepperianum* on the invasive tree *Acacia saligna* in South Africa: 15 years of monitoring. Biol. Control 41(1):68-77.

Woodhead, S.H. 1981. Field efficacy of *Phytophthora palmivora* for control of milkweed vine [abstract]. Phytopathology 71(8):913.

Woudt, L.P., A. Neuvel, A. Sikkema, M.Q.J.M. van Grinsven, W.A.J. de Milliano, C.L. Campbell, and J.F. Leslie. 1995. Genetic variation in *Fusarium oxysporum* from cyclamen. Phytopathology 85(11):1348-1355.

Wright, M.G., M.P. Hoffmann, T.P. Kuhar, J. Gardner, and S.A. Pitcher. 2005. Evaluating risks for biological control introductions: A probabilistic risk-assessment approach. Biol. Control 35(3):338-347.

Wymore, L.A., C. Poirier, A.K. Watson, and A.R. Gotlieb. 1988. *Colletotrichum coccodes*, a potential bioherbicide for control of velvetleaf (*Abutilon theophrasti*). Plant Dis. 72(6):534-538.

Yandoc Ables, C.B., E.N. Rosskopf, and R. Charudattan. 2007. Plant pathogens at work: Progress and possibilities for weed biocontrol classical versus bioherbicidal approach. Plant Health Progress [online]. Available: http://www.plantmanagement network.org/pub/php/review/2007/bioherbicide/ [accessed Apr. 5, 2011].

Yang, X.B., and D.O. TeBeest. 1992a. Rain dispersal of *Colletotrichum gloeosporioides* in simulated rice field conditions. Phytopathology 82(10):1219-1222.

Yang, X.B., and D.O. TeBeest. 1992b. Green treefrogs as vectors of *Colletotrichum gloeosporioides*. Plant Dis. 76(12):1266-1269.

Yang, X.B., and D.O. TeBeest. 1992c. Dynamic pathogen distribution and logistic increase of plant disease. Phytopathology 82(4):380-383.

Yang, X.B., and D.O. TeBeest. 1995. Competitiveness of mutant and wild-type isolates of *Colletotrichum gloeosporioides* f.sp. *aeschynomene* on northern jointvetch. Phytopathology. 85(6):705-710.

Yang, X.B., D.O. TeBeest, and R.J. Smith, Jr. 1994. Distribution and grasshopper transmission of northern jointvetch anthracnose in rice. Plant Dis.78(2):130-133.

Zhang, W.M., M. Sulz, K.L. Bailey, and D.E. Cole. 2002. Effect of epidemiological factors on the impact of the fungus *Plectosporium tabacinum* on false cleavers (*Galium spurium*). Biocontrol Sci. Technol. 12(2):183-194.

Zhan, J., C.C. Mundt, and B.A. McDonald. 2007. Sexual reproduction facilitates the adaptation of parasites to antagonisitic host environments: Evidence from empirical study in the wheat-*Mycosphaerella graminicola* system. Int. J. Parasitol. 37(8-9):861-870.

Appendix A

Biographic Information on the Committee on Mycoherbicides for Eradicating Illicit Drug Crops

Raghavan Charudattan (*Chair*) is professor emeritus in the Department of Plant Pathology of the University of Florida with over 35 years of service. His major field of interest is the biological control of weeds with plant pathogens. Dr. Charudattan has produced substantial scholarly work, including five books, 25 book chapters, and over 90 peer-reviewed journal articles. He served on the National Research Council Committee on Pest and Pathogen Control through Management of Biological Control Agents and Enhanced Cycles and Natural Processes. His more recent recognition includes being named Outstanding Weed Scientist by the Florida Weed Science Society in 2006, receiving the Career Achievement Award from the Florida Phytopathological Society in 2007, and receiving the Outstanding Research Award from the Weed Science Society of America in 2009. Dr. Charudattan was elected a fellow of the Weed Science Society of America in 2000 and a fellow of the American Phytopathological Society in 2005. He is a founder of the journal *Biological Control, Theory and Application in Pest Management*, was the journal editor from 1991 to 2006, and continues to serve as a member of its Editorial Board. He is a holder or coholder of several U.S. patents, including the patent for *Phomopsis* species fungus as a broad-spectrum bioherbicide to control several species of pigweeds and the patent for the use of tobacco mild green mosaic virus-mediated lethal hypersensitive response as a novel method of weed control. Dr. Charudattan received his PhD from the University of Madras, India.

