Theoretically tested remediation in response to insect resistance to Bt corn and Bt cotton:

a new paradigm

By

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Various models of density dependence predicted different evolutionary outcomes for *Helicoverpa zea*, *Diabrotica virgifera*, and *Ostrinia nubilalis* using simple and complex resistance evolution models, different dose assumptions and refuge proportions. Increasing available refuge increased durabilities of pyramided Plant-Incorporated-Protectants (PIPs), especially between 1-5%. For some models of density dependence and pests, additional refuge resulted in faster adaptation rates. Significant considerations should be given to a pest's intra-specific competition in simple and complex theoretical models when designing insect resistance management plans.

Life-history, refuge, and dose characteristics of a PIP had different effects on the adaptation rate of a generic pest of Bt, and unexpected outcomes occurred. Intrinsic growth rate 'R_0' was the strongest evolutionary force, and large R_0's reduced time to resistance for a high dose PIP to similar levels as projected for a low dose PIP. This was caused by differential density dependent effects in refuge and Bt fields that elevated generational resistance increases beyond those from selection alone. Interactions between density dependence and R_0 were always present and further affected the life-time of the

PIPs. Varying ‘average dispersal distance’ did not affect evolutionary outcomes; however, increasing the proportion of the population engaging in dispersal often increased the durability of high dose PIPs. When resistance genes spread from a hypothetical hotspot, local resistance phenomena developed in the immediate surroundings. Higher growth rates lead resistance to spread faster through the landscape than lower rates. Increasing available refuges slowed adaptation rates to single PIPs and low dose pyramids, although non-linear trends were possible.

Integrated Pest Management (IPM) practices at the onset of PIP commercialization slowed pest adaptation rates. For corn rootworm, interspersing non-selective periods with IPM+IRM delayed resistance evolution, yet crop rotation was the best strategy to delay resistance. For bollworm inclusion of isoline corn as an IPM tool did not increase the life-time of the PIP. A local resistance phenomenon for rootworm was maintained immediately surrounding the hotspot; random selection of mitigatory strategies in the landscape slowed adaptation rates while mitigation in the hotspot alone did not. Mitigation extended the life-time of the pyramid minimally for both corn rootworm and bollworm.

DEDICATION

This dissertation is dedicated to my children, Rori, the free spirit, and Marcus, the pragmatist, who are a continuous source of inspiration.

"What you do makes a difference, and you have to decide what kind of difference you want to make." J. Goodall

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LIST OF ABREVIATIONS

AD	*Adult dispersal proportion*
BCa	*Bias corrected and accelerated boostrap*
Bt	*Bacillus thuringiensis*
CC	*Contest competition*
CRW	*Corn rootworm*
D	*Diffusivity or redistribution constant*
DD	*Density dependence*
DNA	*Deoxyribonucleic acid*
ECB	*European corn borer*
EPA	*Environmental Protection Agency*
FIFRA	*Federal Insecticide, Fungicide, and Rodenticide Act*
h	*Degree of dominance of resistance gene*
Ha	*Hectare*
HD	*High dose*
IPM	*Integrated Pest Management*
IRAF	*Initial resistance allele frequency*
IRM	*Insect Resistance Management*
K	*Carrying capacity*
LD	*Low dose*

Log	*Logistic equation*
OPP	*Office of Pesticide Programs*
PIP	*Plant-Incorporated Protectant*
R_0	*Intrinsic growth rate*
RIB	*Refuge-in-the-Bag*
RR	*Homozygous resistant genotype at locus 1*
RS	*Heterozygous resistant genotypes at locus 1*
s	*Mortality of susceptible genotype*
SAI	*Soil Applied Insecticides*
SAP	*Scientific Advisory Panel*
SC	*Scramble competition*
SS	*Susceptible genotype*
US	*United States*
UXD	*Unexpected damage*
W_{SS}	*Fitness of susceptible genotype*
W_{RS}	*Fitness of heterozygous resistant genotype*
W_{RR}	*Fitness of homozygous resistant genotype*
XX	*Susceptible genotype at locus 2*
XY	*Heterozygous resistant genotypes at locus 2*
YY	*Homozygous resistant genotype at locus 2*

CHAPTER I

LITERATURE REVIEW AND SCIENTIFIC ISSUES

1.1 Introduction

Over the last 50 years, scientists have explored the evolution of resistance in agricultural pests to insecticides in general (Georghiou and Taylor 1977, Comins 1977a, 1977b, Tabashnik and Croft 1982, and others). Over the past 20 years in particular, simple population genetics models (Mallet & Porter 1992; Tabashnik 1994b; Alstad & Andow 1995; Onstad & Gould 1998; and others) gradually evolved into more complex simulation models (Caprio 1998; Caprio & Suckling 2000; Storer et al. 2003; and others) with the purpose of identifying Insect Resistance Management (IRM) strategies that aided in extending the life-time of *Bacillus thuringiensis* (Bt) corn and cotton Plant-Incorporated Protectants (PIPs). The latest simulation models have gotten more sophisticated and include any or all of following components: population biology, ecology, and behavior of pest, multiple toxins, cross-resistance, agricultural practices, landscape crop diversity, grower behaviors, explicit space, stochasticity, and probability analyses (Storer 2003; Caprio et al. 2009; Caprio and Glaser 2010; Ives et al. 2011; Pan et al. 2011).

In response to the Scientific Advisory Panel's (SAP) recommendation (1998), the U.S. Environmental Protection Agency's (US EPA) Office of Pesticide Programs (OPP) began to require that industry submit resistance risk analyses based on simulation

modeling with their PIP application requests. The purpose of this change in requirements was for biotechnology companies to demonstrate that EPA's mandated IRM program would extend the durability of the PIPs beyond the time it would take for resistance to develop in absence of a refuge or, as of late, that a new proposed IRM strategy (*i.e.* Refuge-in-the-Bag, RIB, aka seed blend) was superior to the previously established block refuge paradigm.

While a lot of effort went into developing IRM models to test new strategies aimed at delaying resistance, limited theoretical research has been conducted to explore what remedial action strategies could effectively mitigate field resistance to agricultural pesticides or Bt toxins (Gressel et al. 1996; Pittendrigh et al. 2004). The current remedial action plans "on the books" with EPA have not undergone rigorous theoretical testing and scientific analyses by biotechnology registrants or the Agency to assess their degree of success. In fact, it is questionable whether the proposed, generic strategies shared in remedial action plans of different major target pests would be productive mitigatory tools until the Agency and the registrants have agreed on more specific remedial action plans. Recently, scientists at the US EPA (EPA 2013) proposed to the FIFRA SAP (2014) that pest-specific instead of generic remedial action plans should be in place addressing different resistance scenarios (hotspot vs. widespread resistance) so that theoretically tested actions could be initiated to slow the adaptation rate in the landscape at the first signs of resistance. The SAP concurred with EPA on this point.

1.2 Regulatory background and insect resistance management requirements

All registration applications for chemical, biological, and antimicrobial pesticides submitted to the Office of Pesticide Programs are required to undergo a thorough risk

assessment analysis under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to determine that such products would not pose an "unreasonable adverse effect" (or risk) to the environment and/or human health. In addition, the Agency may conduct a separate assessment to decide whether benefits to society outweigh the risks of registering a pesticide. The US EPA (2001) conducted such a benefits assessment for Bt PIPs targeting pests of corn (field and sweet corn), potato, and cotton. It was determined that for BT corn products, the major benefits were increased yields and a reduction in insect damage. For Bt cotton, Bt sweet corn, and Bt potatoes, the major benefits represented a reduction in the use of chemical insecticides yielding private benefits for the farmer and environmental and health benefits for society. The Agency's thorough analysis provided strong support for the earlier established, yet still voluntary, IRM program for PIPs within OPP. Because of the tremendous benefits identified, EPA mandated under FIFRA that IRM became mandatory for Bt crops (despite a still lacking rulemaking process and official IRM guidelines). Registrants were now obligated to fulfill a set of requirements as part of a post-registration process called "condition of registration" to proactively preserve the benefits and Bt technology and provide supporting documentation on a yearly basis (points 5, 6, and 7 discussed below). A new PIP application to EPA requires that the following information and data are submitted: 1) a description of the target pest biology and ecology, 2) cross-resistance data for the proposed new toxin(s) and currently commercialized toxins, 3) dose-mortality data for each proposed toxin and target pest of the new PIPs, 4) a risk mitigation or IRM plan (e.g. the use of non-PIP refuges) supported by predictive simulation modeling and 5) a plan for resistance monitoring, which subsequently is conducted on an annual basis and

baseline susceptibility data for each toxin and target pest, 6) grower education for IRM requirements, 7) a compliance assurance plan to ensure grower adherence to the mitigation (refuge) strategy, and 8) a generic remedial action plan should field resistance be suspected or confirmed in a target pest.

1.2.1 Definition of resistance

Different definitions for resistance are used among various groups of scientists and EPA. When Tabashnik et al. (2008) published that resistance had evolved in *Helicoverpa zea*, the authors referred to a heritable decrease in susceptibility by the pest to Cry1Ac as measured by the LC_{50} (lethal concentration killing 50% of the population) or the resistance ratio (field-derived LC_{50} values/ LC_{50} of lab strains). Ratios that are greater than 10, implicate that a heritable decrease in susceptibility is due to resistance (Tabashnik 1994). This definition does not address or include the performance of resistant individuals in the field and is solely based on laboratory conditions and a statistically significant reduction in susceptibility of field populations compared to the laboratory reference strains (Moar et al. 2008). Tabashnik et al. (2014) also refer to resistance as 'a genetically based decrease in susceptibility to a pesticide', which goes back to a definition by the National Academy of Science (Brent 1986) but also to a definition by Food and Agriculture Organization of the United Nations (FAO 1979). The authors specifically express that the original definitions of resistance do not include whether there is an economic impact visible or not (Tabashnik et al. 2014). OPP of the US Environmental Protection Agency operates under a risk-benefit law (FIFRA) that requires the Agency to consider other non-risk factors when registering pesticides. Those can, for example, include economic impacts on the farming community and consumers

by denying the registration of a pesticide. In the case of PIPs, to declare pest resistance to a Bt toxin and initiate mitigation in absence of visible field effects could be interpreted as an undue burden on the community and contrary to the Agency's directive. Therefore, EPA uses a definition of resistance for PIPs that includes 'a heritable decrease in susceptibility (as measured by the EC_{50}/LC_{50}) leading to greater survival in offspring when exposed to Bt crops in the field'. Field efficacy, Bt crop failure, or economic impact due to resistance, are all assumed in this definition of resistance. One downfall of this definition is that it does not work well for non-high dose toxins, and some field damage should typically be expected even in absence of resistance (e.g. for *H. zea* and *D. virgifera*). One challenge for resistance monitoring and early detection of resistance to non-high dose Bt PIPs has been to differentiate between target pest populations that might have evolved resistance versus populations that have an inherent tolerance to a pesticidal trait (e.g. greater genetic diversity such as *H. zea*) or survive sublethal doses (e.g. *H. zea* and *D. virgifera*). For these types of pests, the US EPA's definition and approach of detecting resistance is unlikely to be proactive, and growers, extension entomologists, and industry will know that resistance has developed before it will be confirmed in the lab. By the time resistance to a particular PIP is confirmed in, for example, *H. zea* and *D. virgifera*, it may be too late to successfully respond with remedial actions under the current regulatory process.

H. zea and *D. virgifera* are agricultural pests with life-cycles and dispersal propensities on the opposite sides of the biological spectrum. *H. zea* has multiple generations per year (3-6) and migrates from the cotton growing regions of the United States into the northern Corn Belt in early summer; in early fall, reverse migrants (from

the Corn Belt) return to the southern regions. Some information about *H. zea* dispersal and geographic origin of populations in the United States is still unresolved to-date. *D. virgifera* has only one generation per growing season (with an obligate diapause) and typically engages in local dispersal, although some data suggest that pre-ovipositional long-distance dispersal in female corn rootworm occurs (Coats et al. 1986 or Naranjo 1990) and could be as high as 25%. In addition, the fast spread of *D. virgifera* across the United States since the 1940s has been partially ascribed to frequent transport of populations by local weather systems that predominantly move from west to east (Grant and Seevers 1986). Additionally, an analysis of allozymes as well as nuclear and mitochondrial DNA revealed low levels of variation within and between populations of *D. virgifera* and suggested either high levels of gene flow or recent geographic expansion (Krysan et al. 1989, Szalanski et al. 1999). Conversely, *H. zea* and *D. virgifera* share that they both have great variability in susceptibility to the Bt toxins currently registered and as supported by diet bioassay data (Ali et al. 2006). This suggests that both pests may be naturally pre-adapted to evolve resistance to Bt toxins, especially to non-high dose PIPs.

1.3 Documented cases of field resistance

Documented Bt field resistance in agricultural pests has now been reported in South Africa, India, China, Australia, Puerto Rico, Brazil, and the continental US. *Busseola fusca*, the African stemborer was reported to have caused severe field damage in Monsanto's Cry1Ab corn (MON810) during 2004 and 2005 (van Rensburg 2007). A closer look into grower management practices revealed that all fields had a history of irrigation and Bt use. Collections of 2000 diapausing larvae occurred in one of the failed fields, and a colony was established. An artificial infestation experiment with Cry1Ab

was conducted in the field during 2006 to measure survival on Bt. The experiment was also replicated in the greenhouse. Larvae of the control strain died after several days of exposure, while larvae of the field strain survived to fourth instar during the exposure period. The field population acquired a significantly greater body mass on Bt than the control population and was determined to be resistant. Two confirmed Bt resistance cases come from India, one for *H. armigera* and the other for *P. gossypiella* (Ranjith et al. 2010). The authors found that individuals of *H. armigera* survived and reproduced successfully on single gene Cry1Ac cotton and pyramided Cry1Ac x Cry2Ab2 (Bollgard II) cotton. Unexpected survival on Bt cotton in India is not unusual and has been previously observed. Until 2009, however, surviving individuals were unable to reproduce. The resistance allele frequency in the population was not determined. In a second and more recent case, an analysis of five spatially distinct population samples of *P. gossypiella* (collected during 2007-2009) revealed based on LC_{50} comparisons that the pest had evolved resistance to Cry1Ac cotton by 2008 (Dhurua and Guar, 2011). By monitoring for resistance, Downes et al. (2010) detected (field sampling and F2-screens) an exponential increase in the frequency of the Cry2Ab resistance allele in *H. punctigera* from 2004 to 2009 (R^2= 0.94) in Australia. The resistance allele was also detected in populations sampled from non-Bt cropping regions, but the observed allele frequency from the F_2-screen was only 12% that of the populations from Bt growing areas. In 2006, unexpected damage from fall armyworm, *Spodoptera frugiperda*, in Cry1F maize was observed in Puerto Rico, and resistance was determined to be recessive (Storer et al. 2010; Blanco et al. 2010). Main factors attributed to resistance evolution were island geography, unusually large populations in 2006, and drought conditions restricting the

pest's ability to feed on alternate hosts. Dow AgroSciences and Pioneer/DuPont, who had commercialized Cry1F maize in Puerto Rico, indefinitely stopped Cry1F maize sales on the island. This action was taken even though migrants from the island (PR) to the United States do not overwinter (exception in Florida where not much Bt field corn is grown). The Cry1F resistance is fixed in the Puerto Rico population. In 2009, Gassmann et al. (2011) collected insect samples in response to unexpected corn rootworm damage in Cry3Bb1 corn in eastern Iowa. On-plant assays revealed that offspring of the collected individuals were resistant to the toxin and that the trait was heri. Gassman et al. further determined that resistance in *D. virgifera virgifera* was incomplete but did not report on progeny survival and potential costs to resistance. Zang et al. (2011) reported that Cry1Ac resistance has been documented in 13 populations of *H. armigera* in northern China. This is a report of decreased susceptibility to one Bt toxin that is not associated with control failure. According to the U.S. regulatory definition of resistance, this report would not be understood as a resistance case but simply as a decrease in susceptibility of the target pest. However, it is worth noting that China does not have a mandated IRM program as is standard practice in Australia and the U.S., and farmers rely solely on natural host plants as a source for refuge insects. This lack of an IRM program could be one cause for more rapid decrease in susceptibility in parts of China. In 2014, the first documented case of fall armyworm resistance has been documented to Cry1F maize in Brazil (Farias et al. 2014). Cry1F corn was commercially released in 2010. The rapid evolution of resistance has been attributed to lack of refuges, year-round cultivation of corn, and the numerous generations of fall armyworms per year.

1.4 Tools for delaying resistance evolution

Resistance evolution to presently registered pesticides is an expected outcome of such human controlled selection experiments and particularly for PIPs because of their high expression levels as well as season-long expression of toxins (great selection factors). Hence, the question should not be whether resistance to Bt PIPs evolves but rather how long resistance can be delayed before the technology is rendered obsolete. In 1998, the SAP recommended the high-dose refuge strategy to help delay the evolution of pest resistance to Bt PIPs; this concept was subsequently adopted by the Agency. Such a high-dose refuge strategy relies on the idea that all susceptible and heterozygous genotypes will die from exposure to the toxin in the Bt field (dominance of resistance gene <0.05). In theory, homozygous resistant genotypes would be the only individuals emerging from Bt fields. Subsequent mating with susceptible genotypes emerging from nearby refuge fields would produce susceptible heterozygous offspring. The SAP (1998) and others (Roush 1998, Tabashnik 2008) also stated that if a high-dose could not be achieved, then the amount of refuge needed would need to be increased in order to delay resistance. EPA has made a decision to registered non-high dose Bt PIPs because the benefits of commercializing these products and reducing chemical pesticide use both outweighed the risk of resistance evolution (*i.e.* single gene products expressing Cry3Bb1, mCry3A, Cry34/35 to control corn rootworm); no requirement was established, however, to increase the non-Bt refuge proportion to greater than the standard 20% largely to maintain consistency between corn refuge approaches (FIFRA requires that EPA consider economic and other impacts on affected communities and stakeholders). Another tactic for delaying resistance evolution is to pyramid two or more Bt toxins that

do not share cross-resistance and independently cause a high degree of mortality to heterozygote (70%) and susceptible genotypes (90-100%) (Roush 1998; Zhao et al. 2003). This strategy results in 'redundant killing' of RS and SS individuals and kills homozygous individuals resistant to one of the toxins.

The SAP (2014) recommended that using IPM upfront for less-than high dose Bt PIPs would extend the durability of the technology, which otherwise could be compromised in just a short time. No modeling research has been conducted yet to theoretically test this recommendation. The IPM tools listed by the SAP to delay, for example, corn rootworm resistance were to not use Bt corn for more than two consecutive years on any field, implement crop rotation with a non-host, use of pyramids, and introducing non-selective periods by planting non-Bt corn with soil applied insecticides (SAI). All these tools should be considered for implementation by individual growers.

Pittendrigh et al. (2004) proposed that negative cross-resistance toxins (a mutant allele conferring resistance to one toxin and hyper-susceptibility to another toxin) of moderate toxicity and deployed in the refuge of the primary PIPs could delay resistance evolution. Even if the resistance gene was fairly common in the population (1%), the negative cross-resistance PIP contributed to a continued decrease in resistance allele frequency over time in their theoretical explorations. The degree of pest dispersal and toxicity of negative cross-resistance, however, affected how quickly the resistance allele frequency decreased. It is worth noting though that no such Bt toxin (or conventional pesticide) has been identified yet to have negative cross-resistance with each other. While these results are intriguing and important, at this point they remain a purely academic

exercise and are not helpful to the US EPA if the Agency has to consider remedial actions for a Bt resistance problem in the field.

1.5 Density dependence and resistance evolution

Scramble and contest competition are two intra-specific interactions that have diametrically opposing mechanisms of population regulation. Nicholson (1954) coined these terms and described the scramble competition as a condition where all individuals in the population have access to a resource and survival is 100% until the resource is exhausted. In contest competition, the winner 'takes all', and the remaining individuals die. Over-compensation and under-compensation describe the "transitional space" between these two extreme forms of density dependence.

Comins underlined the importance of including density dependent interactions in insect resistance management (IRM) models when pest dispersal was considered (1977a and 1977b). He demonstrated how the influx of susceptible migrants from non-pesticide treated areas could slow resistance evolution if the resistance gene was sufficiently recessive - this is analogous to the concept of the "high-dose+refuge" paradigm. Early insecticide applications favored the delay of resistance evolution, and a greater dose had variable effects depending on the type of density dependence modeled. An increased population suppression with perfect density dependence (contest competition) reduced the time to resistance but combined with under-compensation (slightly less stringent conditions than contest competition), the pattern was reversed and time to resistance increased (Comins 1977a).

Not until the 1980s was Comins' recommendation considered in theoretical modeling and density dependence included to assess resistance evolution to pesticides

(Tabashnik and Croft, 1982; Caprio and Tabashnik 1992; Alstad and Andow 1995; Onstad et al. 2001; and Ives and Andow 2002). Despite Comins' abundant theoretical work supporting the importance of including intra-specific competition for a shared resource in IRM models, there are still verbal disagreements among scientists today over the importance of density dependence in regard to resistance evolution with pesticide applications.

1.6 Unexplored factors potentially affecting durability of PIPs

Pests of economic significance typically exhibit high intrinsic net growth rates. No specific threshold value has been proposed in the literature for when a species becomes a pest, but Conway (1979) discussed that economic pests could be characterized as *r*-selected species with great fecundity (e.g. black cutworm, fecundity = 1500 eggs per female) and short generation times (1-2 weeks to 1-2 months). He speculated that the majority of pests in temperate regions were likely r-selected species. Hence, the effect of increased growth rates on the life-time of pesticides should be evaluated. A preliminary analysis has previously been conducted (Caprio and Martinez 2012) and revealed that a high dose PIP lost 30% of its durability if the pest had a high growth rate. A thorough analysis is needed to evaluate effects of growth rates on single and dual gene PIPs with high and low dose expressions. Another missing link is the exploration of other life-history characteristics (such as for example dispersal and density dependence) and their effects on the effectiveness of current IRM strategies for Bt crops.

Different IRM strategies, such as RIBs and block refuges, have shown to predict variable durabilities for the same type of Bt PIP (Tabashnik 1994; Caprio and Glaser 2010; Pan et al. 2011; and others). In some instances blocks have predicted to increase

durability of PIPs; in other cases, RIB strategies were hailed as the best resistance management tool to delay resistance evolution, especially in the face of grower non-compliance with refuge requirements. No integrated theoretical analysis has been published yet to explore joint effects and interactions between growth rates, density dependence, dispersal distance and proportion, dose of toxin, and IRM strategies on the estimated life-time of a PIP product (single and dual gene). The results of such an analysis would need to be validated by empirical research in the field.

1.7 Remediation of resistance

General remedial action plans for PIPs have been submitted by Bt technology providers to the EPA and are all conceptually similar. These are in place for the purpose of responding quickly once pest resistance in Bt corn and cotton has been confirmed. The plans consist of the following mitigation activities: instruct customers to use alternate control measures such as crop residue incorporation after harvest and conventional pesticide use. Stop sales may be initiated EPA if resistance is confirmed. In the case of confirmed resistance, EPA and the technology providers develop a specific and long-term mitigation plan for the affected area(s).

To this date, industry has not provided and the EPA has not requested a scientific rationale or a science based analysis for the current general remedial action plans on-file with the Agency to demonstrate that their proposed mitigatory steps had the potential to slow adaptation rates or geographically containing resistance. It is proposed here that remediation plans should undergo scientific scrutiny (much like IRM plans) and be subjected to simulation modeling to assess whether intended objectives and goals are feasible and achievable. In addition, the approaches to confirm resistance (diet bioassays

in general) and definitions of resistance outlined in EPA's regulatory documents are not proactive enough for low dose toxins, so that field resistance would likely be widespread before resistance could ever (if at all) be confirmed (EPA 2013). It is doubtful that remediation would ever be initiated under the current regulatory program for these types of Bt PIPs. Meanwhile, on-plant assays have been developed for corn rootworm (Nowatzki et al. 2008, Gassmann et al. 2011), which provide greater sensitivity than diet bioassays and allow confirming resistance within a year. The EPA is currently in the process of making changes to the resistance monitoring program of corn rootworm to incorporate a more proactive approach (EPA 2013) including on-plant assays and changes to the annual resistance monitoring (and more). In the U.S., no remedial action plan has been triggered yet, though it has been known since 2011 that corn rootworm have evolved resistance to Cry3Bb1 and that cross-resistance is exhibited with mCry3A (Gassmann et al. 2014).

For extension entomologists and biotechnology providers, the challenge will be to detect pest resistance early in cases of localized phenomena. If this can be accomplished, then the implementation of a remedial action plan in areas of documented resistance may have a greater probability of success. This brings up the need to clarify what is meant by success. I suspect it is unlikely that even quick remediation would contain resistance in a hotspot if the pest disperses by flight and/or wind currents. Some resistant individuals are likely to always escape, even with the most efficacious chemicals since insects typically emerge over a period of time and insecticide applications must follow application intervals prescribed on the EPA approved label. Rapid mitigation response in reaction to a visibly detected resistance phenomenon can result in population suppression in a

hotspot, however, but by itself will not reduce the resistance allele frequency. A faster rather than delayed mitigatory response should minimize the subsequent escape of resistant migrants and the effect of the resistance spread in the surrounding regions. Hence, population suppression with various IPM approaches can be expected to slow the adaptation process in the landscape, but this needs to be tested and the best available tools evaluated. The timing of detection and successful population suppression in hotspots will depend to a great degree on efficient communication between scouting experts, extension entomologists, industry, and EPA but also on the lifecycle and mobility of the pest.