Joan W. Bennett is a professor in the Department of Plant Biology and Pathology of Rutgers University, where she is also associate vice president for promoting women in science, engineering, and mathematics. Her research interests include fungal genetics and mycotoxins, fungal biodegradation and biotechnology, fungal genomics, bioethics, and history of microbiology. In collaboration with

scientists at the Southern Regional Research Laboratory of the U.S. Department of Agriculture, Dr. Bennett has pioneered research on the genetics and biosynthesis of aflatoxins. Her laboratory has been involved in genome projects related to *Aspergillus flavus*, *A. fumigatus,* and *A. oryzae*. She has held a number of leadership roles in the scientific community, including being chair of the biology division of the American Association for the Advancement of Science, president of the Society for Industrial Microbiology, and president of the American Society for Microbiology. She was elected to the National Academy of Sciences in 2005. Dr. Bennett earned her PhD in botany from the University of Chicago.

Jerome J. Cura is a senior environmental scientist at the Woods Hole Group and was a founding member of Cura Environmental and Menzie-Cura & Associates. His research interests are in ecological risk assessment. He has conducted such assessments in terrestrial environments, freshwater systems, and marine and estuarine habitats. He has developed guidance for conducting risk assessments at dredging sites and has experience in conducting assessments at Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and Resource Conservation and Recovery Act (RCRA) sites. Dr. Cura is chair of the Ecological Risk Assessment Specialty Group of the Society for Risk Analysis. He received his PhD in biological oceanography from the University of Maine.

William E. Fry is professor in the Department of Plant Pathology and Plant-Microbe Biology of Cornell University. He also serves as dean of the Cornell University faculty. His research interests are in plant-disease control and epidemiology, population genetics, host-pathogen interactions, and disease resistance. Of specific interest are the basic biology and management of potato late blight and its pathogen, *Phytophthora infestans,* and the use of genomic approaches and computer simulations to characterize the pathogenicity of populations of *P. infestans*. His laboratory has characterized the role of biopesticides and "green" fungicides in the management of late blight, demonstrated the potential role of petunia in the epidemiology of late blight, and predicted the epidemiologic impact of exotic strains of *P. infestans* in the United States. Dr. Fry received his PhD in plant pathology from Cornell University.

Guy R. Knudsen is professor of microbial ecology and plant pathology at the University of Idaho. His research interests are in using antagonistic bacteria and fungi in the biological control of plant pathogens; soil and rhizosphere microbial associations with indigenous plant species; microbiology of aquatic, riparian, wetland, and soil habitats; fate of genetically engineered microorganisms in the environment; and prediction and management of fungal epizootics of insect pests. Dr. Knudsen was a member of the National Research Council committee that organized the Workshop on Research to Improve the Evaluation of the Impacts of Genetically Engineered Organisms on Terrestrial and Aquatic Wildlife and Habitats. He received his PhD in plant pathology from Cornell University and his JD from William Howard Taft University.

John F. Leslie is professor and head of the Department of Plant Pathology of Kansas State University. He has been teaching researchers from around the world how to identify *Fusarium* species, the diseases that they cause, and the toxic compounds that they can produce. His broader research interests include biochemical, molecular, and population genetics of model and plant pathogenic fungi. He was a senior Fulbright scholar at the University of Sydney, Australia. He is a fellow of the American Phytopathological Society and an honorary fellow at St. Paul's College, University of Sydney, Australia. He is an adjunct professor at Seoul National University in Korea and a research associate at the Royal Botanical Gardens in Sydney, Australia. Dr. Leslie earned his PhD in genetics from the University of Wisconsin-Madison.

Nu-May Ruby Reed retired in 2010 after over 20 years as a staff toxicologist with the California Environmental Protection Agency (Cal/EPA) Department of Pesticide Regulation. She was the lead scientist on risk-assessment issues, evaluating health risks and developing risk-assessment guidelines for pesticides. She has been on several Cal/EPA working groups that initiate research and revise risk-assessment guidelines and policies, and she represented her department in task forces on community concerns and emergency response, risk-management guidance, and public education. Dr. Reed was a member of the National Research Council Committee on Risk Analysis and Reviews and is a current member of the Committee on Acute Exposure Guideline Levels. She received her MS in botany and her PhD in plant physiology from the University of California, Davis.

Judith C. Rhodes is professor in the Department of Pathology and Laboratory Medicine of the University of Cincinnati College of Medicine. Her research interests are in understanding the pathogenesis of fungal infections, with a focus on infection caused by *Aspergillus fumigatus*. Recent efforts have been directed toward establishing in vitro tissue-culture assays to assist in isolating genes that are potentially involved in virulence. Dr. Rhodes is a fellow of the American Academy of Microbiology, a past president of the Medical Mycological Society of the Americas, and a former chair of the medical-mycology division (Division F) of the American Society for Microbiology. She received her PhD in microbiology and immunology from the University of California, Los Angeles.

John W. Taylor is a professor in the Department of Plant and Microbial Biology of the University of California, Berkeley. He studies the pattern and process of fungal evolution with a long-term goal of making fungi the best models for evolutionary biology. His more recent research emphasis involves using genetics and genomics to find genes that maintain species and facilitate adaptation. He is a president of the International Mycological Association, a former president of the Mycological Society of America, and a fellow of that society, of the American Academy of Microbiology, and of the American Association for the Advancement of Science. Among Dr. Taylor's other honors are the award for Distinguished Mycologist from the Mycological Society of America, the Rhoda

Benham Medal from the Medical Mycological Society of the Americas, and the Lucille K. Georg Medal from the International Society for Human and Animal Mycology. He received his PhD in mycology from the University of California, Davis.

David O. TeBeest is a professor in the Department of Plant Pathology of the University of Arkansas. His research interests are in the molecular ecology, epidemiology, and genetics of fungal plant pathogens of grain, sorghum, and rice, especially *Colletotrichum* species, rice blast, and smuts of rice. He also continues to study the biological control of weeds to provide continued refinement of Collego, the world's first bioherbicide developed for use in row-crop agriculture. Recent work has focused on the dynamics of interstrain competition and host-selection factors in strain competition of effective strains. Dr. TeBeest was the recipient of the U.S. Department of Agriculture Award for Superior Service for Pioneering Research and the University of Arkansas Gamma Sigma Delta Award of Merit and the John W. White Award for Research. He received his PhD in plant pathology from the University of Wisconsin.

Ariena H.C. van Bruggen is a plant-disease epidemiologist and professor of plant pathology at the Emerging Pathogens Institute and the Institute of Food and Agricultural Sciences of the University of Florida. She is involved in fundamental and applied research on the health of ecosystems in relation to invasion by plant and human pathogens. Recent work has focused on pathways and mechanisms through which plant pathogens and microorganisms move through successive ecosystems. Other work has examined the ability of some organisms in agroecosystems to disperse over time and across space. Her work contributes to the understanding of how some diseases progress, and she is credited with discovering a new plant pathogen, *Rhizomonas suberifaciens*. Dr. van Bruggen is the recipient of the Ciba-Geigy Award from the American Phytopathological Society and the Jakob Eriksson Gold Medal from the Royal Swedish Academy of Sciences. She received her PhD in plant pathology from Cornell University.

Maurizio Vurro is a senior researcher at the Institute of Sciences and Food Production of the National Research Council in Italy. His studies have focused on the production, purification, identification, and biological characterization of toxins produced by plant pathogenic fungi and on their role in plant-pathogen interactions. Since 1991, Mr. Vurro has been studying the biological control of weeds, using fungal plant pathogens and their phytotoxins. His fields of specialization include the isolation and identification of new weed biocontrol agents; production, purification, and biological characterization of phytotoxins produced by weed pathogens; the role of toxins in plant-pathogen interactions; evaluation of the herbicidal potential of phytotoxins; the integration of biological and chemical control strategies; and biological control of parasitic weeds. He coordinated the European research project on enhancement and exploitation of soil biocontrol agents for bio-constraint management in crops. Mr. Vurro has

served as the coordinator of the Working Group on Parasitic Weeds of the European Weed Research Society since 2006. He is the senior editor of *Phytopathologia Mediterranea* and serves on the Editorial Board of *Food Security*. Mr. Vurro earned his BSc from the University of Bari Faculty of Agricultural Science.

Alan K. Watson is professor in the Department of Plant Science of McGill University. His research is broadly based in integrated pest management and sustainable agricultural systems while focusing on weed management. His major research interests are in the use of indigenous plant pathogens as bioherbicides, assessment of exotic plant pathogens as biological control agents of introduced weeds, population dynamics of weed species with emphasis on herbaceous perennials, and development of integrated weed-management systems in temperate and tropical regions. His research program seeks to understand the processes involved in host-pathogen interactions, to investigate mechanisms involved in disease development and weed host response, and to use this knowledge to select, develop, and implement effective, safe, and sustainable means to reduce the adverse effects of major noxious weeds. His studies span research biocontrol methods for less-developed nations and subsistence farmers. His research team successfully developed effective biocontrol strategies for several weeds in rice in Asia and a *Fusarium oxysporum*-based bioherbicide against *Striga hermonthica*, a root parasitic weed that is considered the scourge of African cereal crops. Dr. Watson earned his PhD in weed science from the University of Saskatchewan.

Charles P. Woloshuk is a professor in the Department of Botany and Plant Pathology of Purdue University. His research interests include corn-mycotoxin pathology and the genetics, biochemistry, and physiology of mycotoxin biosynthesis. His long-range research program goals are to understand the biosynthesis of various mycotoxins and to develop novel approaches to eliminating the risk of mycotoxin contamination of food sources. He uses mycotoxin-producing fungi as model systems to advance the understanding of secondary metabolism in fungi. Dr. Woloshuk is also involved in the evaluation of mycotoxins associated with grain harvest, storage, and management practices with an objective of educatinge grain producers, handlers, and processors about mycotoxin effects. He also leads workshops on the use of mycotoxin test kits. He received his PhD in plant pathology from Washington State University.