1.8 Research questions

This research focused on three pests of concern of which two have very distinct dispersal propensities as larvae as well as adults (*H. zea* and *D. virgifera*) and for which there are currently no high dose single toxins commercially available. The third agricultural pest, *Ostrinia nubilalis*, has a dispersal behavior that is intermediate to the other two pests, and contrary to *H. zea* and *D. virgifera,* high-dose single toxins are available for control. The first aspect of my research explored whether the same density dependent assumptions for the above mentioned pests of Bt (*H. zea*, *D. virgifera*, and *O. nubilalis*) could elicit non-uniform effects on time to resistance in theoretical models (spatial and non-spatial) when the proportions of refuge was varied. I will discuss which form of density dependence is most appropriate for each of the three pests. Second, using a hypothetical, generic, diploid arthropod pest of Bt with sexual reproduction, I explored effects and interactions between life-history characteristics, IRM strategies, and dose of toxin on time to resistance to a single Bt PIP with a spatially explicit model. In addition, I investigated the spread of the resistance gene from a hotspot one and three generations

after resistance was first visually detected to simulate a delay in response time based on the current EPA process of confirming resistance before remedial action is trigger. For the generic pest of Bt, I examined for the various life-history parameters, refuge configurations, and dose of PIP whether increasing refuge to 50% could slow the spread of resistance across the landscape, as was previously suggested (Tabashnik & Gould 2012, Andow et al. 2014). Since this work was conducted with a single PIP, I included separate analyses for a dual gene PIP when interesting patterns became apparent in the single PIP analyses. Lastly, this research provides a scientific approach to testing remedial action strategies for resistance in corn rootworm and bollworm and Integrated Pest Management (IPM) strategies that prolong the life-time of either compromised or newly commercialized Bt PIPs. First, I explored what specific IPM actions could prolong the life-time of a newly commercialized, low dose pyramid for a corn rootworm and bollworm scenario and what proportion of IPM participation was needed to significantly extend the durability of the Bt pyramid compared to "no action" (IRM only). Second, I investigated what individual remedial actions would be most effective to reduce the adaptation rate in both pests, how much more durability could be gained from the second gene in the pyramid with one gene compromised (50% resistance) under different mitigatory response times and mitigation participation percentages. I further explored how the resistance allele at one locus in the pyramided PIP spread through the landscape assuming conservative pest dispersal propensities. Ultimately, this research will make recommendations to the US EPA how the current resistance management and remedial action plans could be improved. The last part of this theoretical work will be in direct

contrast to current approach (no theoretical testing of proposed remedial actions) and represents a paradigm shift.

1.9 References

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CHAPTER II

EFFECTS OF VARIOUS MATHEMATICAL MODELS OF DENSITY-DEPENDENCE ON EVOLUTION OF RESISTANCE TO PLANT-INCORPORATED PROTECTANTS WITHIN THE CONTEXT OF STOCHASTIC AND SPATIALLY IMPLICIT AND SPATIALLY EXPLICIT MODELS OF POPULATION GENETICS

2.1 Abstract

Comins (1977a, 1977b) demonstrated the importance of including density dependent interactions in theoretical models when predicting evolutionary outcomes for insect resistance to conventional pesticides. In the past couple of decades, population regulation has also been more frequently included in IRM models for Bt Plant-Incorporated Protectants (PIPs). However, no analysis has been conducted yet to determine whether evolutionary outcomes are affected by different assumptions of density dependence and various refuge proportions in simple theoretical models. Here I showed that various mathematical models of density dependence could result in significantly different evolutionary outcomes with simple and complex IRM models for three pests of Bt corn, namely, *Helicoverpa zea*, *Diabrotica virgifera*, and *Ostrinia nubilalis*, when dose assumptions and the proportion of non-Bt refuge were varied. Typically, increasing refuge proportion increased the durability of both low dose and high dose PIPs especially between 1-5% refuge proportions. For some models of density dependence and for some pests, however, increasing refuge proportion resulted in lower

durability estimates. In these cases, the "high dose + refuge" paradigm failed to function as expected.

I report that spatial models incorporating various refuge proportions, dispersal, and models of density dependence generally support results obtained with the simple models. But some deviations were observed. For example, spatial models tended to estimate greater durability than non-spatial models, while non-spatial models tended to predict greater durability gains between refuge increases tested here. I found that increasing dispersal distance increased the rate of adaption for *H. zea* and *D. virgifera*. In contrast, for *O. nubilalis*, varying dispersal distance did not lead to significant differences in durability for the pyramid. I recommend that significant consideration be given to a pest's intra-specific competition in both simple and complex theoretical models when the aim is to design insect resistance management plans to extend the durability of Bt technologies.

2.2 Introduction

The idea of population regulation by density-dependent mechanisms was publicly formulated by Nicholson (1933) and spurred debates over its applicability in natural systems for many decades. In 1974, May introduced the importance of various mathematical assumptions of density dependence in theoretical models that resulted in drastically different population dynamics for organisms with discrete, non-overlapping generations (May 1974). As a result, a wave of ecological research followed to quantify density dependent relationships in natural populations. Arguments over the relevance of density dependence as a population regulating mechanism continued well into the 1990s at which point ecologists seemed to converge on a general agreement: intra-specific

density dependence was an intrinsic part of population regulation, and a population equilibrium could best be described as "a stationary probability distribution of population density" (Turchin 1994) or a "stochastic equilibrium probability distribution" (May 1973).

Although Comins underlined the importance of including density dependence in insect resistance management (IRM) models when pest dispersal was considered (1977a), when evaluating cost to pesticide resistance and effects on evolution (1977b and 1979), and when using multiple toxin tactics to delay resistance evolution to conventional pesticides (1986), many IRM scientists still did not include density dependent interactions in their resistance models (but see Tabashnik and Croft 1982, Caprio and Tabashnik 1992). When IRM models were first developed for *Bacillus thuringiensis* (Bt) Plant-Incorporated Protectant (PIP) corn to explore options for managing the evolution of resistance, density dependence was not included (Mallet & Porter 1992; Tabashnik 1994; Roush 1998, and Onstad & Gould 1998). The prevailing assumption appeared to be that density dependent factors would be unimportant to the overall evolutionary process during low population densities, especially shortly after pesticide exposure.

Tabashnik and Croft (1982) and Caprio and Tabashnik (1992) were among the first to adopt Comins' premise that density dependence played an important role in the evolutionary process of adaptation to pesticides. Alstad and Andow (1995), Onstad et al. (2001), and Ives and Andow (2002) extended this premise to PIP IRM models. Onstad et al. (2001) derived their model for density dependence by fitting an equation to data obtained from three field studies; and Ives and Andow (2002) used the Hassell equation (1985). Though Ives et al. (2011) stated that the exact nature of mathematical algorithms

underlying density dependent effects was unimportant in simple insect resistance management models and used the expression $1/(1+x)$ to regulate population density (x being the total number of survivors in a field), Ives (*pers. com*.) expressed that, in more complex IRM models, the type of density dependence model used may affect evolutionary outcomes.

Initially, I used three simple IRM models to test the null hypothesis that there was no difference in expected durability with various models of density dependence (Ives et al. 2011). These simple models were spatially implicit in nature with no stochasticity for parameters except for fecundity and dominance of the resistance gene. Though I acknowledge that natural variability should otherwise be included in advanced modeling, I wanted to exclude any potential noise introduced by stochasticity and/or parameter variability that could complicate testing the proposed hypothesis and possibly confound results.

I expanded on the first hypothesis by using a stochastic and spatially explicit stepping stone IRM model (based on the 1-field model) to determine whether dispersal and various mathematical assumptions of density dependence including various refuge percentages predicted different evolutionary outcomes (as measured by the time to resistance) for the same pest exposed to high dose and low dose pyramided Bt PIPs (*ceteris paribus*). Comins (1977a) showed that when dispersal occurred between treated and untreated populations that the level of density dependence became relevant. The situation in my simulations differed from that of Comins as all fields experienced a level of selection. Furthermore, I assessed whether results predicted with the same model of density dependence but using the spatial and non-spatial model varied significantly.

In these simulations, I used four models of density dependence: Logistic, Ricker, scramble competition, and contest competition; the latter two were modeled using the Hassell equation (1975). For corn rootworm, I also used an empirical model of density dependence developed by Crowder and Onstad (2005). I was interested in the question whether refuges could delay resistance to both high-dose as well as low dose toxins with different models of intra-specific density dependence. Second, I discussed which form of density dependence was more realistic considering information known about each pest's biology. The currently registered PIPs targeting European corn borer express a high dose, while single and pyramided PIPs targeting corn rootworm and bollworm all express less-than-high dose. The dual gene pyramids in my model were not representative of any currently commercialized Bt PIPs. Reported results should be viewed in an overall context, and relative differences in durability and overall trends should be more informative than actual numerical results.

2.3 Materials and methods

2.3.1 General model structure

A two-locus, deterministic, spatially implicit and frequency-based model was written in Java (using NetBeans IDE 7.0.1) to explore the effects of various mathematical assumptions of density dependence on the resistance evolution of *H. zea* (bollworm), *D. virgifera* (corn rootworm), and *O. nubilalis* (European corn borer). The landscape for this spatially implicit model consisted of two compartments which together accounted for a 50 ha field. For corn rootworm and European corn borer, the landscape was occupied by continuous corn (4 million plants) consisting of 95% Bt and 5% non-Bt plants. For the first two generations of bollworm in spring, the landscape consisted of an

early natural hosts. In early summer and for generation three and four, the landscape consisted of continuous corn with 80% planted to Bt and 20% to non-Bt plants, and in late summer/early fall and for generation five and six, the landscape is divided into 80% Bt cotton and 20% wild host plants (natural refuge). At the beginning of each year of bollworm simulations, 20% of the total landscape was assigned to early natural hosts where the other 80% represented non-sui habitat. The plant density of the natural host was 1/10 that of the cultivated crops. This estimate for wild host plant density was based on observed numbers of bollworms during this time period (Parker 2000). I adjusted the plant density of the late natural hosts to include the 13% of land area covered by soybean in Mississippi (Gustafson et al. 2006) and then increased the natural host area by another 10% to account for other cultivated and uncultivated wild hosts (Stadelbacher 1972, Stadelbacher et al. 1986, Blanco et al. 2007). The refuge percentages for the cultivated corn in the southern U.S. as well as the Corn Belt was concordant with EPA's current block refuge requirements for pyramided PIPs (EPA 2010) and current assumptions of available natural refuge for Bt cotton in some areas of south. I varied refuge percentages between 5% and 50% for European corn borer and corn rootworm and 20% to 50% for bollworm, but in some cases also explored lower and higher values and effects on the durability of the PIPs. Refuge non-compliance by growers was not considered in these simulations because the main interest was to assess effects of various density-dependent assumptions on the durability of a hypothetical, pyramided PIP (low and high dose). Likewise, non-Bt expressing host plants were always available to local *H. zea* in spring (natural host system) or late summer and fall (cotton system). Here, I note that a portion of the Bt market has been converted over the past several years from PIPs requiring a

structured refuge to PIPs integrating the non-Bt seed into the seed bag (Refuge-In-The-Bag, RIB). Nonetheless, there are still single Bt PIP products sold that require the planting of a separate block refuges, and there is the standing requirement in the cotton growing region that separate block refuges need to be planted irrespective of the type of PIP product planted (RIB or block).

The simple simulation models were discrete time step models with one generation per year for *D. virgifera*, six generations per year for *H. zea*, and two generations per year for *O. nubilalis.* The life-cycles were simulated in discrete sub-models, which progressed through egg stage, larval/selection stage, density dependence, pupa stage, adult stage, mating, and oviposition. The models started with the first generation of eggs in spring for each pest. The pupa life-stage for corn rootworm was excluded in the simulations because survival was assumed to be close to 100% (Onstad 2006 and Onstad et al. 2006).

In this model, as well as others (Alstad and Andow 1995, Ives et al. 2011), density dependence occurred post Bt selection, so refuge and Bt individuals experienced dissimilar levels of density dependent mortality. Specifically, lower intensity population regulation was experienced in Bt fields where densities were reduced by toxin or pesticide mortality. If density dependence occurs before Bt selection, then individuals in both compartments would experience a similar degree of density dependence. This is essentially what Onstad simulated when density dependence was calculated based on egg densities present per hectare (Onstad et al. 2006). Under the first scenario, durability of the PIP should be reduced because the effective relative refuge size is reduced. This has been mathematically confirmed by Friedenberg & Shoemaker (2013). Based on what is currently known about the three pests under investigation, it is reasonable to apply

density dependent mortality after Bt selection. For example, Hibbard et al. (2004) demonstrated that effects of density dependence did not occur until corn rootworm larvae had matured to late-instars. The same assumption can be made for bollworm where cannibalism (a form of contest competition) becomes evident mostly after the neonate stage and grows more predominant as larvae mature into older instars (Dial and Adler 1990, Chilcutt 2006). Approximately 50% of neonate European corn borer disperse immediately after hatching (SAP 2011) and irrespective of density (Hellmich, *pers. com*., Ross and Ostlie 1990), which should reduce early effects of density dependence and allow for Bt mortality to occur first.

After density dependent mortality took place, the model proceeded through the pupa sub-model and adult life-stage before transitioning into the mating sub model. Hardy-Weinberg frequencies were calculated for the eggs produced. For corn rootworm, where a degree of assortative mating was assumed, I assigned a fraction (k = 0.4) from the Bt fields to mate at random with the refuge population. In absence of any empirical data, I concluded that this is a best first approximation based on the limited dispersal reported for corn rootworm (Nowatzki et al. 2003, Caprio & Glaser 2012). Additionally, since I was not interested in actual years of durability predicted but rather relative durabilities and trends caused by different assumptions of density dependence, I had no concerns with the actual value assigned to assortative mating for rootworm. I intend to discuss the impact of non-random mating on durability by varying this fraction in the sensitivity analysis. A future analysis could include *k* in the PERT-Beta analysis to address the uncertainty that exists with this parameter. Typically though, I would expect a decrease in durability with increasingly greater non-random mating between Bt and non-

Bt insects and in absence of sufficient immigration by susceptible insects. The remaining proportion, (1-k), mated at random in the Bt field. Then egg genotypic frequencies (weighted based on refuge and Bt adult numbers) were calculated, and eggs were uniformly distributed across the 50 ha landscape at the beginning of the next generation. After the first generation was completed, either a new year began (*i.e.,* corn rootworm) or the generation counter was increased within the same year (*i.e.,* bollworm and European corn borer).

The stochastic stepping stone model consisted of a matrix of 10 x 10 fields (1 field = 50 ha) and was essentially a replica of each species specific model with the addition of dispersal. Each field was identical to the one-field-landscape described in the simple deterministic model. The model was designed as a torus so that dispersing insects leaving the edge of the square landscape wrapped around to the opposite side and reentered a field in the landscape. Dispersal of adults was simulated before mating occurred and was based on a predetermined field dispersal distance. Of the fraction dispersing (m) one-fourth moved an assigned number of fields away from the natal field in each of the four cardinal directions. The assigned dispersal distances in the model did not represent actual distances but rather represented relative dispersal capacities of the three pests in relation to the landscape. Since the landscape matrix was ten fields wide, yet existed on a torus, the largest linear dispersal distance away from a given natal field was five fields. This dispersal distance was assigned to bollworm with a dispersal proportion of m = 50% (due to lack of empirical data, this seemed a good first approximation). European corn borer as assumed to disperse a shorter distance than bollworm and moved three fields away from the field of origin. The proportion of

population engaging in dispersal was also lower than that assumed for the bollworm: m = 30%. For corn rootworm, dispersal occurred into the adjacent four fields to simulate local dispersal (Nowatzki et al. 2003); and m = 30%. Post-mating, long distance dispersal of female corn rootworm (Coats et al. 1986; Naranjo 1992) was achieved by moving a fraction of eggs (mode 15%) into new fields some five fields removed from the natal site. A sensitivity analysis for different dispersal distances and their effect on time to resistance was also included. Likewise, it was explored whether different dispersal proportions affected time to resistance differently.

For the stepping stone models, I add stochasticity at the beginning of each simulation by initializing the population size in each field with an egg number randomly drawn from an interval between 0 and 60,000,000. A simulation was terminated when the average resistance allele frequency in all fields reached 0.5 or higher. At that point, the number of generations and average resistance allele frequency in the matrix were recorded. I assumed that resistance was governed by two major resistance genes with no cross resistance between them (though the model was set up to evaluate cases of epistasis) and two alleles (R and Y for resistance and S and X for susceptibility) and three genotypes (RR, RS, and SS; YY, YX, and XX) at each locus. This assumption was reasonable for high dose toxins but could be unrealistic for less-than-high dose toxins where multiple genes could regulate resistance (FIFRA SAP 2009 & 2014).

A hypothetical high dose scenario with recessive inheritance was modeled for both loci and all three pests, although no such pyramided PIP is currently commercialized or registered for *H. zea* and *D. virgifera*. Benchmark mortality rates for homozygous susceptible, heterozygous and homozygous resistant genotypes were 99% (w_{SS}=0.01,

s=0.99), 96.92% (w_{RS}=0.03078, *h*=0.021), and 0% (w_{RR}=1.0) for homozygous susceptible, heterozygous, and homozygous resistant genotypes, respectively. These values simply provided a starting point for the proposed analyses. I was less interested in actual numerical estimates made by the different models and more interested in relative differences between various models of density dependence. Dominance, '*h',* was varied from 0.01 to 0.05 in the PERT Beta analysis in this model and described the level of fitness for the heterozygous resistant genotype based on the fitness of the homozygous susceptible and resistant genotypes when exposed to the insecticidal PIPs, and referred to the population genetic definition given by Bourget et al. (2000). If there are no empirical values for dominance, but fitness values for all genotypes are known, then *h* can be calculated as follows:

$$h = (Wrs - Wss)/(Wrr - Wss) \tag{2.1}$$

When dominance *h* is known (or determined, as in this model) and the heterozygous fitness needs to be estimated, the following formulas can be utilized:

$$\mathrm{Wss} = 1 - \mathrm{s} \tag{2.2}$$

$$Wrs = (1 - \mathrm{s}) + h\,(Wrr - (1 - s)) \tag{2.3}$$

$$Wrr = 1 \tag{2.4}$$

A less-than high-dose scenario was also modeled for all three pests. In this case, the benchmark (mean) mortality rates were 80% (w_{SS}=0.20, s=0.8), 68% (w_{RS}=0.32, *h*=0.06), and 0% (w_{RR}=1) for homozygous susceptible, heterozygous and homozygous resistant genotypes, respectively. Dominance of the low dose toxin was varied from 0.05

to 0.15 in the PERT Beta analysis. This upper range could have been further increased to 0.20, but I settled on the lower value for a starting point.

No fitness cost of resistance was assumed for any of the pests, which is the typical and conservative assumption in IRM risk assessments. Cost of resistance would slow the adaptation process in the pest in the presence of a non-Bt crop and lead to greater pesticide durability. There is evidence from several Bt selection studies under laboratory conditions that the assumption of no-cost-of-resistance could be violated in some cases for *H. zea* (Anilkumar et al. 2008) and *D. virgifera* (Oswald et al. 2012; Meihls et al. 2012). Whether the genes selected under laboratory conditions could also be selected in the field has yet to be determined. Some evidence, however, seems to support the contrary, namely that resistance evolution in the field may act on different genes than artificial selection in the laboratory (FIFRA SAP 2009). The assumption of 'no fitness cost' in my simulations remains, for the time being, a good null hypothesis. The initial resistance allele frequencies (IRAF) were set to 0.005 for all pests.

Although the theoretical model was frequency based (keeping track of genotypic frequencies throughout the simulations), it was possible during the density dependent mortality sub-routine to calculate the relative reductions in population sizes in the model compartments and then change the genotypic frequencies accordingly. For the spatial model with dispersal, I recalculated the population densities after population regulation to have a correct fraction of individuals dispersing. Calculating the number of individuals during the population regulation sub-routine and applying intra-specific density dependence with different models was accomplished as follows: I used the number of eggs (at time 't_n') at the start of each generation (N_t), the pest-specific, uninhibited

growth rate 'R_0', and various density dependent functions of population growth to estimate the number of eggs that would be present at the beginning of the next generation (time 't_{n+1}') if no additional mortality (e.g. selection) had occurred. The *projected number of eggs* at time t_{n+1} '*NextGenEggs'* was multiplied by the proportion of Bt *PropBt* and refuge *PropRef* and divided into the actual number of eggs predicted at time t_{n+1} in the different compartments in the landscape (*BtEggs* and *RefugeEggs*). The *actual number of eggs* was calculated based on number of adults that had survived at time t_n, divided by two (to account for females only) and multiplied by fecundity and any additional pupa and adult survival (if applicable). The ratio of projected number of eggs and actual number of eggs provided a coefficient of population regulation (density dependent survival) in the refuge, '*DDSurviv'*, and in the Bt field, '*DDBtSurviv*'. The coefficients were multiplied by the genotypic frequencies in the Bt and refuge compartment and modified the gene frequencies accordingly. If the projected number of eggs in the next generation was greater than the predicted number of eggs during the current generation run, then the coefficient was set equal to one and the population density further increased. If the coefficient was <1, density dependent mortality took effect. The closer *DDSurviv* was to zero, the greater the population regulation effect.

The first analysis used the logistic model (aka Verhulst-Pearl equation) and assumed linear (decreasing) per-capita growth rates as the population density increased. The following is the solution of the differential equation used to calculate density dependent effects for all three pests.

$$NexGenEggs = \frac{NrEggs}{\left[1+\frac{NrEggs\cdot(R_0-1)}{KEggs}\right]\cdot {R_0}^{-1}} \qquad (2.5)$$

'*NrEggs*'is the number of eggs at the beginning of each generation and before any kind of mortality has been incurred. The variable '*Ro'* is the intrinsic (uninhibited) growth rate of each pest (obtained from the literature), and '*Keggs*', is the egg carrying capacity for a plant. For corn rootworm, the egg carrying capacity per plant was estimated by dividing the maximum number of adults produced per corn plant (~ 30, Hibbard et al. 2010) by the lower range of adults reported to emerge per plant (3-8%). This converted into a carrying capacity of 1100 eggs per corn plant. For bollworm (e.g. 2.6 eggs per corn ear) and European corn borer (285.7 eggs per corn plant), the egg carrying capacity was obtained by dividing the number of larvae sustained per plant by the egg viability and larval survival.

The second analysis used the Ricker model, which was first developed for estimating stock recruitment in fisheries (Ricker 1954) but has also been used to model density dependent growth in bacteria (Lay et al. 1998) and nematodes (Chavarria & de la Torre 2001). The Ricker model allowed for non-linear effects on the per-capita growth rate as population size increased. This model was proposed for use in species that exhibited some scramble competition dynamics (Brannstrom & Sumpter 2005). The Ricker model also represents a special case of Hassell's discrete time (logistic) population model (1975). The following form of the Ricker equation was used:

$$NextGenEggs = NrEggs \cdot R_0^{\left(1-\frac{NrEggs}{Keggs}\right)} \quad (2.6)$$

The Hassell model (1975) was used to explore a gradient of effects ranging from contest to scramble competition. Contest and scramble competition are concepts first introduced by Nicholson (1954). Scramble competition implies that larvae have equal

access to a resource resulting in complete survival with an abrupt threshold of 100% mortality when the resource is exhausted. Contest competition implies that the "winner takes all" and has sufficient resources to survive and reproduce, while the remaining competitors obtain insufficient resources and die. The Hassel equation with my variable names is:

$$NextGenEggs = NrEggs \cdot R_0 \cdot (1 + a \cdot NrEggs)^{-b} \quad (2.7)$$

The variable 'b' is responsible for making the transition from scramble to contest competition. When $0 < b > 1$, the system returns to equilibrium when perturbed and models exclusively over-compensation. When $b = 1$ (and $R_0 >> 1$), there is perfect compensation (contest competition). The condition where $2 \leq b < \infty$, (and $R_0 >> 2$), under-compensation and scramble competition take place. Since not all the growth values of the pests modeled here may satisfy the requirement that $R_0 >> 1$ (for contest competition) or >>2 (for scramble competition), I set b to satisfy the following constraint for contest and scramble competition, respectively:

$$b(1 - Ro^{-1/b}) = 1, 2 \quad (2.8)$$

A value for the parameter 'a' in the Hassell equation can be obtained at equilibrium assumptions with Ra, the actual, realized growth rate: Sample text after figure.

$$Ra = Ro(1 + a \cdot Nt)^{-b} \quad (2.9)$$

At equilibrium, $Ra = 1,\ Nt = K$, therefore the equation simplifies to:

$$1 = Ro(1 + a \cdot K)^{-b} \quad (2.10)$$

$$a \cdot K = Ro^{\frac{1}{b}} - 1 \quad (2.11)$$

$$a = (Ro^{1/b} - 1)/K \quad (2.12)$$

A fourth mathematical model of density dependence was developed by Crowder and Onstad (2005) and incorporated into the corn rootworm models and expanded upon. Their model was derived with a regression analysis based on published empirical data obtained from several egg infestation studies. Their equation calculated the proportion of survivors based on egg densities before larval selection and, therefore, applied equal population regulation across the field. This equation was later modified by Pan et al. (2011) to estimate surviving adult corn rootworm based on young larval densities that survived Bt exposure. Like Pan et al., I modified the Crowder-Onstad equation by applying density dependence to larvae in the refuge compartment and larvae that survived Bt exposure. Onstad et al. 2006 noted that the original equation adjusted the egg density per ha by m^2/ha and incorporated a divisor of 10,000. Since I simulated 50 ha fields, during the density dependent routine, I had to adjust the larval densities in each compartment (*PropComp*, e.g., 0.05 for refuge and 0.95 for Bt, etc.) by 50*10,000.

$$DDSurviv = \frac{1}{(2.59+1.29(NrLarv/\ (PropComp*50*10{,}000))^{0.88})} \quad (2.13)$$

Figure 2.1 shows the curves for the five proposed models of density dependence in a non-Bt environment using the corn rootworm example and the models' behavior across a range of population densities (population size is expressed as a fraction of the carrying capacity, N/K). The line $Ln(N_{t+1}/K) = Ln(N_t/K)$ denotes the carrying capacity with a slope of 1, where the population proportion in the following year is always equal to the population proportion the prior year. The nature of Crowder-Onstad's equation

applies the least amount of density dependence at high population densities (≥ K) compared to other models of density dependence. This becomes evident when I examine the slope of the density dependent curve at, for example, the carrying capacity ([0, 0] - the equilibrium line crosses lines of all models of DD), where its slope is the most positive but still less than 1 (see Figure 2.1); this model results in damped oscillations as the population density continues to increase. When the population size is less than K, the Crowder-Onstad equation applies the least amount of population regulation strength. The Ricker model applies the greatest degree of density dependence of all the models used shortly before and after the carrying capacity (but the least amount when densities are much less than K), and the slope of the line at the equilibrium point is the most negative. This type of model results in s limit cycles. The slope of the line at the equilibrium point for the Hassel scramble competition model is negative as well but the strength of density dependence is not as great as for the Ricker model; this model also results in s limit cycles. Hassell's contest competition has a slightly negative slope at K and the Logistic model a slightly positive slope; these models also result in damped oscillations over time and increasing population density.