Appendix B

EPA Requirements and Guidelines under 40 CFR 158, Subpart V

TABLE B-1 Microbial Pesticides Product Analysis Data Requirements

Guideline Number	Data Requirement	All Use Patterns	Test Substance		Test Notes[a]
			MP	EP	
Product Chemistry and Composition					
885.1100	Product Identity	R	MP	EP	—
885.1200	Manufacturing process	R	TGAI and MP	TGAI and EP	—
	Deposition of a sample in a nationally recognized culture collection	R	TGAI	TGAI	—
885.1300	Discussion of formation of unintentional ingredients	R	TGAI and MP	TGAI and EP	—
Analysis and Certified Limits					
885.1400	Analysis of samples	R	TGAI and MP	TGAI and EP	1
885.1500	Certification of limits	R	MP	EP	—
Physical and Chemical Characteristics					
830.6302	Color	R	TGAI	TGAI	—
830.6303	Physical state	R	TGAI	TGAI	—
830.6304	Odor	R	TGAI	TGAI	—
830.6313	Stability to normal and elevated temperatures, metals and metal ions	R	TGAI	TGAI	—
830.6317	Storage stability	R	TGAI and MP	TGAI and EP	—
830.6319	Miscibility	R	MP	EP	2
830.6320	Corrosion Characteristics	R	MP	EP	3
830.7000	pH	R	TGAI	TGAI	—

(Continued)

TABLE B-1 Continued

Guideline Number	Data Requirement	All Use Patterns	Test Substance		Test Notes[a]
			MP	EP	
Physical and Chemical Characteristics					
830.7100	Viscosity	R	MP	EP	4
830.7300	Density/relative density/bulk density (specific gravity)	R	TGAI	TGAI	—

Abbreviations: EP, end-use product; MP, manufacturing-use product; R, required; TGAI, technical grade of the active ingredient.

[a]Test notes. The following test notes are applicable to the data requirements for microbial pesticides product analysis as referenced in the last column of the table contained in paragraph (c) of this section.

1. Required to support registration of each manufacturing-use product and end-use product. This analysis must be conducted at the point in the production process after which there would be no potential for microbial contamination or microbial regrowth. For full registration, generally an analysis of samples is a compilation of batches, over a period of time, depending on the frequency of manufacturing.
2. Only required for emulsifiable liquid forms of microbial pesticides.
3. Required when microbial pesticides are packaged in metal, plastic, or paper containers.
4. Only required for liquid forms of microbial pesticides.

Source: CFR 40 Part 158 Subpart V.

TABLE B-2 Microbial Pesticides Residue Data Requirements

Guideline Number	Data Requirement	All Use Patterns	Test Substance Data to Support MP or EP	Test Notes[a]
885.2100	Chemical Identity	CR	EP	1
885.2200	Nature of the Residue in plants	CR	EP	1
885.2250	Nature of the Residue in animals	CR	EP	1
885.2300	Analytical methods – plants	CR	TGAI	1
885.2350	Analytical methods – animals	CR	TGAI	1
885.2400	Storage Stability	CR	EP	1
885.2500	Magnitude of residue in plants	CR	EP	1
885.2550	Magnitude of residues in meat, milk, poultry, eggs	CR	EP	1
885.2600	Magnitude of residues in potable water, fish, and irrigated crops	CR	EP	1

Abbreviations: CR, conditionally required; EP, end-use product; TGAI, technical grade of the active ingredient.

[a]Test notes. The following test note is applicable to the data requirements for microbial pesticides residue as referenced in the last column of the table contained in paragraph (c) of this section.

1. Required when the results of testing:
   i. Indicate the potential to cause adverse human health effects or the product characterization indicates the microbial pesticide has a significant potential to produce a mammalian toxin; and
   ii. The use pattern is such that residues may be present in or on food or feed crops.

Source: CFR 40 Part 158 Subpart V.