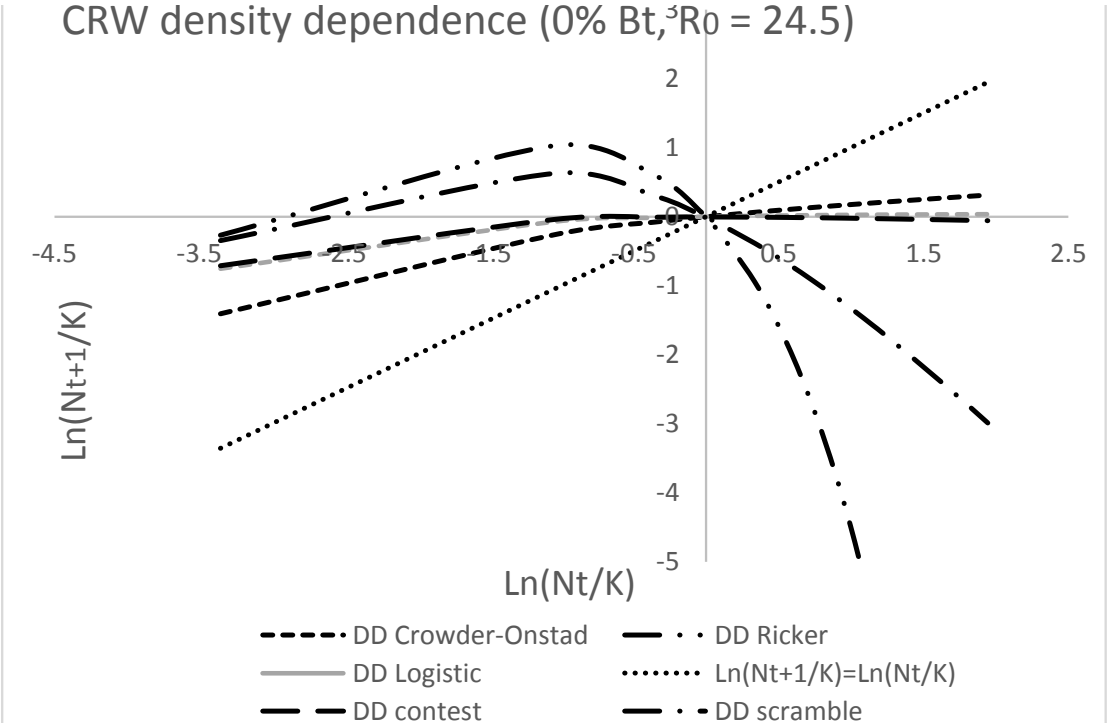

Figure 2.1 Density dependent curves for five models of population regulation in a non-Bt environment.

Notes: $Ln(N_t/K)$ denotes the fraction of the population with respect to the carrying capacity at time t; $Ln(N_{t+1}/K)$ is the fraction of the population during the following generation. $Ln(N_{t+1}/K) = Ln(K_t/K)$ denotes the equilibrium line, the Carrying capacity.

2.3.2 Corn rootworm parameter assumptions

Assortative mating was incorporated for the population of *D. virgifera* in a 50 ha landscape because of reported limited daily and intra-field movement of adult corn rootworm (Spencer et al. 2003; Spencer et al. 2009; but also see Caprio and Glaser 2010). Non-random mating in this context refers to a fraction of the population emerging from the Bt portion of the field that randomly mated with the population emerging from the refuge. This fraction was a function of field size (Caprio and Glaser 2010) and set equal to 40% for the 50 ha fields used in the simulations.

Egg overwintering survival was set to 0.50 (Godfrey & Turpin 1983; Onstad et al. 2006), and pupa and adult survival were assumed to be 1.0 (Onstad et al. 2006). The estimated range for egg viability was 0.029 to 0.084 and based on a multi-year field study (Hibbard et al. 2010). Levine et al. (1992), however, reported viability values observed in the lab that ranged from 0.84 to 0.92 (Hibbard et al. 2010). I set egg viability in the model to 0.10 to achieve an overall 4.5% recruitment of egg to adulthood. Likewise, mean larval survival was set to 0.90 based on back-calculations that lead to 4.5% egg survival to adulthood. Overall corn rootworm survival was then 0.45 and slightly higher than Onstad et al.'s (2006) estimate (max. 0.35 using Crower-Onstad equation). Average fecundity was reported as 1087 eggs oviposited in 13 clutches (Hill 1975). Therefore, the net multiplication rate per generation (R_0 = overwintering survival x egg viability x larval survival x fecundity/2) in this model was 24.5, although this value was higher than the growth rate used by Caprio and Glaser (2010) (R_0 = 11.0). In this model, the larval carrying capacity on a corn plant equaled the total number of adults (~30) that can be sustained by a plant (Hibbard et al 2004) because pupa survival was assumed to be 1.0. This translated into 2.4 million adults per ha and was similar to what was assumed by Crowder and Onstad (2005). This was also equivalent to 1000 eggs per plant assuming the lower range of survival (3%) or 80 million eggs per ha in our model. Given R_0=24.5, K = 4.44 E^{+10} (per 50 ha), and the values for b (1.05 contest comp; 2.999 scramble comp), the values for 'a' were derived using the equation in section 2.3.1. Figure 2.2 gives the schematic for the flow of the model.

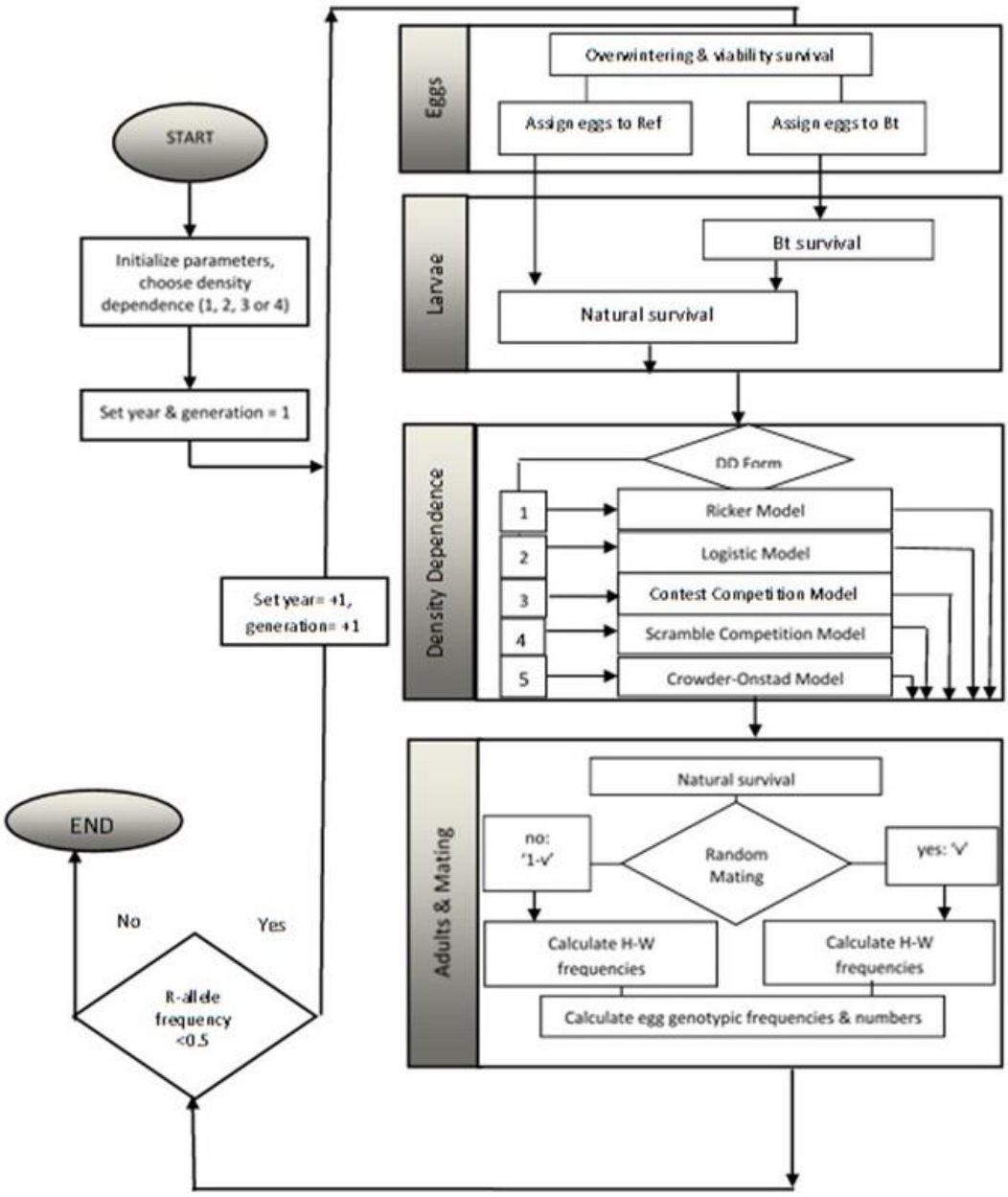

Figure 2.2 Flow chart for non-spatial corn rootworm model

2.3.3 Bollworm parameter assumptions

Random mating was assumed in each field for the entire population of *H. zea* because of its high propensity to disperse (Han & Caprio 2002, Gould et al. 2002, Sparks

et al. 1986). It has been observed that ovipositing females did not lay eggs in natal or neighboring fields but dispersed much further (Isley 1935). Random mating on the scale of a 50 ha field appears a defensible assumption in North America.

An average egg viability of 0.8 on corn, 0.6 on cotton, and 0.5 on natural hosts was selected (Caprio et al. 2009); overwintering survival for pupae was set to 0.05 but has been reported as low as 0.026 (Stadelbacher & Pfrimmer 1972) . Larval survival on natural hosts, corn, and cotton was 0.35, 0.48, and 0.14 and was the product of young and mature larval survival listed in Caprio et al. (2006). Pupal and adult survival were each set to 0.8 in all environments (Kring et al. 1993).

Reported average fecundity for bollworm ranges from 300 to 600 eggs/female (Caprio et al. 2009). In this model, I adjusted fecundity to achieve a net multiplication rate R_0 that was reflective of values reported in the literature. For example, females coming off corn had a fecundity of 200 eggs per individual to achieve a rate of 24.6, which was close to that reported on silking corn (Caprio et al. 2006). In the cotton simulations, I set fecundity to 500 eggs/female to achieve an intrinsic growth rate of 13.4 (R_0 =14.2, Caprio et al. 2006); R_0 for the last generation in cotton was much lower because of the low overwintering survival reported (R_0 = 0.672). Likewise, in the natural host simulations, the value for fecundity was set equal to 600 eggs/female so that the intrinsic growth rate reached a value of 33.6. For wild hosts, Caprio et al. (2009) reported R_0 values ranging from 44 (general mid- to late-season hosts) to 58.8 (on wild geranium). The calculated values for R_0 were, therefore, similar or equal to what has been reported for bollworm in southern cropping and natural host systems. If, however, the reported R0 numbers reported by Caprio et al. (2009) in the different cropping systems already

included intra-specific density dependent effects, then the *H. zea* growth rates used in these simulation analyses reported here would underestimate the actual growth rates in the field.

Bollworm larval carrying capacity on corn was set to 1 larva/ear (two per plant for whorl stage, one per plant for silking stage) and 2 larvae for cotton and natural hosts because of the pest's cannibalistic behavior (Chilcutt 2006). Anecdotal evidence might support a carrying capacity of up to 2 larvae per corn ear (based on exit holes observed), up to three and four on sorghum and possibly on cotton during high pressure situations (Dr. Musser, MS State, *pers. com.*). The carrying capacities in the model might represent a conservative underestimate and would keep overall population densities lower than under high insect pressure in the natural systems. Uncertainties about effects of carrying capacity was discussed in the sensitivity analysis. The values for 'a' were calculated using the respective R_0's in the different environments, adjusted values for *b* in contest competition and scramble competition simulations, as well as host specific carrying capacities. However, given that the R_0 of the last generation bollworm was very low, I considered the effects of competition on in-season generations only and used the fifth generation b- and a-values calculated for the Hassell equation for the sixth generation. Figure 2.3 represents the flow of the model for bollworm.

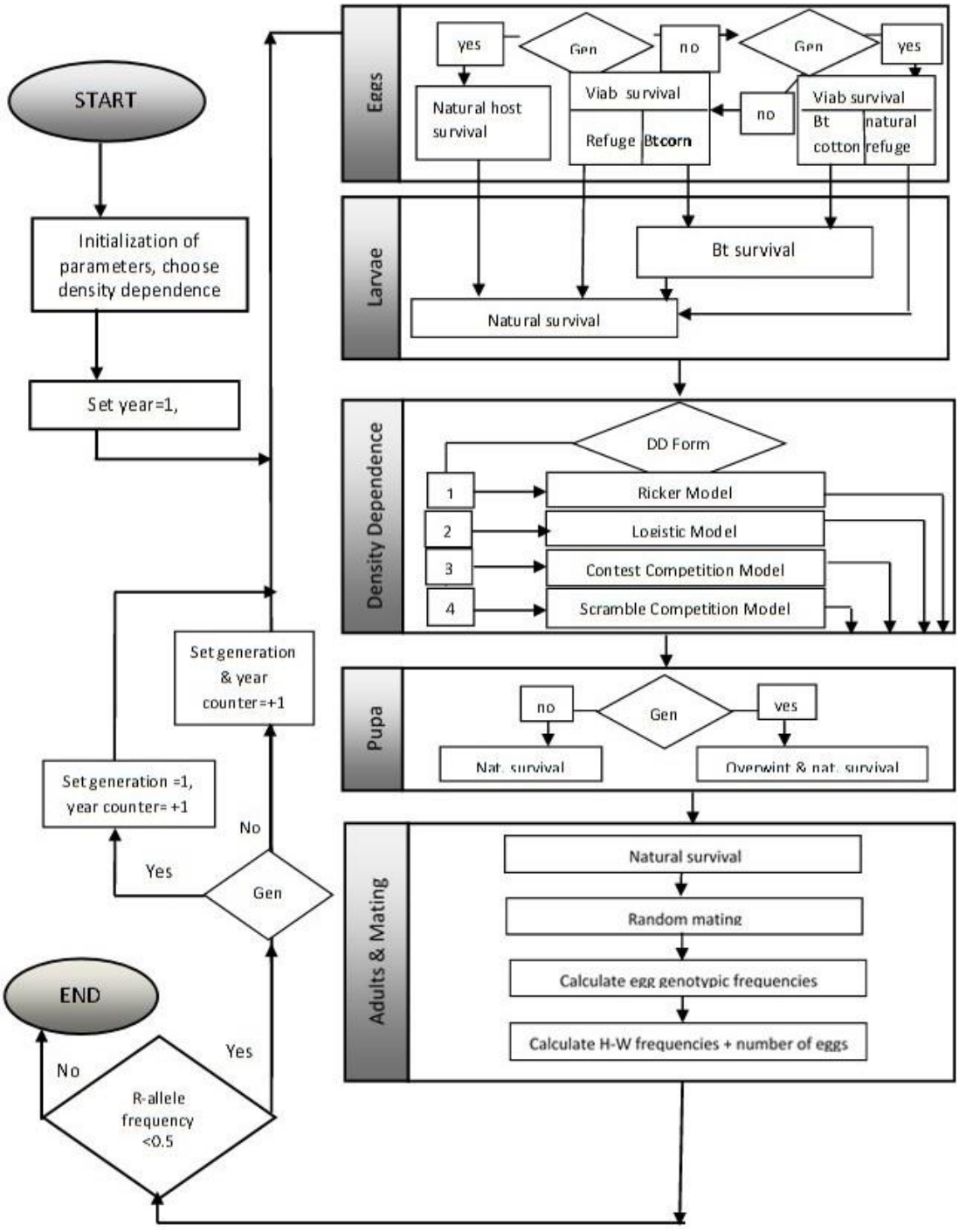

Figure 2.3 Flow chart for non-spatial bollworm model

2.3.4 European corn borer parameter assumptions

Consensus among scientists converges on a value of >100 km long distance dispersal per generation for European corn borer (Bourget et al. 2000; Krumm et al. 2008; Kimm et al. 2009; and others), although it is unknown what exact proportion of a European corn borer population engages in this type of movement. Short range movement seems to predominantly occur on the scale of several hundred meters up to a few kilometers as is supported by mark-release-recapture studies (Showers et al. 2001). Adults typically move out of cornfields on a daily basis to aggregate in sites with more suitable microclimatic conditions. This behavior is followed by subsequent dispersal back into cornfields the following morning (FIFRA SAP 2011; Showers et al. 2001) and could further support the random mating assumption made in the ECB model here. The random mating on the scale simulated here may be further supported by Chiang & Hodson (1958) and Shelton et al. (1986).

Late-instar overwintering survival was set to 0.18, natural survival for larvae from egg stage to last instar was estimated at 0.077, and fecundity was 290 eggs/female (Onstad 1988). Survival at the egg stage was reported as high as 0.95 but was ignored together with pupa and adult mortality much like in Guse et al. (2002) because they were negligible. The carrying capacity was 22 larvae per plant for both generations of corn borers (Onstad 1988). The intrinsic growth rates estimated were 11.2 for the first generation ECB and 2.0 for the second (overwintering) generation. These values (especially for the second generation) likely represent an underestimate and do not represent a pre-Bt commercialization growth rate. Pests of economic significance typically have an intrinsic net growth rate that is high - although no specific lower

threshold value has been proposed. Conway (1979) stated, however, that economic pests could be characterized as *r*-selected species when exhibiting a high fecundity (e.g. black cutworm, fecundity = 1500 eggs per female) and short generation time (1-2 weeks to 1-2 months). Given that the R_0 estimate of the second generation ECB is very low, I considered the effects of scramble and contest competition on the in-season generation only and used the first generation b- and a-values calculated for the Hassell equation also for the second generation. Given the larval carrying capacity K on a corn plant and R_0 of 11.2, *b* and *a* for contest and scramble competition simulations were obtained following the previous description. (See Figure 2.4 for model flow chart of European corn borer)

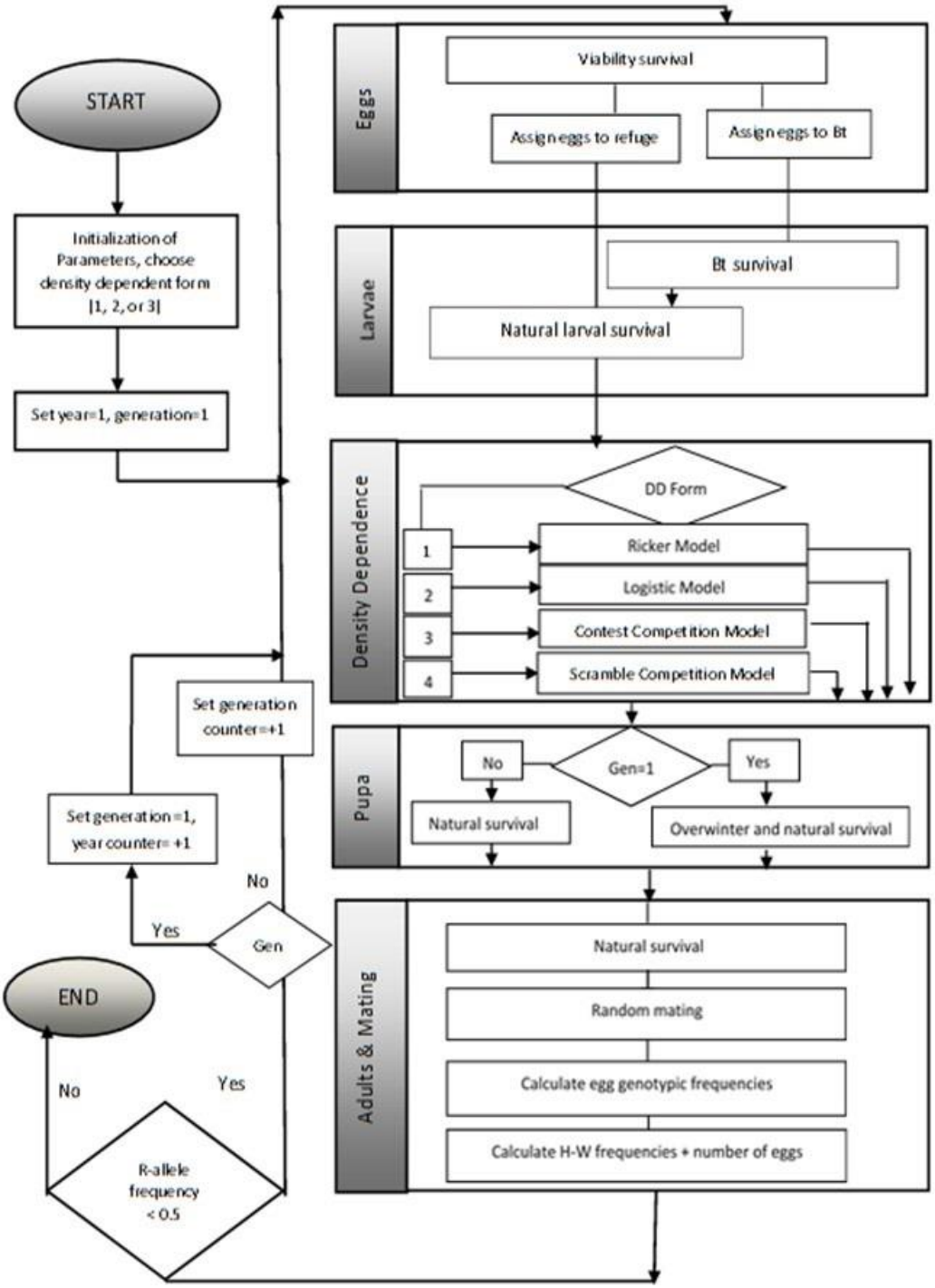

Figure 2.4 Flow chart for non-spatial European corn borer model

2.3.5 Quality control in model development

Validation of the spatially implicit and explicit models included multiple steps. In general, the following steps were taken for all models: division by zero was protected against; Hardy-Weinberg frequencies were hand-calculated and compared against values computed by the model; the population dynamics (without selection) were compared to known (published or anecdotal) dynamics in the field (Alstad & Andow 1995; Caprio et al. 2009, Dr. Musser, MS state, *pers. com.)*. All spatially implicit models were run with selection but without density dependence to compare only the population genetics part of the models (Table 2.1). Keeping the genetics assumptions equal, all models predicted the same durability for the hypothetical dual gene PIP. With a high dose and 5% refuge, the estimated durability was 352 years; with a low dose and 5% refuge, the estimated durability was 31 years for all pests and models.

Table 2.1 Estimated years to PIP failure with no density dependent mortality

H. zea		***D. virgifera***		***O. nubilalis***	
HD	**Less than HD**	**HD**	**Less than HD**	**HD**	**Less than HD**
Bt/non-Bt 95/5	Bt/non-Bt 95/5	Bt/non-Bt 95/5	Bt/non-Bt 95/5	Bt/non-Bt 95/5	Bt/non-Bt 95/5
352	31	352	31	352	31

Notes: *H. zea* was modeled with two extra generations/year with no Bt selection; results (1259 and 47 generation) were, therefore, adjusted by a factor of 4/6. 95% Bt and 5% non-Bt refuge was assumed. *D. virgifera* and *O. nubilalis* were modeled with 1 and 2 generations/year, respectively

The deterministic, spatially implicit model for *Helicoverpa zea* was verified by using toxin-specific information and effective refuge contribution of C3-plants reported by Gustafson et al. (2006). My simulations predicted five years of durability for a

Bollgard cotton equivalent PIP (using Logistic model of density dependence), while Gustafson et al. obtained six years of durability with their model. A comparison of outputs for high dose assumptions and various refuge proportions was also conducted against outputs generated by a simple population genetics model by Caprio. The overall trend between outputs generated by different models was comparable: at high dose, a 5% refuge reduced the durability of the PIP compared to a 20% refuge. The numerical values were lower for the bollworm model (described here) with a 5% refuge than for the Caprio model (Caprio and Glaser 2010), which could be used as a generic pest model. This difference at low refuge proportions was likely attributed to different model structures and complexities. At the higher refuge proportion, the numerical estimates of time to resistance were comparable between the two models.

The deterministic, spatially implicit corn rootworm model was tested by comparing the output against that reported by Pan et al. (2011) for a single locus model with 20% and 5% refuge, initial resistance allele frequency of 0.005, and assuming same genetic parameterizations as well as reduced fecundity for susceptible genotypes (ss) when emerging from Cry34/35 fields. My model predicted 17 and 7 generations durability for 20% and 5% block refuge (fixed location), respectively, while Pan et al. (2011) reported 11 and 7 generations. The trend in my simple model was similar to the Pan et al., whose model was much more complex and which likely contributed to the observed differences between the two simulations with a 20% block refuge.

The deterministic, spatially implicit model for European corn borer was compared to the results of Guse et al. (2002) using the same genetic and life-history information listed there. A main difference between the two models was that I used a specific density

dependent function, while Guse et al. (2002) set an upper limit to larval density per plant. With an initial resistance allele frequency of 10^{-3}, a low dose PIP deployed with a 10% refuge, and dominant expression of the resistance allele, both models estimated 2.5 years until the resistance allele frequency reached 0.03 (level of resistance set by Guse et al. (2003)). For a high dose PIP with a 10% refuge and full recessive expression of the resistance allele, the estimated time to resistance in my model was 26.0 years, while Guse et al. reported a durability of 56 years. This difference may be attributed to how population regulation is dealt with in two models: in my model, strength of density dependence gradually increases with increasing density, and Guse et al. (2002) applied a cap to the number of larvae sustained by a corn plant. In the latter case, density dependence did not occur until the carrying capacity was reached. A better way of comparing the two models would be to look at the relative differences predicted by each for various IRM scenarios (FIFRA SAP 1998).

2.3.6 Data Analysis

For each pest, a comparison of means (ANOVA) was conducted to determine significant differences in estimated durability results obtained with different models of density dependence at each level of refuge percentages (5% - 50% for *O. nubilalis, D. virgifera* and 20% - 50% for *H. zea*), and low dose /high dose assumption (significance level $p < 0.05$ (R software, version 3.0.2). Linear regression analyses were used to look for significant effects caused by 1) fecundity and 2) different refuge proportions on the durability estimates obtained with the same model of density dependence. ANOVA was also used to determine whether significant differences in durability occurred between spatial and non-spatial models. I used a PERT-Beta probability distribution for the

dominance values of the resistance gene at locus 1 and locus 2, and a Poisson distribution for fecundity values on Bt and non-Bt plants. This kind of sampling strategy generated variability between replicate simulations but kept the fecundity and dominance values constant for different generations within a simulation. The PERT- Beta distribution is "often used to describe the uncertainty about the probability of the occurrence of another event" (Vose 2001) and uses the same three parameters as the triangle distribution (min, mode, and max). In case of the PERT distribution, the mode is assigned a weight of four, while the minimum and maximum receive a weight of one. The distribution can be a normal but need not be so. If the mode is not centered between the minimum and maximum values, the distribution is skewed. In other cases, the distribution can take on various shapes. The parameterization of the three values is set by expert knowledge or, if no information is available, by best guesses. The minimum, mode, and maximum values for my PERT-Beta analyses and mean values for the Poisson analyses were listed in Figure 2.2. I ran 30 simulations for each scenario (dose, % refuge, and DD model) and sampled for each parameter from the appropriate distribution. The average time to resistance was calculated for each set of simulations.

Table 2.2 Parameters and their values used in PERT-Beta and Poisson distribution

Pert-Beta Distribution Values for all Pests					
	Parameters	Min	Mode	Max	Comments
High Dose	Dominance – Locus1	0.002	0.021	0.05	N/A
	Dominance – Locus2				
Lower Dose	Dominance – Locus1	0.05	0.06	0.15	N/A
	Dominance – Locus2				
Poisson Distribution					
Natural host fecundity		600			*H. zea* *
Corn fecundity		350			
Cotton fecundity		500			
Nat. Fecundity		1087			*D. virgifera*, Hill 1975
Bt Fecundity		1087			
Nat. Fecundity		290			*O. nubilalis*, Onstad 1988
Bt Fecundity		290			

Notes: * = values adjusted to achieve intrinsic growth rates (R_0) reported by Caprio et al. (2009).

2.4 Results

2.4.1 Spatially implicit models

2.4.1.1 *H. zea*

For bollworm simulations with high dose assumptions, the durability of the dual-gene PIP increased with increasing refuge percentage for simulations with Logistic and contest competition model (Table 2.3); estimated times to resistance differed significantly with each subsequent refuge proportion tested (R^2 >0.9). For simulations with the scramble competition and Ricker model, durability estimates first increased up to 30% and

subsequently decreased at 40% refuge until they were lowest at 50% refuge for scramble competition and 70% for the Ricker model (R^2 Ricker = 0.8179; R^2 SC = 0.1774) .

A between-density dependent model comparison showed that durabilities at 20% refuge were highest with the Ricker model, followed by scramble competition, contest competition, and finally the Logistic model (p-value <<0.05). At 30% refuge, contest and scramble competition estimated the highest durabilities, while the Ricker and Logistic model estimated lower durabilities (p-value <<0.05). At 40% and 50% refuge, the contest competition model predicted the greatest durability for the high dose pyramid, while the Ricker and scramble competition estimated lower durabilities (p-value <<0.05).

Figures 2.5 and 2.6 show that durability increased linearly for the Ricker model from 1% to 10% refuge, then leveled off until a 20% and dropped continuously towards a 60% refuge before slowly increasing again at 80% refuge. The pattern obtained with scramble completion followed that of the Ricker model, but durability estimates remained mostly unchanged between a 20% and 40% refuge before dropping off toward a 50% refuge. Thereafter, the durability began to slowly increase with greater refuge percentages. The estimates obtained with the Logistic model at the various refuge percentages increased mostly linearly with increasing refuge. In absence of density dependence, estimated durabilities followed a sigmoidal pattern with increasing refuge proportions; the durability increase was greatest between a 1-5% refuge, after which the rate of increase slowed with more available refuge. These graphs show that various models of density dependence can predict different evolutionary outcomes for *H. zea*, especially at greater refuge proportions when density dependent effects become more prominent because of greater population densities.

A closer look at the density dependent mortality in different cropping systems of my model revealed that early on, population regulation occurred in refuge corn only and not on Bt corn. When resistance reached higher levels (>0.3) in the population, population regulation occurred also in Bt corn. The whorl stage generation of bollworm experienced greater density dependent mortality on refuge corn than the second generation on the silking stage because of an influx of individuals from the early natural host system. Density dependent mortality did not occur on Bt cotton until r-frequencies reached higher levels in the population and resistant individuals became more abundant. Density dependent mortality was greater in the second generation of bollworm on early spring hosts and in the second generation of late fall hosts. Population regulation became stronger as the percent refuge was increased.

For less-than-high dose assumptions, the overall durability of the two-gene PIP was a fraction of what was predicted for the high dose PIP with all models of density dependence. Each increasing step in refuge proportion lead, however, to a significantly greater durability for all models of population regulation. Linear regression analyses for percent refuge and durability informed that the percentage of variation explained for the response variable was very high (R^2 >0.9 for four models of DD; R^2 = 0.8179 for Ricker) (Table 2.3). The scramble competition model predicted the greatest durabilities across the range explored (20%-50%) (p-values <<0.05), though Figure 2.7 shows that the durability decreased after 50% due to greater density dependent effects. The Ricker model, which is a type of scramble competition, predicted the second highest durabilites for low dose simulations. The lowest durabilities were predicted using the Logistic model (p-value <<0.05); with each 10% increase in refuge, only one additional year in durability

was gained. Figures 2.7 and 2.8 reinforce the message that IRM models need to include intra-specific forms of density dependence in order to not overestimate the projected life-time of a PIP.

Table 2.3 Average years to resistance for *H. zea* using a spatially implicit model with different assumptions of density dependence

Species	**Dose**	**DD**	**% Bt : % Refuge**			
			80:20	**70:30**	**60:40**	**50:50**
H. zea	HD	R	97.9 [d]	77.8 [a]	52.0 [a]	21.4 [a]
		L	64.0 [a]	80.0 [a]	99.0 [c]	115.7 [c]
		CC	70.6 [b]	90.7 [b]	108.7 [d]	130.8 [d]
		SC	85.6 [c]	89.0 [b]	87.7 [b]	59.4 [b]
	< HD	R	9.0 [c]	10.7 [c]	12.1 [c]	13.3 [c]
		L	7.1 [a]	8.0 [a]	9.0 [a]	10.0 [a]
		CC	8.2 [b]	9.7 [b]	11.3 [b]	12.9 [b]
		SC	9.6 [d]	11.7 [d]	15.7 [d]	21.1 [d]

Notes: ANOVA results are reported for between DD model comparisons at each refuge proportion (red letters). Mean natural life-time fecundity= 500, 350, and 500 (natural host, corn, and cotton, respectively); CC = contest competition, b_{nh} = 1.05, b_c = 1.1, b_{ct} = 1.15, b_{lnh} = 1.05; SC = scramble competition, b_{nh} = 3.0, b_c = 3.4, bct = 4.6, b_{lnh} = 3.0; R_{0nh} = 33.6, R_{0c} =21.5, R_{0ct} =13.4; HD= high dose scenario; W_{SS}=0.01, W_{RS}=0.03079, W_{RR}=1.0, h=0.021 (mode). Less-than-high dose scenario: W_{SS}=0.2, W_{RS}=0.248, W_{RR}=1.0, h=0.06 (mode), IRAF L_1, L_2= 0.005

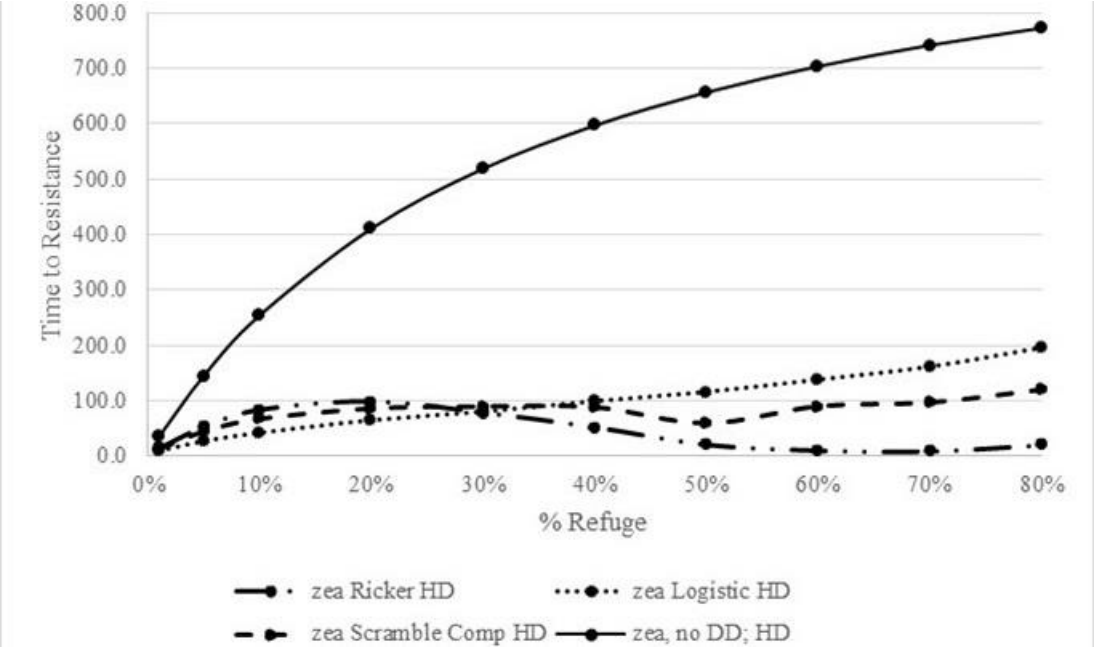

Figure 2.5 Graph of durability versus refuge percentage for *H. zea* using the Ricker, Logistic, and scramble competition model (HD)

Notes: *b*=2.0, as a reference, the projected durability without density dependence is included from 1%-80% refuge.

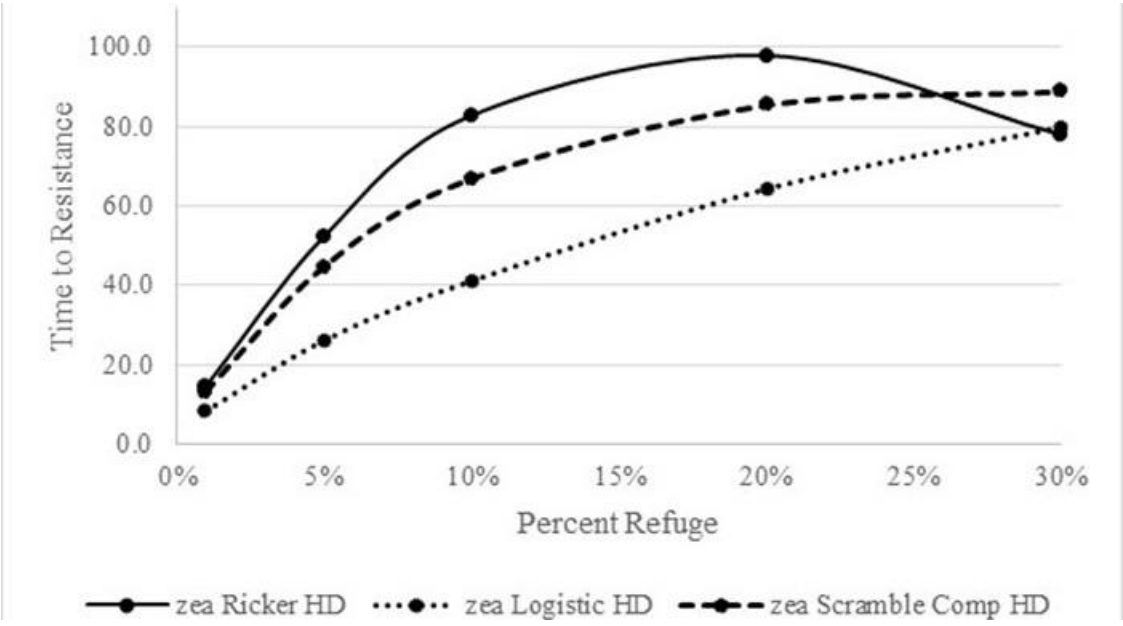

Figure 2.6 Graph of durability versus refuge proportion (1-30%) for *H. zea* using the Ricker, Logistic, and scramble competition model (HD)

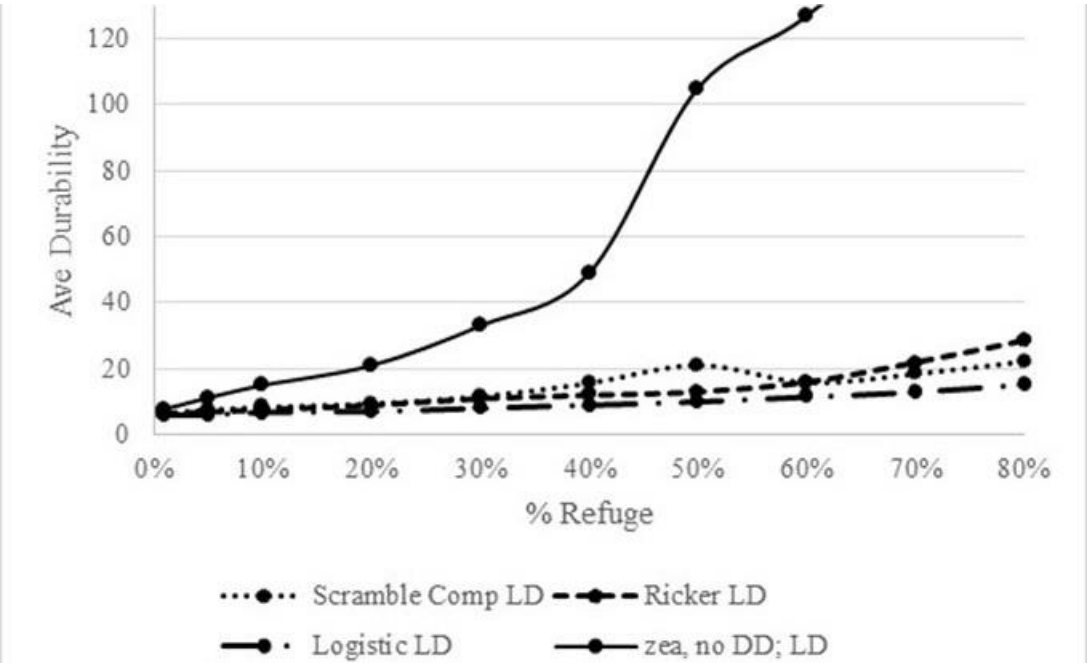

Figure 2.7 Graph of durability versus %refuge for *H. zea* using the Ricker, Logistic, and scramble competition (LD)

Notes: As a reference, the projected durability without density dependence is included and visible from 1%-60% refuge.

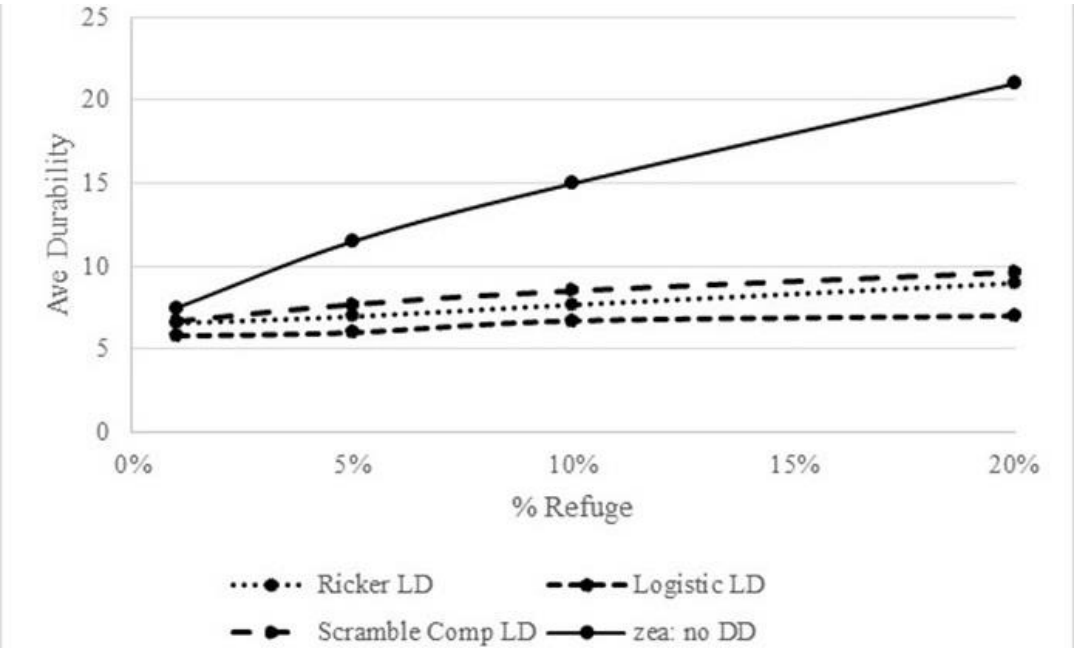

Figure 2.8 Graph of durability versus refuge proportion (1-20%) for *H. zea* using the Ricker, Logistic, and scramble competition model (LD)

Notes: As a reference, the projected durability without density dependence is included and visible from 1%-20% refuge.

2.4.1.2 *D. virgifera*

For corn rootworm simulations with a hypothetical high dose for the pyramid, the estimated average durability increased for four out of five models of density dependence (except Ricker model) as the percent refuge increased from 5% to 50% (p-values <<0.05, R^2 >0.9) (Table 2.4, Figure 2.11). For the Ricker model, durability estimates increased with increasing refuge proportions up to 30%, after a 40% and 50% refuge, a decrease in durability was observable. For the Crowder-Onstad modified equation, the durability roughly doubled between 5% and 10% (from 352 generations to 735 generations); this represented the biggest gain in durability from increasing the refuge percentage across the range explored. Furthermore, the Crowder-Onstad model predicted the greatest durability gain at each refuge proportion compared to the other models of density dependence (P-

value <<0.05). For the remaining models, the increase in durability was more moderate across the tested refuge range (exception Ricker model after 30% refuge).

ANOVA analyses at each refuge proportion and for all models of density dependence, showed that at low refuge percentage (5% and 10%), there was less differentiation between the various models, and the average time to resistance for the two-gene PIP was not significantly different for three out of four models ($p<0.05$). The durability lines for the Logistic equation, scramble and contest competition in Figure 2.11 are super-imposed indicating that the differences observed between the three models were minimal for *D. virgifera* simulations. As a reference, simulations results were included in Figure 2.11 where density dependence was excluded. It becomes obvious that the durability of PIPs is greatly overestimated without the inclusion of some population regulation mechanism.

Typically, trend for density dependent mortality in the refuge across time was affected by the available amount of refuge (Figure 2.9). Increasing the refuge proportion is equivalent to increasing the growth rate of the pest. Hence, at low refuge proportions when there are fewer individuals present, there should be less density dependent mortality. The observed relationship between density dependent mortality and generations can be best described by a sigmoidal curve (dotted lines in Fig. 2.9). As the refuge increased from 10% to 20%, the curve moved upward and shifted to the left (population regulation occurred earlier, effects became greater and leveled off faster at a higher equilibrium mortality). As the refuge further increased, density dependent mortality began to oscillate from a 2-point cycle at 30% and 40% refuge to a four point cycle at 60% refuge. More available refuge meant greater fluctuations in density

dependence in the refuge, where at some point the population during one generation was reduced so greatly that the next generation experienced much lower population densities and population regulation. When the refuge population decreased during a particular year, the resistance allele frequency increased greatly the following generation because less refuge insects were available to mate with the resistant individuals coming off the Bt (data not shown).

Scatterplots for 30 samples of refuge insect fecundity (obtained from Poisson distribution) versus estimated time of resistance (see Figure 2.10, SC) show that for simulations with 20% and 50% refuge proportion (for example), durability of the pyramid sharply decreased with increasing fecundity; this was observed for all models of density dependence. I conducted a linear regression where durability was the response variable and refuge fecundity and percent refuge were the explanatory variables. Fecundity was highly but negatively associated with durability (p-value = $2e^{-16}$), while a strong and positive association was detected between percent refuge and the response variable ($R^2 = 0.9693$) – as previously discussed. The negative association between fecundity and life-time of high dose PIP was a result of greater density dependent mortality in the refuge compartment (decreasing susceptible pool of insects) with higher fecundity values that increased the adaptation rate of CRW.

For less-than high dose scenarios predicted durability estimates were much lower than for high dose scenarios, irrespective of the models of density dependence used. Nonetheless, a similar trend was visible here in that increasing the refuge proportion resulted in greater durability for the pyramided PIP ($R^2 > 0.9$ for four out of five models; $R^2 = 0.789$ with Ricker model). The greatest gain in durability occurred by switching from

a 40% to 50% refuge with Ricker, Logistic, scramble and contest competition models. At lower refuge percentages the gain in durability was moderate or minimal. At lowest and highest refuge proportions, the greatest statistical variability was observed between estimates obtained with different models of density dependence. With the Crowder-Onstad modified equation, the greatest gain in durability occurred by switching from a 40% to a 50% refuge (23.5% increase). Typically the Crowder-Onstad modified equation estimated durabilities for the low dose pyramid that were approximately ≥2 times greater than estimates obtained with other models of density dependence. The Ricker equation predicted the second greatest durability estimates at high and low refuge proportions.

Table 2.4 Average years to resistance for *D. virgifera* using a spatially implicit model and different assumptions of density dependence

Species	Dose	DD	% Bt : % Refuge					
			95:5	90:10	80:20	70:30	60:40	50:50
CRW	HD	R	287.5 [b]	308.1 [b]	342.7 [a]	377.9 [a]	241.2 [a]	181.0 [a]
		L	286.9 [b]	302.6 [ab]	343.0 [a]	391.2 [b]	458.6 [b]	547.9 [b]
		CC	279.9 [a]	300.8 [a]	340.0 [a]	389.7 [ab]	452.4 [b]	544.6 [b]
		SC	288.7 [b]	307.5 [b]	343.1 [a]	391.0 [b]	451.7 [b]	545.0 [b]
		CO	352.9 [c]	735.6 [c]	883.6 [b]	1014.0 [c]	1180.8 [c]	1408.7 [c]
	< HD	R	19.1 [c]	19.8 [b]	21.2 [b]	23.6 [b]	33.3 [c]	46.8 [c]
		L	18.9 [b]	19.8 [b]	21.5 [b]	23.6 [b]	26.5 [b]	30.4 [b]
		CC	17.0 [a]	18.1 [a]	20.1 [a]	22.3 [a]	25.3 [a]	29.3 [a]
		SC	19.0 [c]	19.8 [b]	21.2 [b]	23.3 [b]	26.1 [b]	30.2 [b]
		CO	28.9 [d]	36.3 [c]	44.2 [c]	53.4 [c]	64.7 [d]	79.7 [d]

Notes: ANOVA results are reported for between DD model comparisons at each refuge proportion (red letters). CC = contest competition, b=1.05; SC = scramble competition, b=2.999; CO = Crowder-Onstad modified equation; Mean natural life-time fecundity= 1087 (viable and non-viable eggs); R_0= 24.5; HD= high dose scenario; W_{SS}=0.01, W_{RS}=0.03079, W_{RR}=1.0, h=0.021 (mode); less-than-high dose scenario: W_{SS}=0.2, W_{RS}=0.248, W_{RR}=1.0, h=0.06 (mode), IRAF L_1, L_2 = 0.005

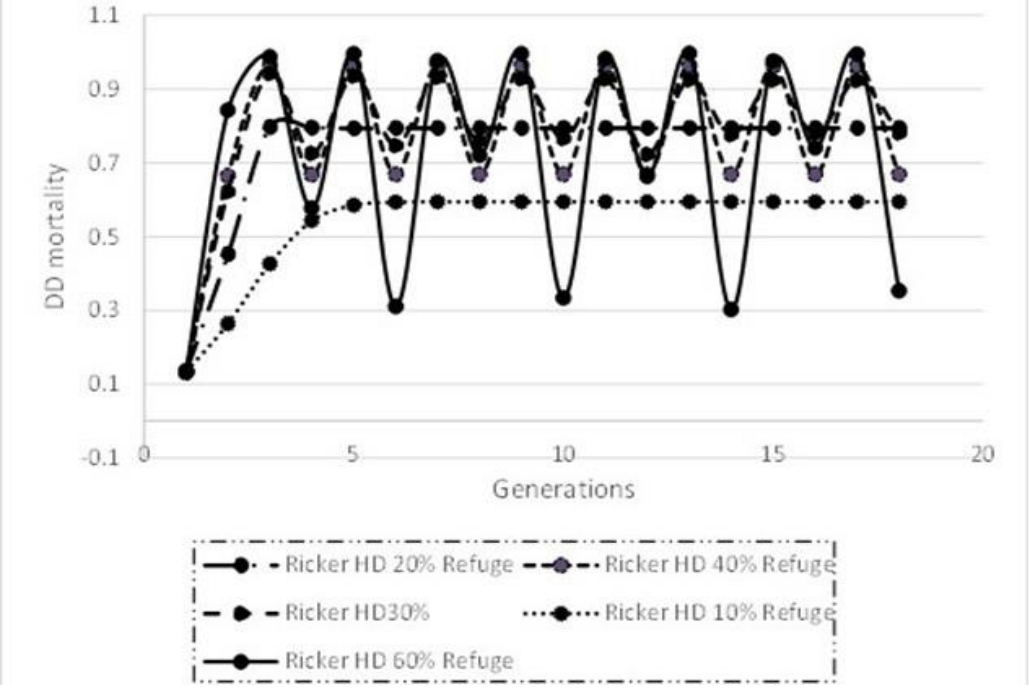

Figure 2.9 Density dependent mortality vs generations of selection for CRW with different refuge proportions (HD)

Notes: Ricker model was used in simulations

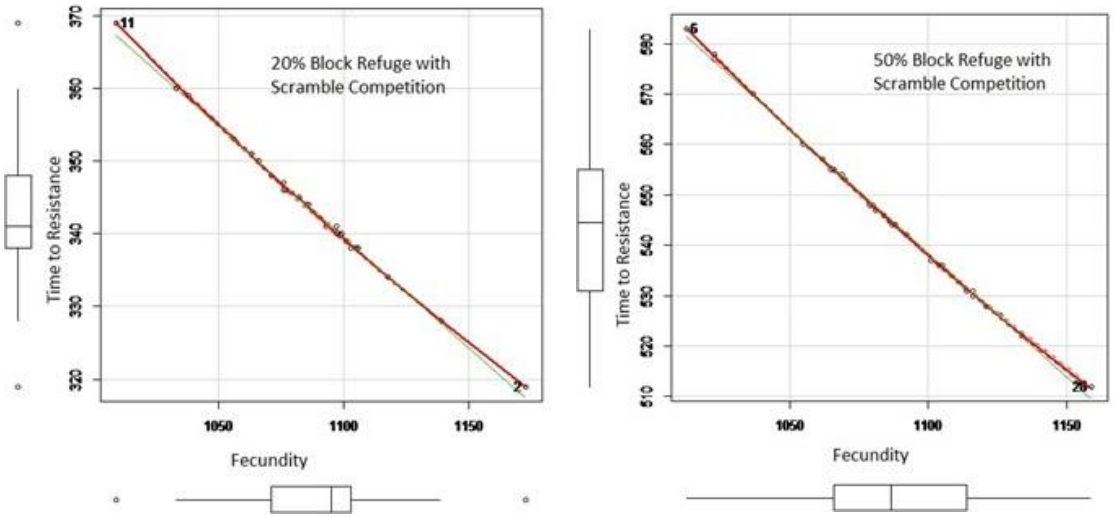

Figure 2.10 Corn rootworm fecundity values vs. resulting durability values using scramble competition with a 20% and 50% refuge (HD)

Notes: values sampled from Poisson distribution. Box plot (Q1, Q2, and Q3) and whiskers are displayed for both variables.