TABLE B-3 Microbial Pesticides Toxicology Data Requirements

Guideline Number	Data Requirement	All Use Patterns	Test Substance	Test Notes[a]
Tier I				
885.3050	Acute oral toxicity/pathogenicity	R	TGAI	1
885.3150	Acute pulmonary toxicity/pathogenicity	R	TGAI	—
885.3200	Acute injection toxicity/pathogenicity/(intravenous)	R	TGAI	2
	Acute injection toxicity/pathogenicity/(intraperitoneal)			
885.3400	Hypersensitivity incidents	R	All	3
885.3500	Cell culture	R	TGAI	4
870.1100	Acute oral toxicity	R	MP , EP	1, 5
870.1200	Acute dermal toxicity	R	MP , EP	5
870.1300	Acute inhalation toxicity	R	MP , EP	5, 6
870.2400	Acute eye irritation	R	MP , EP	5
870.2500	Primary dermal irritation	R	MP , EP	5
Tier II				
885.3550	Acute toxicology	CR	TGAI	7
885.3600	Subchronic toxicity/pathogenicity	CR	TGAI	8
Tier III				
885.3650	Reproductive fertility effects	CR	TGAI	9, 13
870.4200	Carcinogenicity	CR	TGAI	10, 13
870.7800	Immunotoxicity	CR	TGAI	11, 13
885.3000	Infectivity/pathogenicity analysis	CR	TGAI	12, 13

Abbreviations: CR, conditionally required; EP, end-use product; MP, manufacturing-use product; R, required; TGAI, technical grade of the active ingredient

[a]Test notes. The following test notes are applicable to the data requirements for microbial pesticides toxicology as referenced in the last column of the table contained in paragraph (c) of this section:

1. The acute oral toxicity/pathogenicity study is required to support the TGAI. However, it can be combined with the unit dose portion of the acute oral toxicity study, with an EP or MP test material to fulfill the requirement for the TGAI and the MP or EP in a single study, if the new protocol is designed to address the endpoints of concern.
2. Data not required for products whose active ingredient is a virus. For test materials whose size or consistency may prevent use of an intravenous injection, the intraperitoneal injection procedure may be employed.
3. Hypersensitivity incidents, including immediate type and delayed-type reactions of humans or domestic animals, occur during the testing or production of the TGAI, MP, or EP, or are otherwise known to the applicant must be reported if they occur.
4. Data must be submitted only for products whose active ingredient is a virus.
5. The 870 series studies for the MP and EP are intended to provide data on the acute toxicity of the product. Waivers for any or all of these studies may be granted when the applicant can demonstrate that the combination of inert ingredients is not likely to pose any significant human health risks. Where appropriate, the limit dose approach to testing is recommended.

6. Required when the product consists of, or under conditions of use would result in, an inhalable material (e.g., gas, volatile substances, or aerosol particulate).
7. Data required when significant toxicity, in the absence of pathogenicity and significant infectivity, is observed in acute oral, injection, or pulmonary studies (Tier I). Route(s) of exposure correspond to route(s) where toxicity was observed in Tier I studies. The toxic component of the TGAI is to be tested.
8. Data required when significant infectivity and/or unusual persistence is observed in the absence of pathogenicity or toxicity in Tier I studies. Routes of exposure (oral and/or pulmonary) correspond to routes in Tier I studies where adverse effects were noted. Data may also be required to evaluate adverse effects due to microbial contaminants or to toxic byproducts.
9. Data are required when one or more of the following criteria are met:
   i. Significant infectivity of the microbial pest control agent (MPCA) was observed in test animals in the Tier II subchronic study and in which no significant signs of toxicity or pathogenicity were observed.
   ii. The microbial pesticide is a virus which can persist or replicate in mammalian cell culture lines.
   iii. The microbial pesticide is not amenable to thorough taxonomic classification, and is related to organisms known to be parasitic for mammalian cells.
   iv. The microbial pesticide preparation is not well purified, and may contain contaminants which are parasitic for mammals.
10. Data may be required for products known to contain or suspected to contain carcinogenic viruses or for microbial components that are identified as having significant toxicity in Tier II testing.
11. Data may be required for products known to contain or suspected to contain viruses that can interact in an adverse manner with components of the mammalian immune system.
12. An analysis of human infectivity/pathogenicity potential using scientific literature, genomic analysis, and/or actual specific cell culture/animal data may be required for products known to contain or suspected of containing intracellular parasites of mammalian cells for products that exhibit pathogenic characteristics in Tier I and/or Tier II, for products which are closely related to known human pathogens based on the product analysis data, or for known human pathogens that have been "disarmed" or rendered nonpathogenic for humans.
13. Test standards may have to be modified depending on the characteristics of the microorganism. Requirements may vary for these studies depending on the active ingredient being tested. Consultation with the Agency is advised before performing these Tier III studies.
Source: CFR 40 Part 158 Subpart V.

TABLE B-4 Microbial Pesticides Nontarget Organisms and Environmental Fate Data Requirements

		Use Patterns									
		Aquatic		Terrestrial							
						Residential	Greenhouse	Indoor			
Guideline Number	Data Requirement	Food/Feed	Nonfood	Food/ Feed/ Nonfood	Forestry	Outdoor	Food/ Nonfood	Food/ Nonfood	Industrial	Test Substance	Test Notes[a]
Tier I											
885.4050	Avian oral toxicity	R	R	R	R	R	CR	CR	CR	TGAI	1, 2
885.4100	Avian inhalation toxicity/pathogenicity	CR	CR	CR	CR	CR	CR	CR	CR	TGAI	1, 2, 3
885.4150	Wild mammal toxicity/pathogenicity	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	1, 4
885.4200	Freshwater fish toxicity/ pathogenicity	R	R	R	R	CR	CR	CR	CR	TGAI or TEP	1, 2, 5
885.4240	Freshwater invertebrate toxicity/pathogenicity	R	R	R	R	CR	CR	CR	CR	TGAI or TEP	1, 2, 5
885.4280	Estuarine/Marine fish testing Estuarine and marine invertebrate testing	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	1, 6
885.4300	Nontarget plant testing	CR	CR	CR	R	CR	NR	CR	CR	TEP	1, 7
885.4340	Nontarget insect testing	R	R	R	R	R	CR	NR	CR	TGAI	1, 8
885.4380	Honey bee testing	R	R	R	R	R	CR	NR	CR	TGAI	1

Tier II											
885.5200	Terrestrial environmental expression tests	CR	CR	CR	CR	CR	NR	NR	CR	TGAI or TEP	9
885.5300	Freshwater environmental expression tests	CR	CR	CR	CR	CR	NR	NR	CR	TGAI or TEP	10
885.5400	Marine or estuarine environmental expression tests	CR	CR	CR	CR	CR	NR	NR	CR	TGAI or TEP	11, 12
Tier III											
885.4600	Avian chronic pathogenicity and reproduction test	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	12, 13
885.4650	Aquatic invertebrate range testing	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	12, 14
885.4700	Fish life cycle studies	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	12, 14
885.4750	Aquatic ecosystem test	CR	CR	CR	CR	CR	NR	NR	CR	TGAI	15
Tier IV											
850.2500 850.1950	Field testing for terrestrial wildlife and Field testing for aquatic organisms	CR	CR	CR	CR	CR	NR	NR	CR	TGAI or TEP	11, 16
850.2500	Simulated or actual field tests (birds, mammals)	CR	CR	CR	CR	CR	NR	NR	CR	TEP	16, 17, 20

(Continued)

TABLE B-4 Continued

Guideline Number	Data Requirement	Use Patterns: Aquatic: Food/Feed	Aquatic: Nonfood	Terrestrial: Food/Feed/Nonfood	Terrestrial: Forestry	Residential: Outdoor	Greenhouse: Food/Nonfood	Indoor: Food/Nonfood	Industrial	Test Substance	Test Notes[a]
850.1950	Simulated or actual field test (aquatic organisms)	CR	CR	CR	CR	CR	NR	NR	CR	TEP	16, 18, 19, 20
850.2500	Simulated or actual field tests (insect predators, parasites)	CR	CR	CR	CR	CR	NR	NR	CR	TEP	16, 18, 19, 20
850.3040	Simulated or actual field tests (insect pollinators)	CR	CR	CR	CR	CR	NR	NR	CR	TEP	16, 18, 19, 20
850.4300	Simulated or actual field tests (plants)	CR	CR	CR	CR	CR	NR	NR	CR	TEP	16, 18, 19, 20

Abbreviations: CR, conditionally required; NR, not required; R, required; TEP, typical end-use product; TGAI, technical grade of the active ingredient.

[a]Test notes. The following test notes are applicable to the data requirements for microbial pesticides nontarget organism and environmental fate as referenced in the last column of the table contained in paragraph (d) of this section.

1. Tests for pesticides intended solely for indoor application would be required on a case-by-case basis, depending on use pattern, production volume, and other pertinent factors.

2. The preferred species for the avian oral study is either the upland game or waterfowl. The preferred species for the avian inhalation toxicity/pathogenicity study and the avian chronic toxicity/pathogenicity study is the upland game. There is also the option to test the passerine if there is a concern. The coldwater fish is preferred for freshwater fish testing. However, two species (coldwater and warmwater fish species are the preferred species) must be tested for uses involving direct freshwater exposure. Freshwater invertebrate testing is also required.

3. Data required when the nature of the microbial pesticide and/or its toxins indicates potential pathogenicity to birds.

4. Required on a case-by-case basis if results of tests required by §158.2140 are inadequate or inappropriate for assessment of hazards to wild mammals.