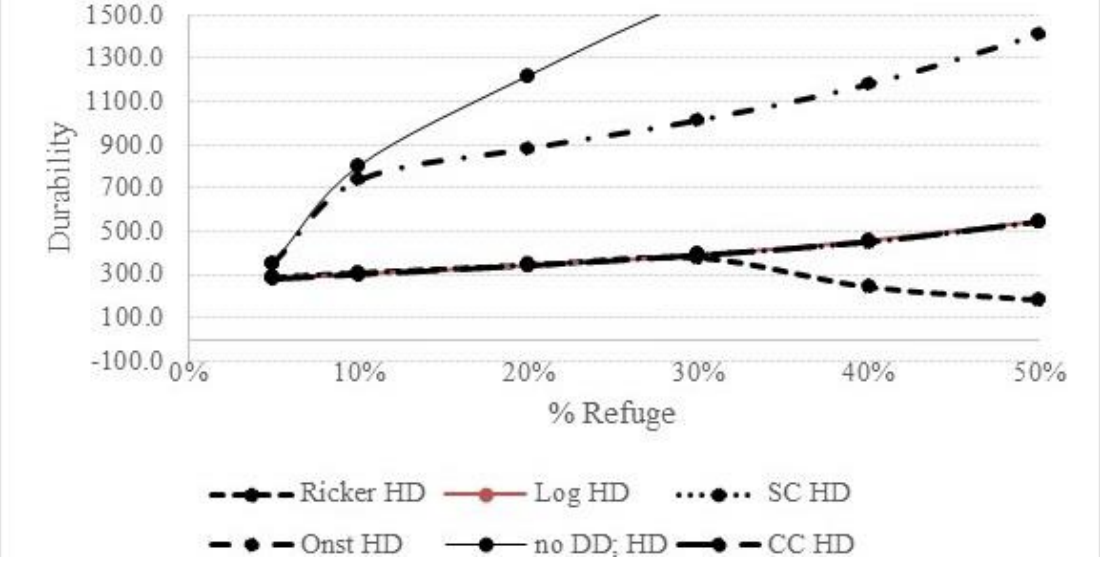

Figure 2.11 Graph of average durability versus % refuge for all five models of density dependence using a spatially implicit model for CRW (HD)

2.4.1.3 *O. nubilalis*

The linear regression analysis for durabilities (response variable) and refuge proportion (factor) identified that the variability of the response variable explained by the factor was greatest for contest competition and Ricker models (R^2 >0.9). The R^2-values were lower for simulations with scramble competition and Logistic models (R^2 for SC = 0.8933; R^2 for Logistic = 0.8944). All results support that increasing the available refuge for ECB results in greater durability for the high dose pyramid (Table 2.5).

Changing the dispersal distance from 3 fields to 5 fields did not significantly affect the durability of the pyramid (high and low dose assumptions, P-value >0.05). When decreasing the dispersal distance to 1 field, again the durability of the PIP was not affected (P-value >0.05).

In Figure 2.12, the durability estimates for the high dose pyramid obtained with different models of density dependence are graphed across a range of refuge proportions for the European corn borer. The durability of the pyramid increased rapidly from 1% to 20%, and the observed trend follows the projected line for durability estimates when no density dependence is included in the simulations. Population densities were close to extinction for the first three refuge proportions, and therefore, density dependent mortality was minimal and close to zero (data not shown). The first observable difference in durability estimates obtained with different models of density dependence became evident at 30% refuge. The Ricker model predicted a greater average durability (965 generations) at 30% than the contest competition and Logistic models (935 generations, 926 generations, respectively) (p-value<0.05), while there was no difference compared to estimates obtained with scramble competition (949 generations) (p-value >0.05) (2.5). At 40% and 50% refuge, the Ricker model continued to predict the greatest durabilities for the pyramid. Estimates obtained with the contest competition model at 40% did not differ from those obtained with other models of density dependence. At 50% refuge, the contest competition model predicted an average durability that was not statistically different from the estimate obtained with the Ricker model (p-value>0.05). The Logistic and scramble competition model predicted the lowest durabilities at 40% and 50% refuge (p-value<0.05).

Density dependent mortality began to oscillate for all models of population regulation after the refuge proportion was increased passed 20%; increasing refuge percentages is equivalent to an increase in R_0. Figure 2.13 shows the difference in trends between the Ricker and Logistic model at 50% refuge for the high dose pyramid. For the

Ricker model, density dependent mortality took several generations more to increase to levels comparable to the Logistic model. The amplitude of oscillations were smaller with the Ricker model, and there were fewer cycles than with the Logistic model. The delay in population regulation is the mechanism behind the greater durability estimates observed with the Ricker model.

A linear regression analysis for durability and refuge proportions (holding density dependent model fixed) informs that as the available refuge increased, the durability of the low dose pyramid increased as well. Three out of four models of density dependence generated R^2-values >0.9; The R^2-value for the analysis with scramble competition was 0.8896. The linear regression results support that increasing refuge for ECB results in greater durability of low dose pyramids.

Though there is less Bt mortality in the landscape with the low dose pyramid, populations are still suppressed up to a 10% refuge proportion. This is a function of the low overall growth rate for ECB (great overwintering mortality) in combination with the Bt mortality incurred. As for the low dose simulations, there is no difference between results obtained with various models of density dependence until the refuge proportion is increased to 30%. At this point, the scramble competition model predicts the lowest durability for the low dose pyramid (42 generations) (p-value <0.05) (Table 2.5). The Ricker model predicts the greatest durability (44 generations) (p-value <0.05), while contest and Logistics model estimate durabilities that are not statistically different from scramble and Ricker model (p-value>0.05). At 40% and 50% refuge, the Ricker model continues to predict the greatest durability for the low dose pyramid (p-value<0.5). The scramble and Logistics model estimate the lowest durabilities at this point. The contest

competition model has an intermediate durability at 40% (p-value>0.5) and, like the Ricker model, predicts the greatest durability at 50% refuge (p-value<0.5).

The observed trends for density dependent mortality for low dose simulations were very similar to those described for high dose simulations (Figure 2.14). The main difference with low dose exposure was that density dependent mortality for the Ricker equation started out sooner but was delayed for the Logistics model. The combined effect was that density dependent mortality for the two models occurred around the same time but with slightly greater magnitudes.

Table 2.5 Average years to resistance for *O. nubilalis* using a spatially implicit model and different assumptions of density dependence

Species	Dose	DD	% Bt : % Refuge					
			95:5	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**
ECB	HD	R	*176.0 [a]	*367.5 [a]	810.2* [a]	965.3 [b]	1108.8 [b]	1287.8 [b]
		L	*176.0 [a]	*367.5 [a]	808.1* [a]	925.9 [a]	1042.1 [a]	1189.2 [a]
		CC	*176.0 [a]	*367.5 [a]	813.1* [a]	934.9 [ab]	1066.4 [ab]	1249.7 [b]
		SC	*176.0 [a]	*367.5 [a]	812.6* [a]	949.1 [ab]	1047.5 [a]	1178.4 [a]
	< HD	R	*15.5 [a]	*23.5 [a]	39.4 [a]	43.6 [b]	48.9 [b]	56.1 [b]
		L	*15.5 [a]	*23.5 [a]	38.6 [a]	42.1 [ab]	46.7 [a]	52.9 [a]
		CC	*15.5 [a]	*23.5 [a]	38.2 [a]	42.4 [ab]	47.8 [ab]	55.3 [b]
		SC	*15.5 [a]	*23.5 [a]	38.8 [a]	42.0 [a]	47.0 [a]	51.7 [a]

Note: ANOVA results reported for DD comparisons at each refuge proportion (red letters). CC = contest competition; SC = scramble competition; significance level p<0.05; * populations suppressed; Mean fecundity= 290; intrinsic growth rate = 11.2. HD= high dose; W_{SS}=0.01, W_{RS}=0.03079, W_{RR}=1.0, h=0.021 (mode), IRAF = 0.005.Less-than-high dose: W_{SS}=0.2, W_{RS}=0.248, W_{RR}=1.0, h=0.06 (mode).

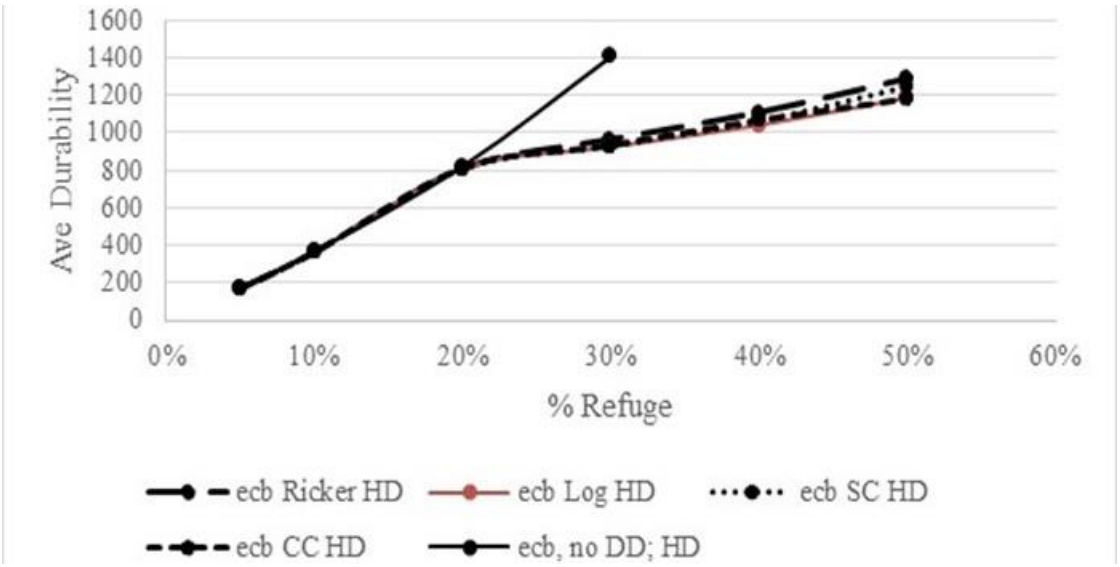

Figure 2.12 Graph of average durability versus % refuge for Ricker, Logistic, Contest and Scramble competition model of density dependence (HD)

Notes: spatially implicit model used; as a reference, the projected durability without density dependence is included and visible from 1%-30% refuge.

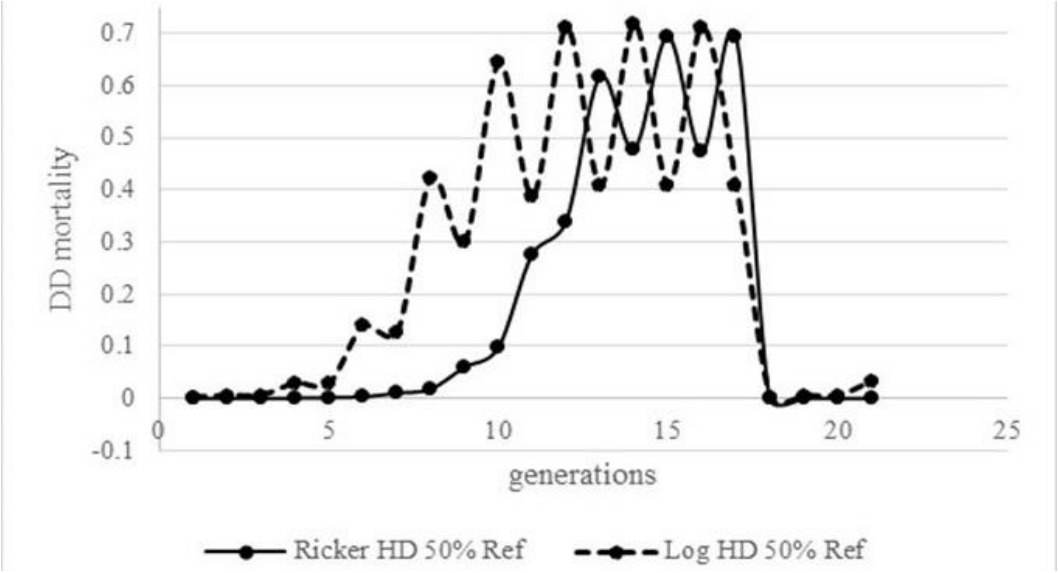

Figure 2.13 Graph of density dependent mortality using Ricker and Logistic models versus generations of ECB with 50% refuge (HD)

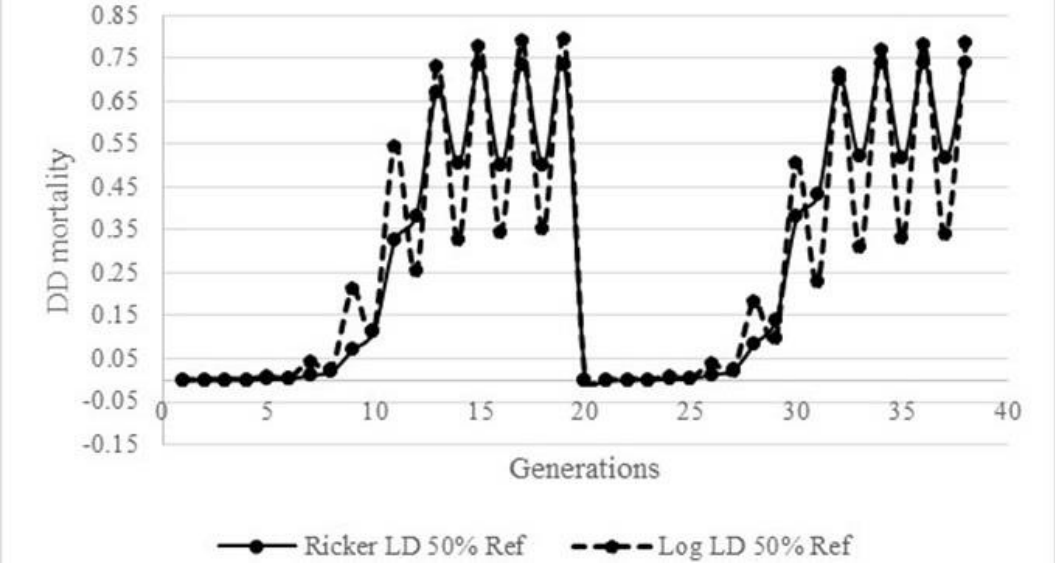

Figure 2.14 Graph of density dependent mortality using Ricker and Logistic models versus generations of ECB with 50% refuge (LD)

2.4.2 Spatially explicit stepping stone model

2.4.2.1 *H. zea*

When dispersal distance for *H. zea* is reduced from five to three fields into each cardinal direction of the landscape, resistance evolved slower and durability of the dual-gene PIP was extended (data not shown) (p-value <0.05). When dispersal distance was held constant but the rate of dispersal was increased from 10% to 90%, resistance typically evolved faster at lower refuge proportions (p-value < 0.05, Figure 2.15, results for high dose pyramid shown only). When the comparison was made with 50% dispersal proportion, then the difference was not statistically significant at 20-30% refuge. For low dose simulations, the difference in durability due to different rates of dispersal was not statistically significant (data not shown, P-value >0.05).

Average time to resistance obtained with the spatial model for *H. zea* showed a similar trend as the results obtained with the non-spatial model for all models of density dependence and different dose assumptions, although numerical results were greater at a particular refuge proportion with the spatial model (holding type of density dependence fixed) (see Table 2.6). A two-way ANOVA (factors: space/no space, refuge proportion) informed that time to resistance differed significantly between the spatial and non-spatial models. Interactions between the factors were significant (data not shown) for high and low dose simulations (p-value <0.05) (exception: Logistic model, low dose).

For two out of four models of density dependence (high dose, there was a significant positive increase in durability of the pyramid when the refuge proportion was increased (R^2 for contest competition >0.9; R^2 for Log = 0.776), which matches the trend observed for the non-spatial model. For simulations using Ricker and scramble competition interactions, there was a significant and decreasing trend in durability of the high dose PIP with increasing refuge proportions (R^2 for Ricker = 0.8179; R^2 for scramble competition = 0.703) (Figure 2.15). For low dose simulations, the durability increased with increasing refuge proportions (Figure 2.16). R^2-values for all linear regression analyses with different models of density dependence (durability ~ percent refuge) exceeded 0.9; the actual time to resistance between each 10% refuge increase was minimal though and typically around one year.

Table 2.6 Average years to resistance for *H.zea* using stepping stone model of dispersal

Species	Dose	DD	% Bt : % Refuge			
			80:20	**70:30**	**60:40**	**50:50**
H. zea	HD	R	87.4 [c]	67.8 [b]	35.5 [a]	12.4 [a]
		L	109.5 [d]	110.6 [d]	123.2 [c]	137.0 [d]
		CC	74.5 [b]	85.7 [c]	97.6 [b]	110.4 [c]
		SC	55.6 [a]	48.6 [a]	41.9 [a]	37.1 [b]
	< HD	R	8.3 [b]	9.3 [b]	10.4 [b]	11.3 [b]
		L	7.8 [a]	8.7 [a]	9.0 [a]	9.7 [a]
		CC	8.7 [c]	9.7 [a]	10.6 [c]	11.7 [c]
		SC	8.7 [c]	9.7 [c]	10.8 [d]	11.8 [d]

Note: landscape is a 10 x 10 matrix of 50 ha fields; ANOVA results are reported for between DD model comparisons at each refuge proportion (red letters). Adult dispersal (50%) is 5 fields away from natal fields in cardinal directions. CC = contest competition; SC = scramble competition. HD= high dose scenario; W_{SS}=0.01, W_{RS}=0.03079, W_{RR}=1.0, h=0.021 (mode). Less-than-high dose scenario: W_{SS}=0.2, W_{RS}=0.248, W_{RR}=1.0, h=0.06 (mode), IRAF = 0.005.

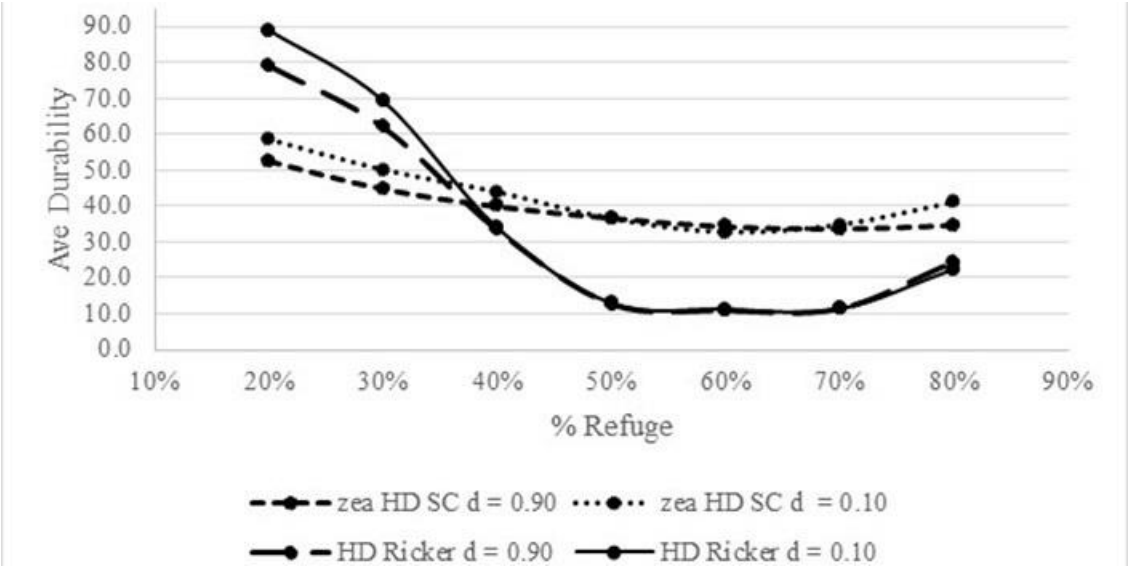

Figure 2.15 Relationship between different dispersal rates for *H. zea* and percent refuge using Ricker and Scramble competition models (HD)

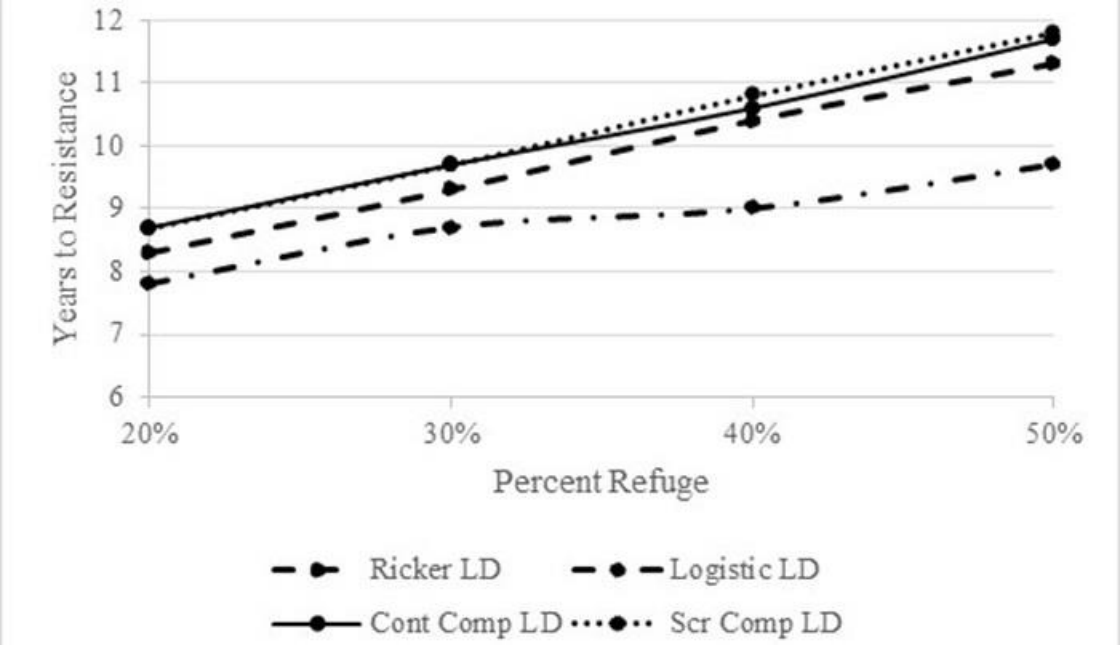

Figure 2.16 Graph of time to resistance vs percent refuge for *H. zea* with intermediate dispersal (LD)

2.4.2.2 *D. virgifera*

For *D. virgifera* (as for *H. zea*), greater dispersal (distance) away from the natal field reduced the overall average durability of the PIP in the landscape (data not shown). But unlike for *H. zea*, two diametrically opposed dispersal rates (d=0.90 and d=0.10) did not result in different durability estimates at any of the refuge proportions tested (Figure 2.19).

The overall trends observed using the spatial model with different assumptions of density dependence were similar to those observed with the spatially implicit model (Table 2.4 vs. Table 2.7). All simulations including density dependent interactions predicted lower durability estimates than simulations excluding intra-specific mechanisms of competition (data not shown). Durability increased with increasing refuge proportions

for high dose PIPs for four of the five density dependent models (R^2 >0.9). The modified Crowder-Onstad equation, however, resulted in a rapid increase in durability after 5% refuge; the durability more than doubled from 5% (352 generations) to 10% (736 generations) and continued to increase more rapidly than predicted with other models tested here. The spatial model with Ricker assumptions predicted increasing durabilities for the high dose PIP up to a 30% refuge and decreasing durabilities thereafter (as the non-spatial model); as refuge percentages increased further, the durability increased once more. A two-way ANOVA identified space as a significant factor that lead to significantly greater durability estimates for all spatial simulations (p-values<0.05); interactions between 'space' and refuge proportions also contributed to observed differences (holding density dependent fixed) (data not shown).

A within-refuge proportion ANOVA identified that the Crowder-Onstad model estimated durability results that differed from those obtained with other models at five out of six refuge proportions reported here ($p<<0.05$). The greatest durability gain for the remaining models occurred by switching from 1% (176 generations) to 5% refuge (346-350 generations), which represented approximately a 2-fold increase in durability. Above 5% refuge, density dependent effects reduced further durability gains, and the durability estimates increased more slowly. There was no significant difference between durability estimates obtained with different models at 1% refuge ($p>0.05$) (Figure 2.17) because intra-specific interactions were minimal with low population densities. As eluded to earlier, the Ricker model predicted a non-linear trend in durability for the high dose PIP with increasing refuge percentages. As the refuge proportion increased to 40 and 50%, the durability decreases rapidly from 442 generations (at 30% refuge) to 261.8 generations (at

40% refuge) and 213.6 generations (at 50%); this was approximately a 40% and 52% loss in durability, respectively, compared to the estimated durability predicted at 30% refuge. The Ricker and scramble competition model describe similar intra-specific competition dynamics, but here they resulted in very different durability estimates after 30% refuge (Figure 2.18). This was likely caused by stronger density-dependent interactions with the Ricker model when the carrying capacity was exceeded as the refuge proportion increased. Density-dependent interactions around the carrying capacity are weaker with Hassell's equation for scramble competition, which is visible in Figure 2.1, and therefore, reduction in durability from density dependent interactions should be comparatively lower.