5. Required when there will be significant exposure to aquatic organisms (fish and invertebrates).
6. Required if the product is intended for direct application into the estuarine or marine environment or expected to enter this environment in significant concentrations because of expected use or mobility pattern.
7. Required if the microbial pesticide is taxonomically related to a known plant pathogen.
8. Data are not required unless an active microbial ingredient controls the target insect pest by a mechanism of infectivity; i.e., may create an epizootic condition in nontarget insects.
9. Required if toxic or pathogenic effects are observed in one or more of the following tests for microbial pesticides:
    i. Avian acute oral or avian inhalation studies.
    ii. Wild mammal studies.
    iii. Nontarget plant studies (terrestrial).
    iv. Honey bee studies.
    v. Nontarget insect studies.
10. Required when toxic or pathogenic effects are observed in any of the following Tier I tests for microbial pest control agents:
    i. Freshwater fish studies.
    ii. Freshwater invertebrate studies.
    iii. Nontarget plant studies (aquatic).
11. Required if product is applied on land or in fresh water or marine/estuarine environments and toxic or pathogenic effects are observed in any of the following Tier I tests for microbial pesticides:
    i. Estuarine and marine animal toxicity and pathogenicity.
    ii. Plant studies - estuarine or marine species.
12. An appropriate dose-response toxicity test is required when toxic effects on nontarget terrestrial wildlife or aquatic organisms (including plants) are reported in one or more Tier I tests and results of Tier II tests indicate exposure of the microbial agent to the affected nontarget terrestrial wildlife or aquatic organisms. The protocols for these tests may have to be modified in accordance with results from the nontarget organism and environmental expression studies.
13. Required when one or more of the following are present:
    i. Pathogenic effects are observed in Tier I avian studies.
    ii. Tier II environmental expression testing indicate that long-term exposure of terrestrial animals is likely.
14. Required when product is intended for use in water or expected to be transported to water from the intended use site, and when pathogenicity or infectivity was observed in Tier I aquatic studies.

15. Required if, after an analysis of the microbial pesticide's ability to survive and multiply in the environment and what ecological habitat it would occupy, the intended use patterns, and the results of previous nontarget organisms and environmental expression tests, it is determined that use of the microbial agent may result in adverse effects on the nontarget organisms in aquatic environments. Testing is to determine if applications of the microbial pest control would be expected to disrupt the balance of populations in the target ecosystem.
16. Tier IV studies may be conducted as a condition of registration as post-registration monitoring if the potential for unreasonable adverse effects appears to be minimal during that period of use due to implementation of mitigation measures.
17. Required when both of the following conditions occur:
    i. Pathogenic effects observed at actual or expected field residue exposure levels are reported in Tier III; and
    ii. The Agency determines that quarantine methods would not prevent the microbial pesticide from contaminating areas adjacent to the test area.
18. Short term simulated or actual field studies are required when it is determined that the product is likely to cause adverse short-term or acute effects, based on consideration of available laboratory data, use patterns, and exposure rates.
19. Data from a long-term simulated field test (e.g., where reproduction and growth of confined populations are observed) and/or an actual field test (e.g., where reproduction and growth of natural populations are observed) are required if laboratory data indicate that adverse long-term, cumulative, or life-cycle effects may result from intended use.
20. Since test standards would be developed on a case-by-case basis, consultation with the Agency and development of a protocol is advised before performing these Tier IV studies.
Source: CFR 40 Part 158 Subpart V.

TABLE B-5 Series 885—Microbial Pesticide Test Guidelines as of January 2010

OPPTS Guideline Number	Guideline Names	Other Reference Numbers			EPA Pub Number	Date Issued
		OPPT	OPP	OECD		
Final 885 Test Guidelines						
885.0001	Overview for Microbial Pest Control Agents	none	150A	none	712-C-96-280	Feb-96
Group A—Product Analysis Test Guidelines						
885.1100	Product Identity	none	151A-10	none	712-C-96–273	Feb-96
885.1200	Manufacturing Process	none	151A-11	none	712-C-96–293	Feb-96
885.1300	Discussion of Formation of Unintentional Ingredients	none	151A-01	none	712-C-96–294	Feb-96
885.1400	Analysis of Samples	none	151A-13	none	712-C-96-295	Feb-96
885.1500	Certification of Limits	none	151A-15	none	712-C-96-296	Feb-96
Group B—Residues Test Guidelines						
885.2000	Background for Residue Analysis of Microbial Pest Control Agents	none	153A-1	none	712-C-96-299	Feb-96
885.2100	Chemical Identity	none	153A-4	none	712-C-96-300	Feb-96
885.2200	Nature of the Residue in Plants	none	153A-6	none	712-C-96–302	Feb-96
885.2250	Nature of the Residue in Animals	none	153A-7	none	712-C-96–303	Feb-96
885.2300	Analytical Methods—Plants	none	153A-8a	none	712-C-96-304	Feb-96
885.2350	Analytical Methods—Animals	none	153A-8b	none	712-C-96-305[a]	Feb-96
885.2400	Storage Stability	none	153A-9	none	712-C-96-306	Feb-96
885.2500	Magnitude of Residues in Plants	none	153A-10	none	712-C-96-307	Feb-96
885.2550	Magnitude of Residues in Meat, Milk, Poultry, Eggs	none	153A-11	none	712-C-96-308	Feb-96
885.2600	Magnitude of Residues in Potable Water, Fish, and Irrigated Crops	none	153A-01	none	712-C-96–309	Feb-96

(Continued)

TABLE B-5 Continued

OPPTS Guideline Number	Guideline Names	Other Reference Numbers			EPA Pub Number	Date Issued
		OPPT	OPP	OECD		
Group C—Toxicology Test Guidelines						
885.3000	Background–Mammalian Toxicity/Pathogenicity/Infectivity	none	152A-1	none	712-C-96–314	Feb-96
885.3050	Acute Oral Toxicity/Pathogenicity	none	152A-10	none	712-C-96–315	Feb-96
885.3100	Acute Dermal Toxicity/Pathology	none	152A-11	none	712-C-96-316	Feb-96
885.3150	Acute Pulmonary Toxicity/Pathogenicity	none	152A-12	none	712-C-96-317[b]	Feb-96
885.3200	Acute Injection Toxicity/Pathogenicity	none	152A-13	none	712-C-96-318	Feb-96
885.3400	Hypersensitivity Incidents	none	152A-15	none	712-C-96-320	Feb-96
885.3500	Cell Culture	none	152A-16	none	712-C-96-321	Feb-96
885.3550	Acute Toxicology, Tier II	none	152A-20	none	712-C-96-322	Feb-96
885.3600	Subchronic Toxicity/Pathogenicity	none	152A-21	none	712-C-96-323[c]	Feb-96
885.3650	Reproductive/Fertility Effects	none	152A-30	none	712-C-96-324	Feb-96
Group D—Nontarget Organism and Environmental Expression Test Guidelines						
885.4000	Background for Nontarget Organism Testing of Microbial Pest Control Agents	none	154A-1, 154A-2, 154A-3, 154A-4, 154A-5	none	712-C-96-328	Feb-96
885.4050	Avian Oral, Tier I	none	154A-16	none	712-C-96-329	Feb-96
885.4100	Avian Inhalation Test, Tier I	none	154A-17	none	712-C-96-330	Feb-96
885.4150	Wild Mammal Testing, Tier I	none	154A-18	none	712-C-96-331	Feb-96
885.4200	Freshwater Fish Testing, Tier I	none	154A-19	none	712-C-96-332	Feb-96

885.4240	Freshwater Aquatic Invertebrate Testing, Tier I	none	154A-20	none	712-C-96-333	Feb-96
885.4280	Estuarine and Marine Animal Testing, Tier I	none	154A-21	none	712-C-96-334	Feb-96
885.4300	Nontarget Plant Studies, Tier I	none	154A-22	none	712-C-96-335	Feb-96
885.4340	Nontarget Insect Testing, Tier I	none	154A-23	none	712-C-96-336	Feb-96
885.4380	Honey Bee Testing, Tier I	none	154A-24	none	712-C-96-337	Feb-96
885.4600	Avian Chronic Pathogenicity and Reproduction Test, Tier III	none	154A-26	none	712-C-96-342	Feb-96
885.4650	Aquatic Invertebrate Range Testing, Tier III	none	154A-27	none	712-C-96-343	Feb-96
885.4700	Fish Life Cycle Studies, Tier III	none	154A-28	none	712-C-96-344	Feb-96
885.4750	Aquatic Ecosystem Test	none	154A-29	none	712-C-96-345	Feb-96
Group E—Environmental Expression Test Guidelines						
885.5000	Background for Microbial Pesticides Testing	none	155A-1,2	none	712-C-96–056	Feb-96
885.5200	Expression in a Terrestrial Environment	none	155A-10	none	712-C-96–338	Feb-96
885.5300	Expression in a Freshwater Environment	none	155A-11	none	712-C-96-339	Feb-96
885.5400	Expression in a Marine or Estuarine Environment	none	155A-12	none	712-C-96-312	Feb-96
DRAFT 885 Test Guidelines						
Please note those guidelines labeled as “Public Draft” are not yet available in final form. Although you may consult these guidelines, please check with the appropriate office before you use a draft guideline to generate data for submission to EPA under FIFRA, FFDCA or TSCA.						

Reserved: We use the phrase “[Reserved]” in the title column as a placeholder whenever the number has been assigned, but the FINAL Guideline has not yet been issued.

[a]Document incorrectly says publication Number ends in “-304”.

[b]Document incorrectly says publication Number ends in “-318”.

[c]Document incorrectly says publication Number ends in “-232”.

Source: CFR 40 Part 158 Subpart V.