Low dose durability estimates obtained with the spatial and non-spatial model were very similar and did not differ significantly across most refuge proportions explored (two-way ANOVA). Typically at larger refuge proportions, the differences observed between spatial and non-spatial results were more likely to be significant than at lower refuge proportions where density dependence was not as predominant (data not shown).

Increasing the refuge proportions for the low dose pyramid resulted in a significant increase in durability for all models of density dependence (R^2 for Ricker = 0.688; all others R^2 >0.9). The increase in durability was low for four out of five models between 5% and 20% refuge and was greatest between 40% and 50% refuge (Figure 2.17). This increase in durability was most pronounced for the Crowder-Onstad modified equation, Logistic and Ricker model. Overall, the Crowder-Onstad equation predicted much greater durabilities for the low dose pyramid than the other models of density dependence. Durability estimates obtained with the Logistic model decreased after 50% refuge before increasing once again after 60% refuge (data not shown). Scramble and contest

competition simulations resulted in almost identical durability estimates across the entire range of refuge proportions for the low dose pyramid targeting corn rootworm; the durability lines were mostly superimposed.

Table 2.7 Average years to resistance for *D. virgifera* using a stepping stone model of dispersal

Species	Dose	DD	% Bt : % Refuge					
			95:5	**90:10**	**80:20**	**70:30**	**60:40**	**50:50**
CRW	HD	R	329.2 [a]	347.0 [a]	393.9 [a]	442.2 [a]	261.8 [a]	213.6 [a]
		L	326.7 [a]	348.4 [a]	392.9 [a]	449.0 [a]	519.9 [b]	622.9 [b]
		CC	325.1 [a]	346.4 [a]	386.9 [a]	446.6 [a]	515.3 [b]	620.7 [b]
		SC	330.5 [a]	350.1 [a]	389.8 [a]	446.0 [a]	523.0 [b]	619.7 [b]
		CO	352.0 [b]	736.0 [b]	1004.2 [b]	1147.7 [b]	1347.8 [c]	1622.6 [c]
	< HD	R	19.7 [c]	20.5 [b]	21.1 [b]	24.1 [b]	27.5 [b]	46.5 [c]
		L	19.3 [b]	20.4 [b]	22.2 [a]	24.7 [c]	27.8 [b]	40.0 [b]
		CC	17.6 [a]	18.9 [a]	20.8 [a]	23.5 [a]	26.7 [a]	31.3 [a]
		SC	19.9 [c]	20.6 [b]	22.1 [d]	24.4 [bc]	27.2 [ab]	31.6 [ab]
		CO	30.0 [d]	43.7 [c]	56.6 [c]	65.4 [d]	75.0 [c]	86.2 [d]

Note: Landscape is a 10 x 10 matrix of 50 ha fields; ANOVA results are reported for between DD model comparisons at each refuge proportion (red letters). Adult dispersal (30%) is 1 field and female post-mating dispersal (15%) is 5 fields into four cardinal directions. CC = contest competition; SC = scramble competition; CO = Crowder-Onstad modified equation; R_0= 24.5; HD= high dose scenario; W_{SS}=0.01, W_{RS}=0.03079, W_{RR}=1.0, h=0.021 (mode); less-than-high dose scenario: W_{SS}=0.2, W_{RS}=0.248, W_{RR}=1.0, h=0.06 (mode), IRAF = 0.005

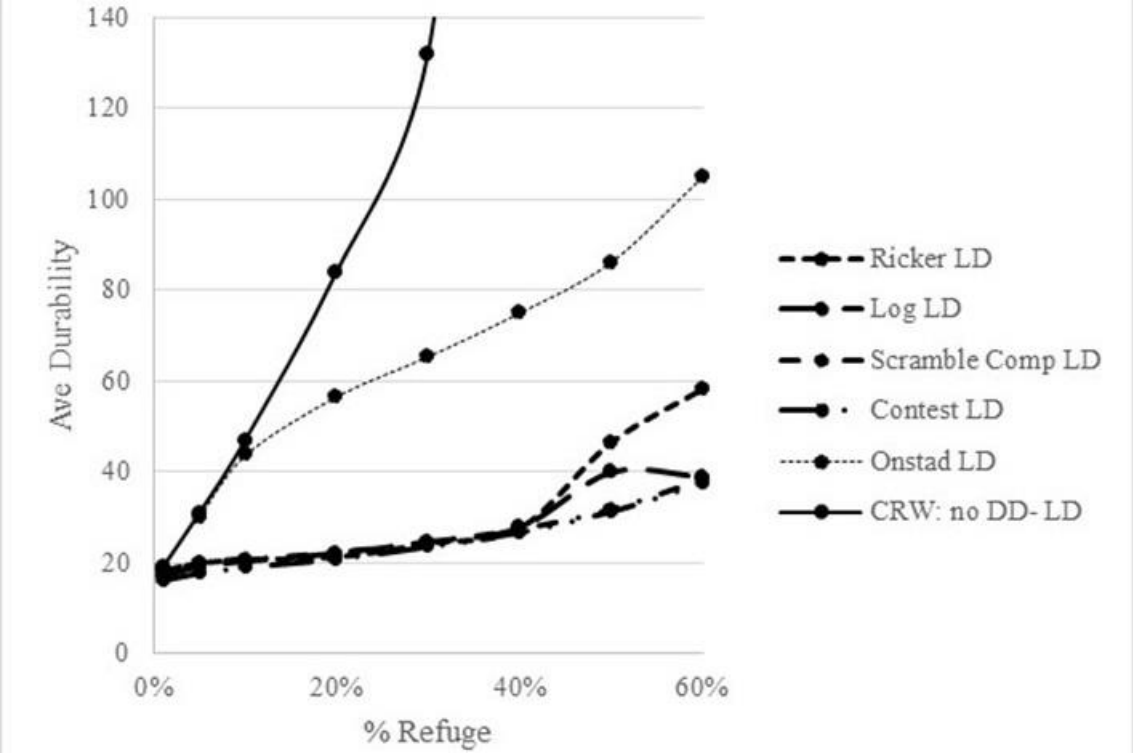

Figure 2.17 Graph of durability for LD PIP vs percent refuge using a stepping stone model of dispersal for *D. virgifera*

Notes: dispersal rate used in simulations was d = 0.3

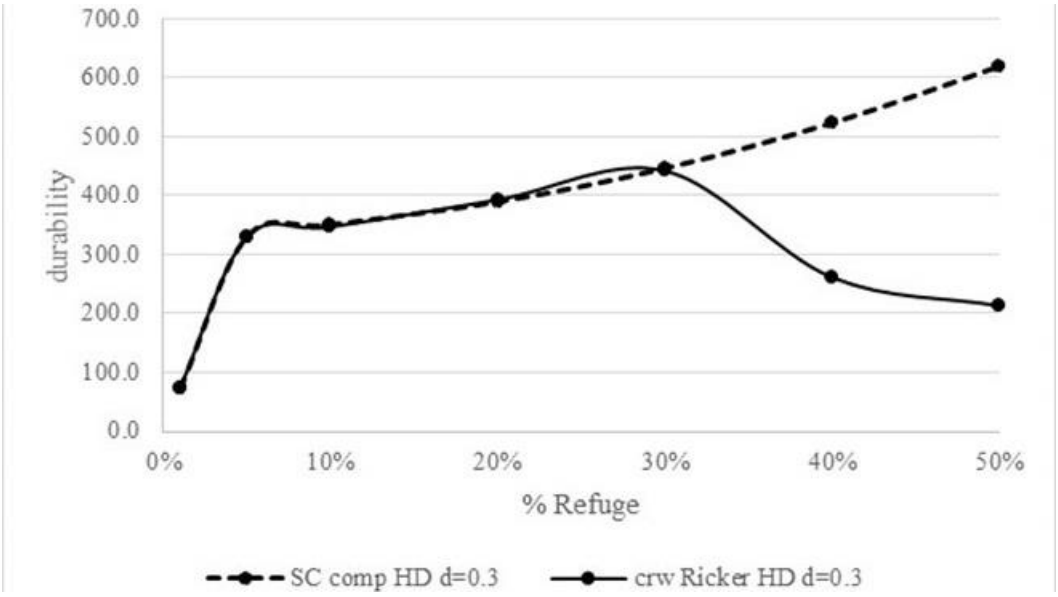

Figure 2.18 Graph of durability for HD PIP vs percent refuge using a stepping stone model of dispersal for *D. virgifera*

Notes: Scramble and Ricker model of density dependence used; dispersal rate d = 0.3.

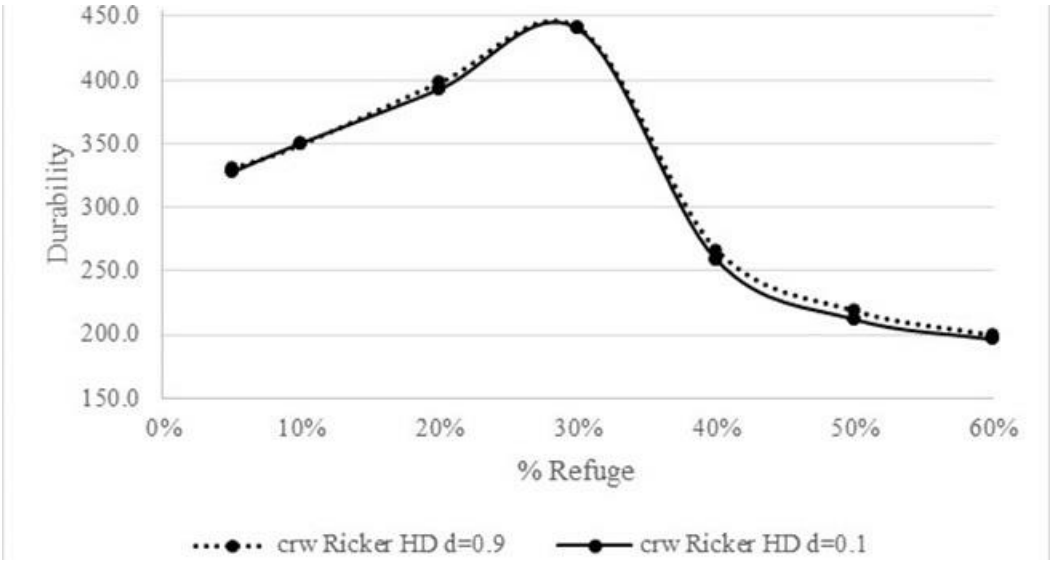

Figure 2.19 Relationship between different dispersal rates for *D. virgifera* and percent refuge using Ricker model of density dependence (HD)

2.4.2.3 *O. nubilalis*

Increasing dispersal distance away from the natal field (from 3 to 5 fields) did not significantly affect durability (high and low dose) at any of the refuge proportions explored in Table 2.8 (P-value >0.05). Likewise, reducing the dispersal distance (from 3 to 1 field) had no significant effect on time to resistance (p-value >0.05). Like for *D. virgifera*, varying the dispersal proportion never affected the durability of the dual gene PIP (p-value >0.05). This is visualized in Figure 2.20 using the Ricker model as an example; the three projected durability lines for low, intermediate, and high dispersal proportion were mostly projected onto each other across the refuge range tested.

When comparing results (high dose, low dose) obtained with the spatial and non-spatial model, it became apparent that significant interactions between the two factors (% refuge and presence/absence of space) were present for all models of density dependence after refuge proportions exceeded 20% and 30%, respectively (data not shown). At this point, percent refuge always had a significant effect on durability, while space had significant effects on durability most of the time (data not shown, p-values <<0.05). Results between the spatial and non-spatial model did not differ at low refuge proportions and when the populations were near extinction. Once density dependent interactions grew stronger at greater refuge proportions (equivalent to an increase in R_0), then there were slight and often significant differences observed between average durability estimates obtained with the spatial and non-spatial model.

A general trend was observed for high and low dose pyramids that durability increased with increasing refuge proportion (R^2 >0.8). For all models of density dependence, each increase in refuge modeled lead to a significantly greater durability

from the previous one. The greatest gain in durability occurred between 10% and 20% refuge for all models of density dependence.

One-way ANOVA informs that for low dose simulations, durability estimates do not differ for various models of density dependence between 5% and 20% refuge (Table 2.8). Density dependent mortality was minimal between 1% and <20% refuge (*DDmort* <0.0005), and population densities were close to extinction below a 20% refuge. Durability estimates obtained with the various density dependent models tracked those obtained without density dependence (results not shown). Visible population regulation did not take place until the refuge proportion increased to 20% and beyond. When the population size began to increase in the block refuge, differences in density dependence became apparent between the various models of population regulation. For example, the Ricker and scramble competition models tended to estimate significantly greater durabilities for the pyramid at 40% (59 generations and 57 generations, respectively) and 50% refuge (51 generations and 49 generations, respectively) than the Logistic and contest competition model (40%, 47 generations and 46 generations, respectively; 50% refuge, 54 generations and 52 generations, respectively) (p-value << 0.05). The growth rates for ECB in the simulations with low refuge percentages suppressed population densities (near extinction) because of the initial assumptions for R_0 in the model and the high Bt proportions in the environment.

Table 2.8 Average years to resistance for *O. nubilalis* using stepping stone model of dispersal

Species	Dose	DD	% Bt : % Refuge					
			95:5	90:10	80:20	70:30	60:40	50:50
ECB	HD	R	176.0* [a]	368.0* [b]	820.8 [a]	1009.8 [c]	1158.2 [b]	1350.8 [d]
		L	175.0* [a]	367.0* [b]	818.0 [a]	998.3 [bc]	1068.4 [a]	1215.4 [b]
		CC	175.0* [a]	367.0* [b]	817.3 [a]	943.9 [a]	1088.4 [a]	1149.3 [a]
		SC	175.5* [a]	367.8 [a]	820.9 [a]	955.9 [ab]	1115.1 [b]	1284.3 [c]
	< HD	R	15.5* [a]	23.0* [a]	38.8 [a]	44.9 [b]	50.8 [b]	59.2 [b]
		L	15.5* [a]	23.0* [a]	38.6 [a]	42.5 [a]	47.2 [a]	53.6 [a]
		CC	15.5* [a]	22.5* [a]	36.7 [a]	40.2 [a]	45.6 [a]	51.0 [a]
		SC	15.5 * [a]	23.0* [a]	38.8 [a]	43.8 [ab]	49.2 [b]	56.7 [b]

ANOVA results are reported for between DD model comparisons at each refuge proportion (red letters). CC = contest competition, b = 1; SC = scramble competition, b = 2.0; significance level $p<0.05$; IRAF = 0.005; * populations suppression. Intrinsic growth rate R_0 = 11.2 first gen, and 2.0 for the overwintering generation. HD = high dose scenario; W_{SS} = 0.01, W_{RS} = 0.03079, W_{RR} = 1.0, h = 0.021 (mode). Less-than-high dose scenario: W_{SS} = 0.2, W_{RS} = 0.248, W_{RR} = 1.0, h = 0.06 (mode). Adult dispersal (30%) in 10 x 10 field matrix is 3 fields into four cardinal directions.

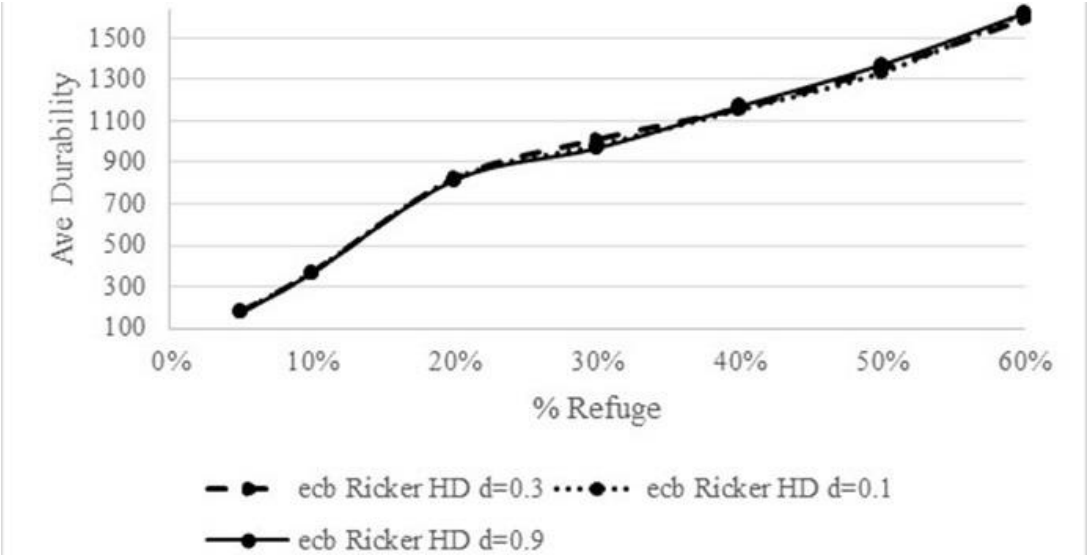

Figure 2.20 Graph of years to resistance vs percent refuge for *O. nubilialis* using a stepping stone model of dispersal (HD)

Notes: Ricker model used in simulations, different dispersal rates graphed (d = 0.1, 0.3, 0.9).

2.5 Sensitivity analysis

A sensitivity analysis was conducted for various life-history and genetic parameters, and a short summary of outcomes is presented here. The initial resistance allele frequency and dominance of the resistance genes are sensitive parameters, and increasing their value, decreased the durability of the pyramids. Greater toxin mortality increased the durability of the PIPs. Fecundity of refuge insects is a sensitive parameter and negatively affected durability. Increasing the refuge proportion was equivalent to increasing the growth rate and lead to slower adaptation rates. When the carrying capacity was lowered, the equilibrium density of the population decreased, which had a slightly negative effect on the durability of the PIP. Increasing the fraction of non-random mating adults was not as sensitive parameter as, for example, dominance and initial

resistance allele frequency. But as non-random mating increased, the durability of the PIPs decreased accordingly.

2.6 Discussion

There are several main points to take away from these explorations. First, when density dependence was excluded from the IRM models, the durability estimates were greatly inflated compared to simulations including mechanisms of population regulation. This was especially true for high dose PIPs (already at low refuge percentages) but also for low dose PIPs (at higher refuge proportions). Second, I reject the null hypothesis that there is no difference in time to resistance with different density dependent models in a simple IRM model when refuge proportions are varied. The type of population regulation chosen by the modeler matters in simple as well as complex IRM models and can significantly impact the predicted durability of PIPs – unless populations are near extinction (or suppressed). In that particular case, the results of a model with and without density dependence should estimate similar durabilities. However, already at low refuge proportions there is the potential for significant differences between simulations with different population regulation models. I show here that different density dependent assumptions can lead to non-identical evolutionary outcomes for both low and high dose expressing PIPs aimed at controlling three agricultural pests with different life-histories. Additionally, I observed that when refuge populations were dramatically reduced in response to population regulation, the resistance allele frequencies in the population targeted by high dose PIPs increased significantly in the following generation. This suggests that after a high pest pressure year the mandated block refuge may not be functioning as envisioned by the high dose + refuge paradigm.

Typically, density dependent effects appeared less predominant at low refuge proportions, and estimated times to resistance were similar between different models of population regulation. Depending on the pest's life-history and the model of density dependence selected, significant differences could still occur at 5% refuge (*e.g.*, *D. virgifera* and Crowder-Onstad equation, see Table 2.7). The results of this research indicate that it is important to understand the type of intra-specific competition of a pest so that an educated decision can be made about which model of population regulation to include in IRM simulations. If this is neglected, then false (relative) durability estimates for a PIP may be obtained, which could result in incorrect management or refuge decisions. Furthermore, at refuge proportions greater than 5%, much greater variation was observed between estimated times to resistance from different models of density dependence. The durability of a low dose PIP increased with increasing refuge proportions (although significant differences may be present between different models of density dependence), but this was not always the case for high dose simulations. For example, the Ricker and/or scramble competition model resulted in lower durability estimates at greater refuge proportions for *H. zea* and *D. virgifera* (but not so for *O. nubilalis*). Since, *H. zea* does not exhibit this type of population regulation, these results are less of concern. For *D. virgifera*, however, where scramble competition interactions are assumed to occur, these results are more disturbing. Hence, if a high dose pyramided PIP should become available for commercialization targeting this particular pest, careful analysis will be needed to assure that the best possible refuge requirements are implemented to extend the life-time of the technology as long as possible. The general recommendation to EPA to increase the refuge for resistant corn rootworm (Tabashnik &

Gould 2010; Andow et al. 2014) may not be an effective strategy in all situations and needs to be more closely evaluated.

Based on what is known about the larval behavior of the three pests investigated here, I recommend that the following models of density dependence be used: the contest competition model for *O. nubilalis* and *H. zea*; and the scramble competition model for *D. virgifera*. The Crowder-Onstad equation can be used as well since it is derived based on empirical data. In this case, however, I would recommend to use a modified form where density dependence is applied to larvae (instead of eggs) and separated between Bt and non-Bt compartments. In the models used here, the modified Crowder-Onstad equation also lead to greater durability estimates for all doses and greater relative differences between refuge proportions, and it may be beneficial to use two different models of density dependence and compare relative durability differences.

Graphs of density dependent mortality (1-*DDSurviv*) vs number of generations showed different trends over time depending on the model of population regulation selected or the kind of pest modeled (Figure 2.9 vs. Figure 2.13). For example, the Ricker model for corn rootworm resulted in a sigmoidal curve for density dependent mortality vs time, while the same model for European corn borer resulted in oscillations (regular or irregular depending on refuge proportion modeled and effective R_0). This strengthens the argument that the type of population regulation for pests with different life-history dynamics can result in different predictions for pest adaptation rates and needs to be considered when developing IRM plans.

The trends observed in spatially explicit and implicit models as a function of density dependence and increasing refuge proportions were typically comparable.

Increasing the dispersal distance for *H. zea* and *D. virgifera* reduced the predicted durability but had no significant impact on the adaptation rate for *O. nubilalis*. Greater dispersal proportions also reduced the durability estimates for PIPs targeting *H. zea* compared to lower proportions, but not so for the other two species.

In conclusion, these results show that the form of density dependence selected can significantly affect the time to resistance for both simple and complex IRM models. Inclusion of space combined with explicit pest dispersal can at times lead to lower or higher PIP durability. Therefore, significant consideration should be given to realistically address the density dependent population growth and regulation of any pest, preferably with empirically obtained dispersal information, to design the most functional insect resistance management plan.

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CHAPTER III

EFFECTS OF VARIOUS LIFE-HISTORY FACTORS, DOSE OF Bt TOXINS, AND REFUGE STRATEGIES ON THE SPREAD OF RESISTANCE GENES: IMPACTS ON POTENTIAL REMEDIAL ACTION STRATEGIES

3.1 Abstract

Different life-history characteristics, refuge strategies, and dose of *Bacillus thuringiensis* (Bt) Plant-Incorporated-Protectants (PIPs) affected the adaptation rate of a generic, diploid pest with sexual reproduction in various ways and at times elicited unexpected results. The life-time of a high dose single PIP was reduced to similar levels as for a low dose single PIP if the pest had a high growth rate. The main cause for these results was the disproportionately occurring population regulation in refuge and Bt fields with high growth rates coupled with simultaneously occurring selection. These results suggest that the high dose + refuge functionality may be compromised when the target pest has a high growth rate.

Presence of density dependence was the second most important parameter affecting resistance evolution, and simulations including population regulation projected much higher resistance allele frequencies in the landscape than those excluding density dependent mechanisms. Significant interactions between density dependence and growth rate were always present and further reduced the durability of the PIPs. Contest competition predicted greater durability for a PIP with low pest growth rates than

scramble competition because population regulation effects were similar on refuge and Bt plants. When the pest growth rate increased, density dependent effects became more discordant with contest competition assumptions, and scramble competition projected significantly greater life-times for the PIPs. Varying the average distance dispersed from the natal field to other cells in the landscape did not affect evolutionary outcomes. The percent of population leaving the natal field often had significant interactions with other parameters such as growth rate and density dependence and, if increased, extended the durability of especially high dose PIPs.

For the majority of the simulations, a 20% block refuge extended the durability of a PIP over that with a 10% RIB. This was a function of the lower effective growth rate in RIBs because of fewer available refuge plants but also greater susceptible genotype mortality caused by inter-plant movement (non-Bt to Bt). When the growth rate was increased, these often visible differences vanished, especially for high dose PIPs.

The spread of the resistance gene was investigated from a hypothetical hotspot throughout the landscape over three generations for single PIPs. Local resistance phenomena were always apparent and spread into the landscape with each passing generation. The average resistance allele frequencies were highest in areas immediately surrounding the resistant site with a low pest growth rate yet lower in the remainder of the landscape. With a high pest growth rate, the average resistance allele frequency around the hotspot was lower compared to simulations with low growth rate assumptions but higher in the fields farther removed. These results imply that resistance genes can be expected to spread fastest through the landscape if the pest has a high growth rate and is exposed to a high dose PIP. Increasing the refuge percentages for RIBs and blocks from

the standard 10% and 20% to 50% slowed the adaptive process for the pest when exposed to single PIPs and low dose pyramids in the landscape over the three generations tested. For a high dose pyramid, this phenomenon was not observed in the matrix. This was a result of using two pyramided Bt toxins that kept the resistance population from building up large numbers and consequently reduced the likelihood of dispersing resistant genes.

3.2 Introduction

The US Environmental Protection Agency's Office of Pesticide Programs requires Bt technology providers (registrants) to conduct monitoring for resistance to target pests as part of the terms and conditions of registration for Bt corn and cotton (US EPA 2010). One aspect of monitoring involves following up with unexpected damage reports from growers, extension agents, consultants, or company agronomists. Unexpected damage to Bt crops can reveal localized cases of resistance or be the result of favorable environmental conditions. The Agency leaves it to industry to decide what threshold constitutes unexpected damage from Lepidoptera feeding in Bt corn and cotton. Typically growers and companies use regional economic thresholds to identify unexpected damage. For *Diabrotica* species, the terms and conditions of the Bt corn registration try to address what constitutes 'unexpected damage', and threshold triggers have been put in place by the Agency. When plants expressing a single Bt PIP have one or more nodes removed from the root system, then the terms and condition state that 'unexpected damage' has occurred. For pyramided PIPs (two or more toxins targeting the same pest system), this threshold is set at $\geq$ 0.5 nodes removed. Unexpected damage (UXD) from *D. virgifera virgifera* is identified in the Bt field by growers as an area with an unusual amount of lodged plants. When registrants receive reports of unexpected

damage, they are required to investigate the cause of lodging. The Agency's intent here is to discover, confirm, and contain resistance in localized areas before it can spread across the landscape. The general criticism, however, is that the current regulatory process is too protracted and that resistance can spread and establish before it can be confirmed (EPA 2013). For example, when companies visit damaged fields, they first have to rule out the possibility that other factors could have caused the lodging (*i.e.,* environmental factors). If the damage stems from *D. virgifera*, then registrants have to collect insect samples for rearing and subsequent bioassays to either refute or confirm resistance in that pest population (EPA 2011). Often though, adults have already dispersed when unexpected damage is investigated, or growers may have already taken alternate measures to treat high target pest abundance (*e.g.,* chemigation). In these cases, technology providers must collect insect samples at the site of concern the following season and start the process of confirming or refuting resistance the next season. Obligate diapause of *D. virgifera* eggs is a further factor that delays the process of confirming resistance. Diet bioassay methods currently lack sensitivity to clearly discern between resistant and susceptible populations and have not shown to be a proactive resistance detection tool (US EPA 2013). On-plant assay methods have, however, been developed (Gassmann et al. 2011, Nowatzki et al. 2006) and show promise at identifying resistant populations with greater certainty (US EPA 2013).

'Confirmed resistance' is another regulatory action trigger defined by the Agency that must be met before remedial action is required (EPA 2010). Once this has happened, however, registrants must notify the Agency within 30 days of having made the discovery, and the generic remedial action plan in place is initiated. A specific remedial

action plan must then be worked out between the Agency and industry within 90 days of confirming resistance.

When all these delaying factors are considered together, it can (at best) take up to two or (at worst) more years to confirm resistance for *D. virgifera* and before a general remedial action plan is initiated (US EPA 2013). Likewise for Lepidoptera, when unexpected damage has been confirmed by industry, the timing may not allow to collect insects that year, and collections would need to be made the following year. The question has been posed whether a delayed reaction time provides enough opportunity for successful remediation of resistance.

I propose to theoretically explore what impact various life-history and behavioral factors have on the simulated spread of the resistance gene of a generic arthropod pest of Bt in the landscape 2-3 generations after unexpected damage (indicative of resistance) has first been detected in one site. I examine how these factors affect the evolution of resistance to a low and high dose single PIP deployed with two different IRM strategies and simulating a generic insect pest of Bt. The main variables investigated are proportion of population dispersing (AD), redistribution constant (D), intrinsic growth rate (R_0), and type of density dependence (DD). The IRM strategies included in the analysis are block and seed blend refuges (Refuge-In-The-Bag, RIB). Second, given different pest and IRM conditions and their effect on the spread of resistance in the fields surrounding the UXD site, I discuss the timing of remediation as well as the geographic scope in response to resistance. The stochastic, spatially explicit simulation model used in the analyses has a probabilistic and deterministic mode, both of which were employed.

3.3 Materials and methods

3.3.1 Model specifics

A one-locus, stochastic, spatially explicit, and frequency-based population genetics model was written in Java (using NetBeans IDE 7.4) to explore the effects of two refuge strategies, various dispersal rates, dispersal kernels, intrinsic growth rates, and types of density dependence on the time to resistance evolution in the landscape and the spread of the resistance gene for a generic arthropod pest of Bt. The landscape consisted of a 51 x 51 field matrix (one cell represents 50 ha of a hypothetical Bt crop with 80,000 plants/ha) and was designed as a torus, which makes every field the center field of the landscape and all cells are, therefore, identical in the matrix. It also makes this assumption that this torus lies in a system that is surrounded by identical toruses to all sides. For the purpose of simplification, no specific life-history was simulated. Based on the one-locus model, a two-locus model was also developed to explore whether some results of interest for the one-gene PIPs would also apply to a two-gene PIP (high and low dose).

The specific model and structure have been previously described (see Chapter 2). A key difference between these spatially explicit models and the previous ones used is that dispersal was simulated with a two-dimensional redistribution kernel rather than with stepping stone dispersal. The type of Gaussian diffusion used here is the solution to Fick's equation of diffusion in two dimensions (Okubo 1980) and a tool I used to create a probability distribution for those adults that dispersed beyond the natal field boundary (excluding trivial dispersal within a field from the process). To clarify, a fraction of the population '□' left the natal field and engaged in inter-cell dispersal using a single,

discrete convolution of the kernel (repeated application of the kernel to all fields in the landscape and means of neighborhood averaging). For simplicity, I assumed that mortality during dispersal was negligible. The remainder of the population, 1-□, stayed in the natal field and engaged in trivial motion. The model's combination of inter- and intra-field dispersal produced a leptokurtic distribution of movement consistent with general empirical observations among plants and animals where a fraction of individuals in the population engage in greater dispersal than others. All redistribution kernels were normalized by dividing each cell of the kernel by its sum so that the distribution summed to 1 (Slone 2011) and kept the population abundance constant; the model, therefore, represents a closed system, and no individuals were lost (or created) during the dispersal process. The motion of each individual was random and independent of the motion of all other individuals dispersing. This type of movement is also referred to as 'random walk' (or flight) dispersal where no other external factors affect the path an individual takes. Of course, this is an oversimplification of what can occur in the field where environmental conditions as well as chemical signals can facilitate or hamper the dispersal process. The equation for two-dimensional movement provided a probability distribution of individuals through space (distribution kernel function) and when multiplied by the number of dispersants from a particular location (Y_0), assigned a number of individuals (y(x, y)) into the landscape based on the distance from the place of origin:

$$y(x,y) = y_0 \frac{e^{-(x^2+y^2)/2(2D)}}{2\pi(2D)} \tag{3.1}$$

The average distance an individual moved is the standard deviation $\sqrt{2D}$ (or $\sqrt{variance}$) and measured in grid cell units; D represents the redistribution constant (or

diffusivity). Values for this parameter were explored at 3, 10, and 15 resulting in average displacements of 2.5, 4.5, and 5.5 fields/generation (low, medium, and high values for intermediate dispersal). Greater and smaller values for D (and$\sqrt{2D}$, respectively) were explored in the sensitivity analysis. The distribution of individuals during a time step was normal with a variance of 2*D*. If *D* is small (<1), the normal curve has a small variance, and most individuals dispersing will be close to the release point. As the value of *D* increases, the variance increases and the normal curve widens because more individuals will move farther away from the release point. In my model, redistribution of individuals took place after mortality and density dependence occurred (the latter occurred at the juvenile stage) but before adult mating was initiated. It is important to note that the process of movement was intentionally modeled separately from mortality and reproduction, so that other demographic processes could be investigated.

Stochasticity was added at the beginning of a simulation by initializing the population size in each field with an egg density randomly drawn from an interval between 0 and 40,000,000. The upper range translated into an egg density of 10 eggs per plant. The initialization with unequal population densities in each field allowed for potential source sink dynamics in the landscape. The stochasticity added by sampling for initial population can be viewed as adding to 'between field' variation. The remaining variability in the system was introduced by sampling from a PERT-Beta distribution with predetermined, parameter-specific ranges (modes, minimum and maximum values) and held fixed for a particular simulations (Vose 2001). Contrary to stochasticity, this type of sampling added to between-simulation variability. The range and mode values for the parameters that were varied are listed in Table 3.2. The egg carrying capacity was fixed

at 18 million eggs per field and translated into 360,000 eggs per hectare (or 4.5 eggs/plant). The egg carrying capacity was used when the projected population size at time t+1 was calculated in the density dependent submodel (see description of density dependence in Chapter 2). Effects of different types of density dependence (referred to as DD later on) were explored using the Hassell equation (1975, $a = (R_0^{\frac{1}{b}} - 1)/K$)) for contest and stable limit cycle (period = 2) competition (here, referred to as scramble competition), two levels of intra-specific competition:

$$NextGenNrEggs = NrEggs \cdot R_0 \cdot (1 + (R_0^{\frac{1}{b}} - 1)/K) \cdot NrEggs)^{-b} \quad (3.2)$$

The constraints between R_0 and b for contest competition and stable limit cycle competition are given by the following, respectively:

$$1 = (1 - R_0^{\frac{-1}{b}}) \cdot b \quad (3.3)$$

and

$$2 = (1 - R_0^{\frac{-1}{b}}) \cdot b \quad (3.4)$$

Given R_0, the value of b resulting in the desired level of competition was calculated.

The simulations were conducted for a hypothetical low and high dose PIP deployed with a block refuge and RIB. The default refuge proportions were 20% and 10% for the single PIP deployed with a separate block refuge and RIB, respectively, when exploring the effects of the various parameters on the average resistance allele frequency in the landscape; these proportions were concordant with EPA's currently mandated requirements for single Bt PIPs (US EPA 2010). Grower non-compliance for

block refuges was not considered in these simulations but if included, they would have lowered the durability estimates reported for blocks.

Base larval movement was simulated in the RIB according to Mallet & Porter (1992) and oviposition by adults onto Bt and refuge plants represented the first movements step. In a single PIP RIB, 90% of eggs were laid onto Bt plants (80% in Blocks) and 10% of the eggs onto non-Bt plants (20% for Blocks). Larval inter-plant movement occurred once and represented the second movement step. Base larval movement '*M*', or the probability that an immature dispersed in the seed blend, was varied between 10-40% with a mean of 30%. The probability that a larva remained on the plant of origin was '1-*M'*. The parameter '*V*' was the probability that an immature landed on a Bt plant, and '1-*V*' described the probability that it reached a non-Bt plant. The value for this parameter was set by the Bt and refuge percentages, respectively (90% vs. 10%). No movement penalty was included in these simulations, though it seems plausible that a moving individual faces a probability of death because of, for example, environmental conditions or predation while dispersing. The effect of different movement mortalities on the durability of RIBs could be explored in future simulations.

3.3.2 Pest specifics

The generic arthropod pest modeled here was diploid, reproduced sexually, and had one generation per year. It was assumed that resistance was governed by a single locus with a major resistance gene having two alleles (R for resistance and S for susceptibility) and three genotypes (RR, RS, and SS). A hypothetical high dose scenario with recessive inheritance was modeled. Mean mortality for homozygous susceptible, heterozygous and homozygous resistant genotypes was 0.99 (W_{SS}=0.01), 96.92%

(W_{RS}=0.03078, h=0.0211), and 0% (W_{RR}=1.0), respectively. A less-than high-dose scenario was also modeled, and in this case the mean mortality was 80% (W_{SS}=0.20), 72% (W_{RS}=0.28, h=0.1), and 0% (W_{RR}=1) for homozygous susceptible, heterozygous resistant and homozygous resistant genotypes, respectively. Total survival for an individual exposed to a pyramid was determined by multiplying the two fitness components for both loci. Dominance '*h'* in these models described the level of fitness for the heterozygous resistant genotype based on the fitness of the homozygous susceptible and resistant genotypes when exposed to the insecticidal PIPs, and referred to the definition given by Bourget et al. (2000). The dominance and fitness calculations have also been described previously (Chapter 2). No cost to resistance was included in the generic models but can be assumed to slow the adaptation rate and increase time to PIP failure. No specific mortality or life-history stages were modeled (e.g., overwintering mortality, immature stage, etc.) but an overall survivability (0.5) was applied at the egg stage and before selection occurred. Density dependence was explored for contest competition and scramble competition (described in section 3.3.1) and applied after selection (described in Chapter 2). Mating occurred randomly within a cell (50 ha).Fecundity F was calculated as the ratio of the intrinsic growth rate (multiplied by a factor of two to account for females only) and survivorship ($2R/s$) and was used to calculated the number of eggs generated by the female population in the next generation. R_0 was varied between a mean of 10 and 30, which resulted in a mean offspring range of 40-120 individuals per female (50% of which survived to adulthood). Adult dispersal was assumed to be equal for males and females, occurred before mating, and no pre-ovipositional dispersal was considered.

3.3.3 Scenarios

3.3.3.1 Generic exploration of different parameters on Resistance evolution

Thirty simulations were run for each combination of dose of toxin (low and high), refuge strategy (RIB and block), and life-history characteristics (growth rates – three levels, redistribution – three levels, dispersal proportion – two levels, and type of density dependence (contest vs scramble competition, referred to as CC and SC later on)) to determine effects on time to resistance in the generic pest of Bt. All simulations were terminated when the resistance allele frequency reached 0.5 or greater. At that point, the generation time and average resistance allele frequency in the matrix were recorded. For each simulation, the following generational output was also stored: change in egg population size, egg load in the refuge and Bt compartment at the beginning of a generation, larval density after selection and population regulation, density dependent survival in the refuge and on Bt, resistance allele frequencies at the beginning of a generation, after selection, and density dependence, and adult densities.

3.3.3.2 Spread of resistance gene in landscape and effects of mitigation

Refuge proportions for mitigation simulations were modeled at 20% and 50% for Blocks and 10% and 50% for RIBs because it has been suggested in the context of corn rootworm resistance that increasing the amount of available refuge was one of several remediation strategies (Andow et al. 2014, Tabashnik & Gould 2011). The discussion about success of remediation 2-3 years (or generations) after resistance was observed (as UXD and verified with diagnostic assay) was based on results of an analysis of the spread of the resistance gene in the landscape over several generations. The average resistance allele frequency was calculated in seven square field sections, where the respective areas

shared the same average distance from the failed field (1st section = fields immediately surrounding UXD, 2nd section = two fields removed from UXD site, etc.) and compared to the resistance allele frequency expected when no resistance was established in the landscape; the fields were not equi-distant from the hypothetical hotspot. These simulations were run without probability sampling to reduce the amount of variability in the system and to identify the nature of the spread of resistance for each IRM-dose-life-history combination. For these simulations, the egg load was randomly initialized between 0 and 18 million (the carrying capacity) in fields without resistance. A large population size was created by initializing the UXD site with 40 million eggs and as to link visible resistance with a high population density. This egg load resulted in 2.2 times more adults in the field with resistance. Two extreme cases of dispersal and growth rate were evaluated to assess whether the spread of the resistance allele was affected. For a pest with low mobility and intrinsic growth rate, the values were set to $D_1 = 3.0$, $AD_1 = 0.30$, and $R_1 = 10.0$; for a pest with greater mobility and intrinsic growth rate, these values were $D_3 = 15.0$, $AD_2 = 0.50$, and $R_4 = 40.0$. The initial resistance allele frequencies were set to mean value of 0.005 for all fields, except in the site where resistance occurred and unexpected damage became visible. There, I assumed a resistance allele frequency of 10%, which is a level of resistance that could be visually detected with a relatively small sample size and diagnostic bioassay methods (Roush and Miller 1986). The shaded square sections in Figure 3.1 visually depict the locations from which the average frequencies for the resistance allele were calculated. The inner section denotes the location of the hypothetical hotspot ([x, y] = [25][25]) and from where resistance spread (indices for field array started at 0 and ran through 50).

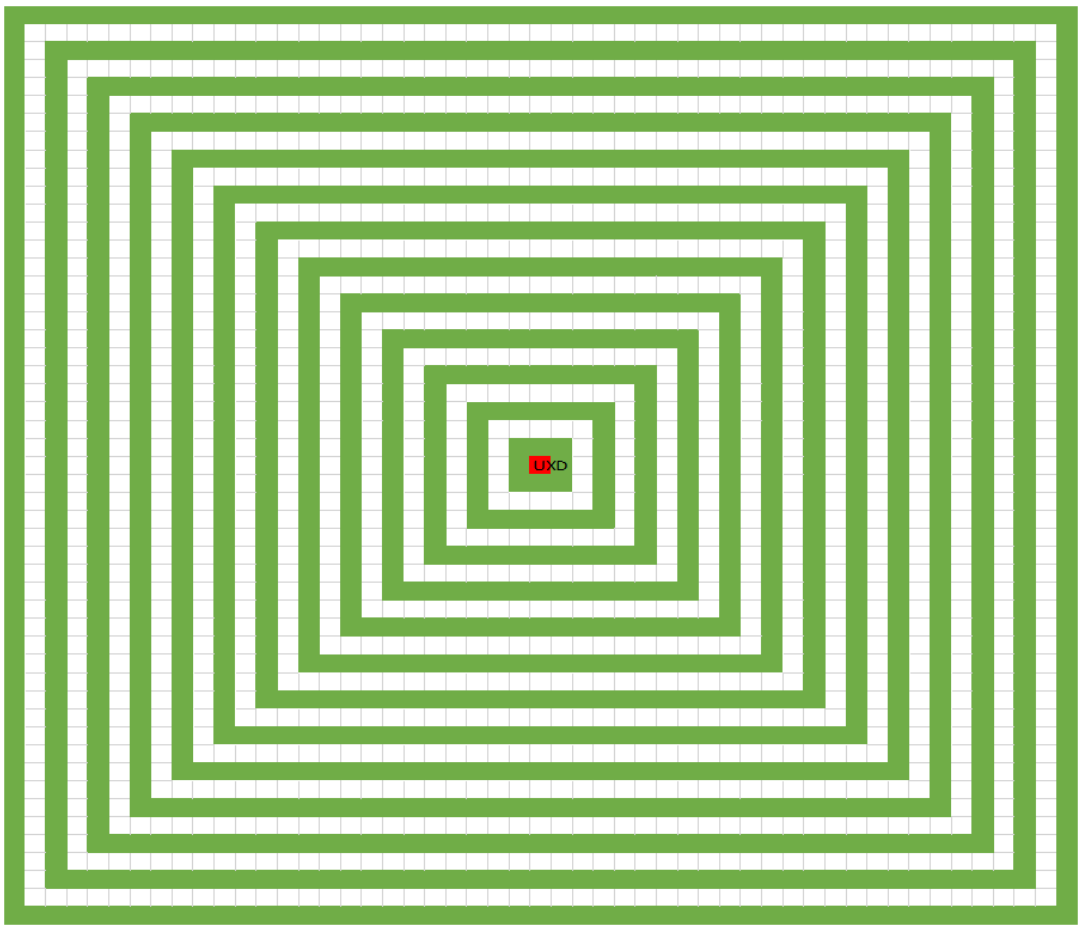

Figure 3.1 Landscape of 51 x 51 fields and rings around the resistant site with 10% resistance and very high population density

Notes: UXD = unexpected damage site with visible resistance

3.4 Quality control in model development

To assure that the generic model behaved according to expectations, I ran scenarios for blocks and RIBs at the highest growth rate R_3, with contest competition assumptions and without density dependence and for a 20% refuge (although this does not reflect the mandated RIB refuge), while all variability in the system was turned off. As expected, simulations without density dependence resulted in greater durability than when population regulation was turned on; this was observed for high and low dose,

RIBs and blocks. RIB simulations predicted lower durabilities than block simulations with density dependence excluded. For high dose simulations at 20% refuge, blocks and RIBs simulations estimated 26 and 22 years of durability for the single PIP (similar relationship for low dose assumptions but lower estimates). This was a function of the added inter-plant movement mortality. When density dependence was turned back on, blocks and RIBs performed approximately equally well because of greater density dependent effects in the block refuge for the single PIP. The overall durability was lower than without density dependence (blocks and RIBs 7 and 8 years for high dose PIP). Why low dose single PIPs performed better than high dose single PIPs during these quality control simulations will be further explored in the following sections.

When the refuge was increased to 50% for both IRM strategies (w/out DD), again block refuges outperformed RIBs. High dose blocks projected 88 years of durability, while RIB deployments resulted in 75 years durability. When density dependence was added into the system, the two strategies performed approximately equally well; block and RIB simulations projected 11 and 12 years, respectively. A similar trend was observed for low dose assumptions.

Table 3.1 Deterministic simulations for IRM strategies including and excluding population regulation and varying growth rates and refuge proportions.

Refuge and Growth Rate	**HD**		**LD**	
	Block	**RIB**	**Block**	**RIB**
20% Ref, R_3, **no DD**	26	22	22	19
20% Ref, R_3, **with DD**	7	8	14	13
50% Ref, R_3, **no DD**	88	75	53	44
50% Ref, R_3, **with DD**	11	12	21	19

3.5 Data analysis

At the beginning of each simulation run, a PERT-Beta probability analysis was conducted by sampling values for the intrinsic growth rate, base larval movement in the RIB, adult dispersal proportion, dominance of the resistance allele, survival of the susceptible genotype, and initial resistance allele frequency from a predetermined range while weighting the mean by four (PERT-Beta process described in Chapter 2). The minimum, mean and maximum values set for each PERT-Beta distribution are listed in Table 3.2.

I analyzed my data using R software (R Core Team, 2013, package version 3.0.2). Multi-way ANOVAs were conducted for each IRM strategy for R x DD x D x AD to determine interactions. Pair-wise comparisons of means were conducted for variables involved in interactions using Tukey contrasts. A bootstrap analysis was used to compare the estimated times to resistance obtained with block refuges and RIBs to determine whether the 95% confidence interval for the ratios of the distributions at the 5% quantile differed significantly when parameters of interest were sampled from identical PERT-Beta distributions. Here, I used the 'adjusted percentile method', or BCa, (Davison and Hinckley, 1997) to estimate confidence limits.

Effects of parameters on the spread and increase in resistance allele frequency in the landscape were assessed with the deterministic mode three years and one year after unexpected damage in Bt was first detected as to avoid noise but keeping the stochasticity for initializing the population density per field intact. This approach allowed detecting patterns of r-frequency distributions in the landscape that were caused by the parameters investigated. The average resistance allele frequency was subsequently calculated in

square sections around the damaged field (see Figure 3.1) and in the entire landscape in the F1 generation of adults responsible for the unexpected damage (year/generation = 1) and in the next two generations (year = 2 and 3) and compared to the frequencies that would be expected in the same locations if no resistance was present (r-freq = 0.005). Block refuge results were compared at 20% and 50% refuge; RIB results were compared at 10% and 50% refuge. The effects of increasing the refuge on the resistance allele frequencies in pests with different growth rates and dispersal propensities were explored with the single and dual gene model.

Table 3.2 Parameters sampled from PERT-Beta distribution

Parameters	Min	Mode	Max	Comments
R_1	5.0	10.0	15.0	Lowest value tested
R_2	15.0	20.0	25.0	Intermediate value tested
R_3	25.0	30.0	35.0	Highest value tested
BLM	0.10	0.30	0.40	Base larval movement in RIB
Dominance –HD	0.001	0.021	0.05	Assumptions for generic single PIP
Dominance –LD	0.05	0.10	0.20	
SS survival –HD	0.90	0.99	0.999	
SS survival –LD	0.75	0.80	0.85	
IRAF	0.001	0.005	0.01	
D_1	1	3	5	Generic species adult redistribution kernel
D_2	8	10	12	generic species adult redistribution kernel
D_3	12	15	18	Generic species adult redistribution kernel
Adult dispersal frequency (1)	0.10	0.30	0.50	Small proportion of population engaging in dispersal
Adult dispersal frequency (2)	0.30	0.50	0.80	Greater proportion of adults engaging in dispersal

3.6 Results

3.6.1 Effects of parameters on time to resistance

3.6.1.1 High dose results

I conducted a four-way ANOVA for a high dose PIP deployed with different IRM strategies and varied growth rate R_0, diffusivity *D* and dispersal proportion AD, and

density dependence (CC and SC) (Tables 3.3). For blocks, I found that there was a significant three-way interaction between proportion dispersing, type of density dependence, and growth rate (AD x DD x R) (p-value = 0.045061) including two additional two-way interactions for AD x DD and DD x R (Appendix A, section 1.1.1). For the RIB analysis, I found that there were also significant interactions between the proportion dispersing and growth rate (AD x R) (p-value = $8.689e^{-05}$) and type of density dependence and growth rate (DD x R) (p-value = 0.0003162) (Appendix A, section 1.2.1). All main factors, except D, were also significant for both RIB and block simulations. Interestingly, the dispersal distance did not affect the results in any of the high dose simulations.

A multiple comparison of means was conducted for all the variables involved in the interactions and are presented in Tables 3.4 (blocks) and Tables 3.5 and 3.6 (RIBs). When the pest exhibited contest competition and was exposed to a Bt/block refuge environment, increasing the dispersal proportion did not affect the time to resistance for any of the growth rate values tested. With scramble competition dynamics, however, at the lower and intermediate growth rate value, an increase in durability could be observed by increasing the proportion of adult pests engaged in dispersal (Appendix 1.1.1). The means comparison for the RIB interaction, DD x R, show that there was no difference in projected time to resistance for the pest at a lower growth rate for either contest or scramble competition assumptions. At the intermediate and high value for growth rate, it was simulations modeling scramble competition that projected greater life-times of the PIP compared to contest competition (Table 3.5). These results support that at a higher pest growth rate, the durability of the PIP deployed with a RIB was greater when the pest

exhibited scramble rather than contest competition. The means comparison for AD x R show that increasing the dispersal proportion from the lower to the higher value did not increase the durability of the PIP (or time to resistance) at the lower growth rate. At the intermediate and higher pest growth rate value, however, the time to resistance (or durability of the PIP) increased significantly (Table 3.6) (Appendix 1.2.1). These results support that greater dispersal proportions with higher pest growth rates delayed resistance evolution in a RIB environment.

I conducted one-way ANOVAs by varying the growth rate and holding diffusivity fixed to determine the effects on the durability of the high dose PIP deployed with different IRM strategies and assumptions of density dependence (a total of 12 comparisons each for blocks and RIBs) (Table 3.3). Results for RIBs and blocks show that in all 24 comparisons the lifetime of the high dose PIP was greatest when the pest had a low growth rate. When the growth rate increased from the lowest to the highest value tested, the durability decreased in some cases up to 245%. In some cases, the durability also decreased when the growth rate was switched from an intermediate to high value (typically at intermediate diffusivity D_2); in other cases, there were no statistically significant differences when R_2 was increased to R_3. For block deployments, the decrease in the life-time of the high dose PIP ranged from approximately 85% to 245% - with contest competition assumptions leading to greater losses in durability than scramble competition (ANOVA results see Appendix A, section 1.1.2). The loss in durability of the PIP was less (yet still high) for RIBs because of the lower available percent refuge. For example, a growth rate of R_1 in a RIB was reduced by a factor of two compared to the growth rate R_1 in a block refuge. The additional mortality from inter-plant movement

further reduced the growth rate in the RIB. The observed loss in durability for a high dose PIP with RIB deployment ranged approximately 20-83%. Once again, the loss in durability was greater if the pest exhibited contest rather than scramble competition dynamics (for ANOVA results see Appendix A, section 1.2.2).

I was interested in determining what caused the durability of the high dose PIP to decrease with higher compared to lower growth rates. I chose five simulations from the data set of the 30 block simulations with contest competition and R_1 D_3 AD_1 as well as R_3 D_3 AD_1 and examined scatterplots of density dependent survival in the refuge at time 't' versus change in population size from time 't-1' to 't' for both scenarios (data for R_3 D_3 AD_1 shown only). By looking at the Figure 3.2, it becomes evident that with a high growth rate, the change in population size underwent greater fluctuations (between 500,000 – 3,500,000 eggs) and density dependent survival ranged from close to zero to 0.25 with a mean of approximately 0.09. Most of the data points were at the lower range of the graph, as is indicated by the box plot and whiskers (between approximately 0.02 and 0.12). Also, the change in population size exponentially decreased as density dependent survival increased. When I took a closer look at resistance allele frequency changes at different times during a simulation (data not shown), I observed that with a high growth rate, the resistance allele frequency increased by approximately 15% during the first three generations of a simulations when measured from the beginning of a generation run until after selection took place. When the increase in resistance allele frequency was measured from 'after selection' to 'after population regulation', however, then the values ranged from 60-90%. This means that the increase in resistance allele frequency was greater due to population regulation (in a selection environment) than due

to selection alone when the pest had a high growth rate, and hence explained the lower durability predictions for the high dose PIP at R_3. Figures 3.3 and 3.4 show the impact of selection and selection + population regulation together, respectively, on the resistance allele frequency in the five randomly selected simulations. It can be seen that population regulation in the refuge (occurring after selection) additionally contributed to the increase in resistance allele frequency, and in most cases, doubled the r-frequencies after selection (earlier generations). During the data mining investigation, I observed that approximately three generations before resistance was declared, the populations in the refuge and Bt compartment reached an equilibrium density in some simulations where no more changes in population density in the cells (fields) of the matrix occurred, and density dependent mortality (or survival) remained unchanged, yet less than 1. The resistance allele frequency at this point was high (>0.15). In these simulations, it was selection that became the main evolutionary force, while no more increases in resistance allele frequency occurred due to population regulation. In other simulations, the populations did not reach an equilibrium density. There, density dependence remained a strong evolutionary force contributing to high yet reduced increases in resistance allele frequency (<50%) that matched those of the selective forces. Interestingly in these high dose scenarios, density dependence in the Bt compartments did not occur until resistance allele frequencies were higher and mostly during the last few generations before simulations were terminated (r-frequency ≥ 0.5).

When the pest growth rate was changed to low (R_1), the change in refuge egg population density fluctuated minimally and remained s between approximately 500,000 and 1,100,000 eggs per field per generation, irrespective of density dependent survival

(graph not shown). Values for density dependent survival ranged from 0.1 to 1.0 with a mean of approximately 0.4. This shows that with a low pest growth rate, less density dependent mortality (greater density dependent survival) occurred in the refuge. I was also interested in the relative contribution between selection and density dependence to resistance allele frequency increases when the pest growth rate was low. The data reveal that early during a simulation, population regulation in a selection environment and selection (before density dependence occurred) contributed approximately equally to resistance increases during a particular generation run. Towards the end of a simulation, it was selection that contributed more to increases and became the major driver for resistance (approximately 50% due to selection vs. 20% due to population regulation). In some cases, an equilibrium population density was reached where there was no longer a contribution from population regulation to resistance evolution, and once again selection became the major evolutionary force. Overall, the results support that the loss in durability of the single high dose PIP was caused by disproportionately occurring density dependent effects in the refuge and Bt field that reduced the susceptible pool in the refuge and lead to greater increases in resistance allele frequency. It was, therefore, the differential in density dependence between refuge and Bt populations that was detrimental to the life-time of the PIP when the pest had a high growth rate – this conclusion holds irrespective of the IRM strategy considered.

A bootstrap analysis between block and RIB simulations with the same model of density dependence is presented in Table 3.7 (AD_1) and Table 3.8 (AD_2). Holding all life-history parameters fixed but varying the IRM strategy, the BCa confidence limits (95%) for the 5% quantiles inform that at the lower dispersal proportion (AD_1) 13 out of 18

comparisons differed significantly. In the majority of simulations, it was the 20% block strategy that predicted greater durability for the high dose PIP rather than the 10% RIB. In the remaining five comparisons, there were no significant differences between the two IRM strategies. Those were cases where the growth rate was intermediate or high (equivalent to an increase in refuge proportion, which benefited the durability of the PIP deployed with a RIB). At the higher dispersal proportion, there were 16 out of 18 comparisons where the block refuge predicted greater durability than the RIB. As was demonstrated in Table 3.2, when the refuge of the RIB was increased to 20% for deterministic low dose simulations, then the durability estimates between the two IRM strategies were dissimilar when density dependence was excluded (blocks more durable). With density dependence included, the estimates for block simulations were reduced to similar levels as the estimates for RIBs. Hence, some of the differences observed between simulations obtained with a 10% RIB and 20% block refuge should be attributed to the reduction in refuge (equivalent to a reduction of growth rate).

Table 3.3 Effects of growth rates on durability of HD PIPs using different refuge strategies and low dispersal proportion

Parameters	10% RIB – HD		20% Block – HD	
	CC	SC	CC	SC
R_1 D_1 AD_1	13.1 [b]	12.6 [c]	22.9 [b]	18.4 [b]
R_2 D_1 AD_1	8.4 [a]	11.8 [b]	9.8 [a]	10.6 [a]
R_3 D_1 AD_1	7.1 [a]	7.8 [a]	8.3 [a]	8.7 [a]
R_1 D_2 AD_1	12.5 [c]	11.6 [c]	25.1 [b]	18.6 [c]
R_2 D_2 AD_1	9.2 [b]	9.2 [b]	10.4 [a]	10.5 [b]
R_3 D_2 AD_1	7.3 [a]	8.3 [a]	8.0 [a]	8.2 [a]
R_1 D_3 AD_1	12.8 [b]	12.9 [c]	25.5 [c]	18.9 [c]
R_2 D_3 AD_1	8.3 [a]	9.3 [b]	11.2 [b]	11.2 [b]
R_3 D_3 AD_1	7.0 [a]	7.8 [a]	7.4 [a]	8.8 [a]
R_1 D_1 AD_2	12.2 [b]	12.6 [b]	24.8 [b]	21.7 [c]
R_2 D_1 AD_2	10.1 [a]	10.8 [a]	12.4 [a]	14.6 [b]
R_3 D_1 AD_2	8.6 [a]	9.4 [a]	10.5 [a]	11.2 [a]
R_1 D_2 AD_2	12.5 [c]	13.0 [b]	23.8 [c]	21.8 [c]
R_2 D_2 AD_2	9.6 [b]	10.5 [a]	13.4 [b]	14.5 [b]
R_3 D_2 AD_2	7.8 [a]	9.9 [a]	9.9 [a]	10.6 [a]
R_1 D_3 AD_2	12.6 [c]	12.0 [b]	24.3 [c]	22.8 [b]
R_2 D_3 AD_2	9.7 [b]	11.0 [ab]	13.4 [b]	12.8 [a]
R_3 D_3 AD_2	8.0 [a]	9.8 [a]	8.9 [a]	10.9 [a]

Note: letters are for ANOVAs varying growth rate only; intrinsic mean growth rate R_1 = 10, R_2 = 20, and R_3 = 30; mean adult dispersal frequency AD_1 = 0.3 and AD_2 = 0.5. Redistribution constants D_1 = 3, D_2 = 10, and D_3 = 15; CC = contest competition; SC = scramble competition. Shaded fields denote statistically significant interactions between CC and SC holding R and D fixed.

Table 3.4 Multiple comparisons of means for interactions in HD block simulations, AD x DD x R

Density Dependence, DD	Mean Dispersal Proportion - AD	Mean Intrinsic Growth Rate - R_0		
		Low (10)	Medium (20)	High (30)
CC	Low	24.5 [a]	10.8 [ef]	7.9 [g]
	High	24.3 [ab]	13.1 [de]	9.8 [fg]
SC	Low	18.6 [c]	10.7 [f]	8.6 [fg]
	High	22.1 [b]	14.0 [d]	10.9 [ef]

Note: standard deviations contained in Appendix 1.1.1

Table 3.5 Multiple comparisons of means for interactions in HD RIB simulations, R x DD

Density Dependence, DD	Mean Intrinsic Growth Rate - R_0		
	Low (10)	Medium (20)	High (30)
CC	12.6 [a]	9.2 [c]	7.6 [d]
SC	12.5 [a]	10.2 [b]	8.8 [c]

Note: standard deviations contained in Appendix 1.2.1

Table 3.6 Multiple comparison of means for interactions in HD RIB simulations, AD x R

Mean Dispersal Proportion - AD	Mean Intrinsic Growth Rate - R_0		
	Low (10)	Medium (20)	High (30)
Low	12.6 [a]	9.2 [c]	7.5 [d]
High	12.5 [a]	10.3 [b]	8.9 [c]

Note: standard deviations contained in Appendix 1.2.1

Table 3.7 Bootstrap comparison between RIB and block simulations with different assumptions of density dependence and low dispersal (HD)

Parameters	Comparison of Distributions (BCa[2])			
	RIB : Block (HD)		RIB : Block (HD)	
	Level of Concern	CC	Level of Concern	SC
R_1 D_1 AD_1	5%	(0.5000, 0.6667)^	5%	(0.6667, 0.9167)^
R_2 D_1 AD_1		(0.6000, 0.6000)^		(0.6667, 0.7500)^
R_3 D_1 AD_1		(0.6667, 1.0000)n.s.		(0.7143, 1.0000)n.s.
R_1 D_2 AD_1		(0.4444, 0.6667)^		(0.6667, 0.8571)^
R_2 D_2 AD_1		(0.7500, 0.8571)^		(0.7778, 0.7778)^
R_3 D_2 AD_1		(0.7143, 1.0000)n.s.		(0.6250, 0.6250)^
R_1 D_3 AD_1		(0.4706, 0.6923)^		(0.5625, 0.8182)^
R_2 D_3 AD_1		(0.6667, 0.8571)^		(0.7500, 1.1429)n.s.
R_3 D_3 AD_1		(0.8333, 1.0000)n.s.		(0.7500, 0.7500)^

Table 3.8 Bootstrap comparison between RIB and block simulations with different assumptions of density dependence and high dispersal (HD)

Parameters	Comparison of Distributions (BCa[2])			
	RIB : Block (HD)		RIB : Block (HD)	
	LoR[1]	CC	LoR	SC
R_1 D_1 AD_2	5%	(0.3810, 0.4444)^	5%	(0.5333, 0.6154)^
R_2 D_1 AD_2		(0.6364, 0.7778)^		(0.6364, 0.7778)
R_3 D_1 AD_2		(0.6667, 0.6667^		(0.6667, 0.8571)^
R_1 D_2 AD_2		(0.5000, 0.6667)^		(0.6429, 0.7500)^
R_2 D_2 AD_2		(0.7000, 0.8750)^		(0.5833, 0.7273)^
R_3 D_2 AD_2		(0.7143, 1.0000)n.s.		(0.8571, 1.1429)n.s.
R_1 D_3 AD_2		(0.4286, 0.5625)^		(0.5333, 0.6667)^
R_2 D_3 AD_2		(0.6000, 1.0000)n.s.		(0.6364, 0.6364)^
R_3 D_3 AD_2		(0.7143, 0.8333)^		(0.6667, 0.6667)^

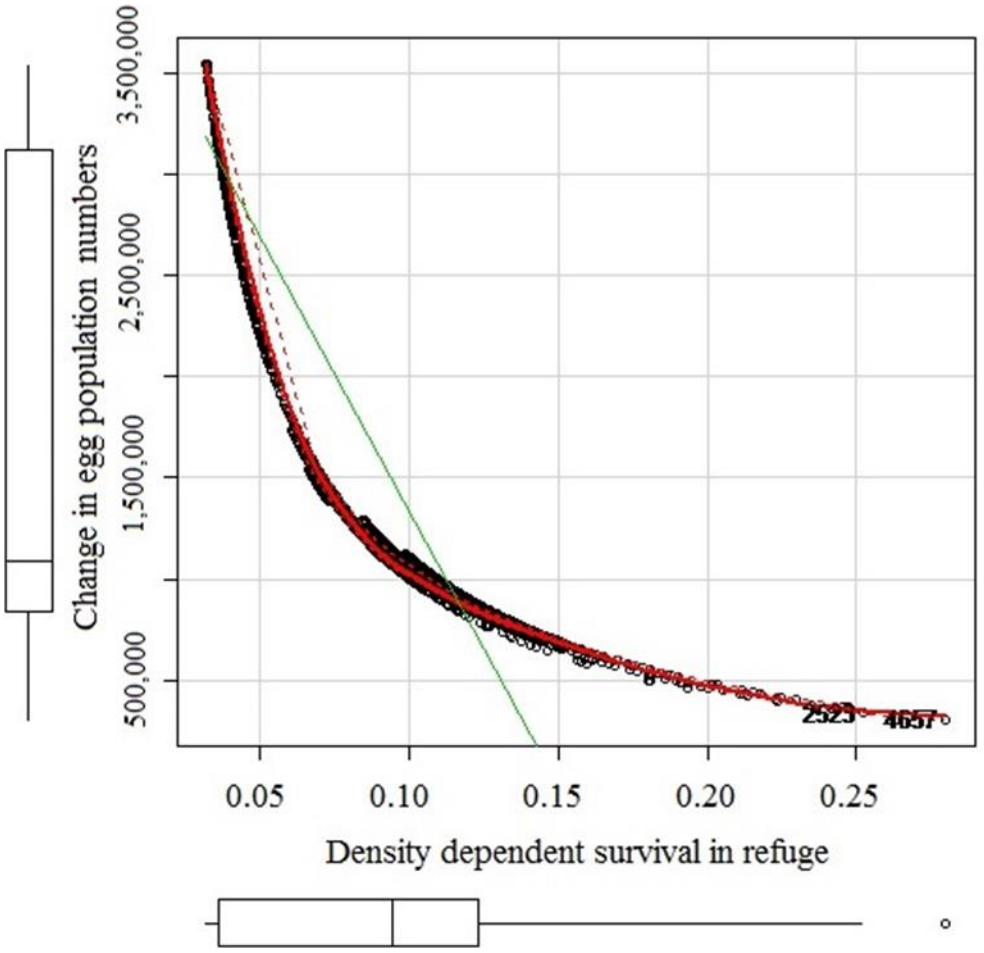

Figure 3.2 Density dependent survival vs. change in egg numbers with high pest growth rate and high redistribution constant (R_3 D_3)

Notes: 20% block refuge strategy; growth rate = R_3 and redistribution constant = D_3; change in population size measured change in egg population after mating between time (t-1) and time (t); straight solid line was the regression line, curved straight line was best fit; dotted lines are standard error lines; black dots are data points from five of 30 simulations; box plots are shown (Q1, Q2, and Q3) with whiskers.

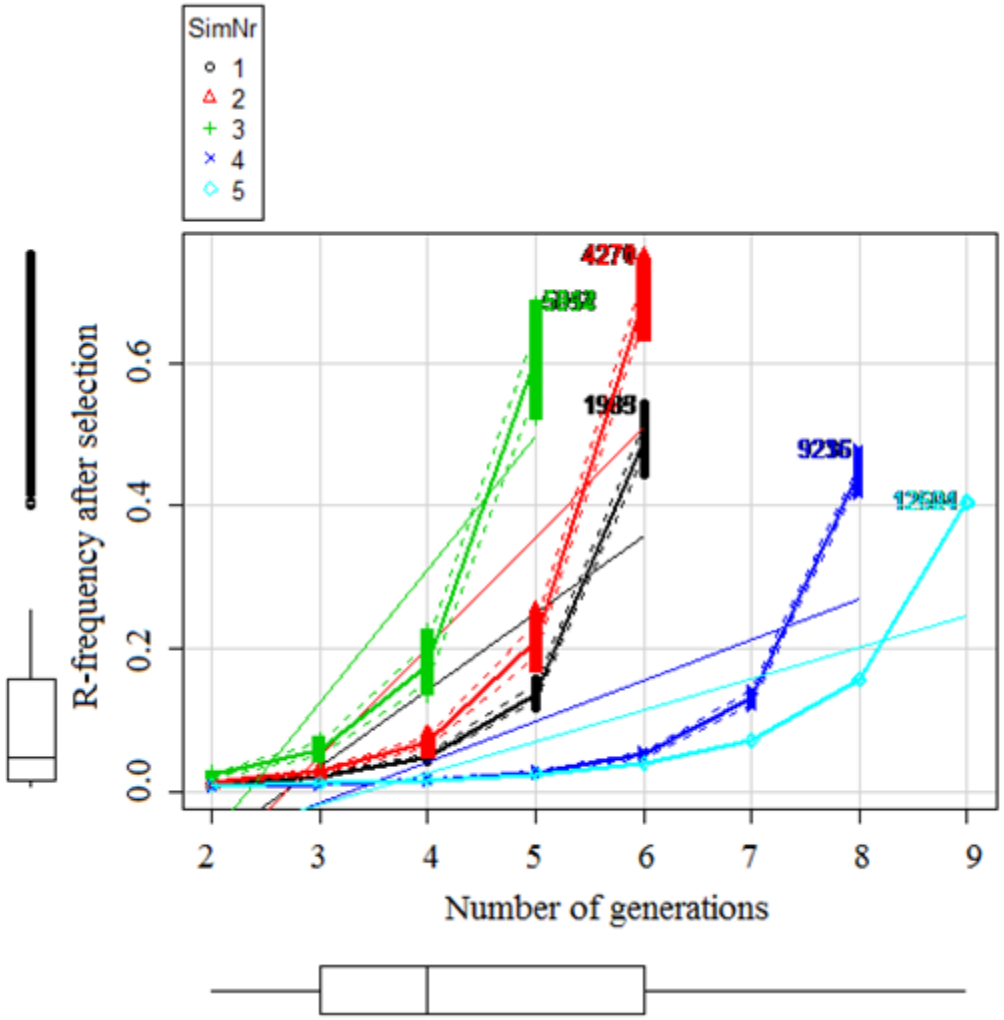

Figure 3.3 Impact of selection on r-allele frequency before DD occurred, high pest growth rate, high diffusivity, and contest competition (HD)

Notes: random selection of 5 simulations; 20% block refuge; growth rate = R_3, redistribution constant = D_3, adult proportion dispersing = AD_1. Box plot and whiskers provide information about the data distribution. Straight, solid lines represent the regression lines; dotted lines are standard error lines. Comparison between Figures 3.3 and 3.4 can only be made between the same simulation run at a particular generation.

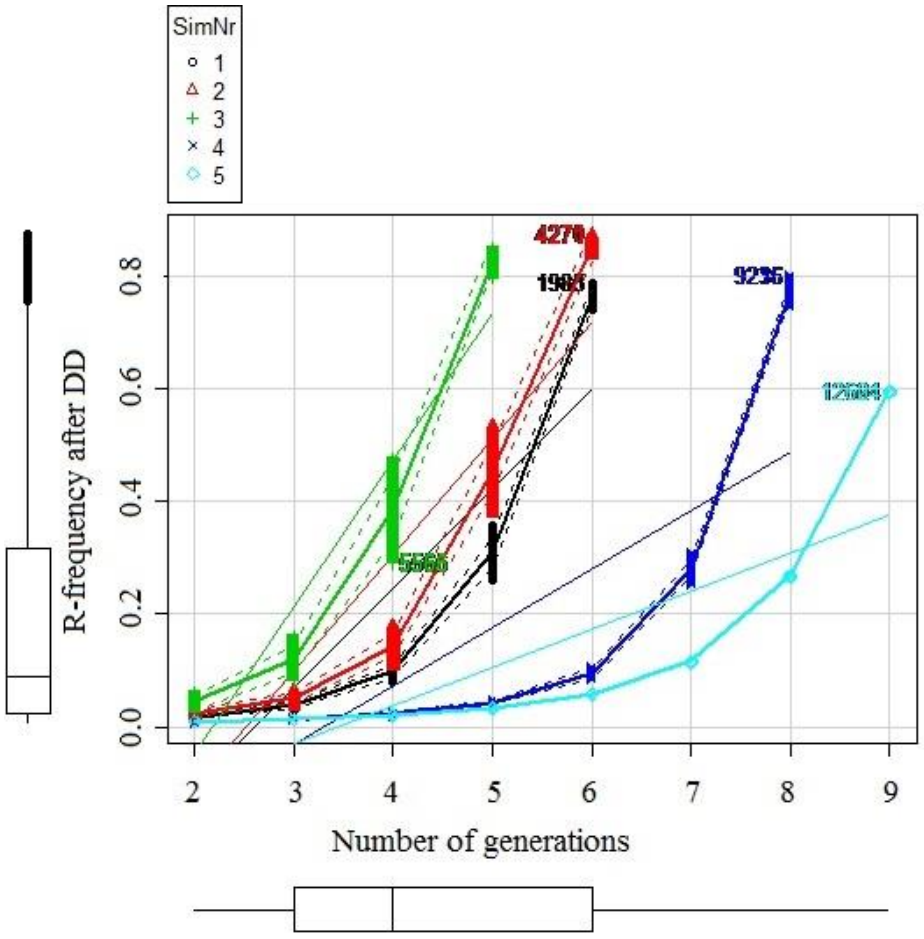

Figure 3.4 Impact of density dependence on r-allele frequency after selection, high pest growth rate, high diffusivity, and contest competition (HD)

Notes: random selection of 5 simulations; 20% block refuge; growth rate = R_3, redistribution constant = D_3, adult proportion dispersing = AD_1. Box plot and whiskers provide information about the data distribution. Straight, solid lines represent the regression lines; dotted lines are standard error lines. Comparison between Figures 3.3 and 3.4 can only be made between the same simulation run at a particular generation.

3.6.1.2 Low dose results

I conducted a four-way ANOVA (DD x R x D x AD) for blocks and RIBs varying density type of density dependence (CC and SC), growth rate R, diffusivity, and dispersal proportion to look for interactions between the four parameters that could affect the durability of the low dose PIP with different life-history assumptions (mean times to

resistance listed in Table 3.9). For blocks, there was one three-way interaction between type of density dependence, diffusivity, and growth rate (DD x D x R) among all the parameters investigated (p-values = 0.04469) and a two-way interaction DD x R. The main factors R and DD, were highly significant (R: $p < 1.13e^{-09}$, DD: $p = 2e^{-16}$), while the other two factors, AD and D, were not ($p > 0.05$) (Appendix 2.1.1). For RIBs, there were two significant interactions apparent between growth rate and density dependence (R x DD) ($p = 0.000184$) and density dependence and diffusivity (DD x D) ($p = 0.03989$) that affected the estimated life-time of the PIP. All main factors with the exception of diffusivity were statistically significant in this analysis (Appendix 2.2.1).

A multiple comparison of means was conducted for all the variables involved in the interactions and are presented in Table 3.10 (blocks) and Tables 3.11 and 3.12 (RIBs). The three-way interaction for block simulations with DD x D x R resulted in greater times to resistance for a pest with contest competition dynamics and a lower growth rate compared to all other combinations of density dependence, diffusivity and R_0. No statistically significant difference was observed between the remaining mean estimates (Appendix 2.1.1). The comparison of means for the RIB interaction, DD x R, shows the greatest durability was projected from simulations with a lower pest growth rate. Contest competition estimates were significantly greater than scramble competition durability projections. All other DD and R combinations resulted in lower durability estimates for the low dose single PIP, and no statistically significant difference was observed between the mean estimates. (Table 3.10). These results support that when the pest had a lower growth rate in a low dose Bt environment, the durability projections were highest. At higher pest growth rates the durability of the low dose PIP decreased, and there was no

difference between the means comparison from the two-way ANOVA. The comparison of means for the RIB interaction, DD x D, shows that the lowest durability estimate was observed when the pest engaged in scramble competition and had an intermediate diffusivity. While diffusivity by itself was never a significant main effect in the ANOVAs, for this particular combination of diffusivity with scramble competition, an effect was observed. All other combinations of DD and D did not result in statistically significantly different mean estimates (Table 3.11) (Appendix 2.2.1).

I was interested in determining what the exact mechanisms were between significant differences of contest and scramble competition simulations at lower growth rates. For this purpose, I chose to further look into the data of block simulations with R_1 D_2 AD_1 because differences appeared to be most pronounced there. I looked at the distributions of density dependent survival in the refuge and Bt compartments and found that survival with contest competition was on average more similar in Bt and refuge compartments and higher than for scramble competition (Figure 3.5). With scramble competition, survival was more dissimilar in the refuge and Bt compartment than for contest competition (Figure 3.6). The overall greater effect of density dependence and differentials in density dependence were responsible for the lower durability observed with scramble compared to contest competition (Table 3.9). Here too, it is unequal effects of population regulation (scramble competition) on Bt and refuge plants together with selection that drive the greater increases in resistance allele frequency and the loss in durability with a low growth rate.

One-way ANOVAs were conducted for intrinsic growth rate holding all other parameters fixed to determine whether varying R_0 would significantly impact the

estimated average durability for RIBs and blocks (letters displayed for means in Table 3.9). Block results show that for a pest with contest competition dynamics (low and high dispersal proportion), the durability of the low dose PIP was significantly greater with a low growth rate (range 17.9-20 generations) than with intermediate and high growth rates (range 13.1 -14.7 generations). The reduction in durability with contest competition ranged approximately from 21-35% as the growth rate increased. For scramble competition, no significant differences were observed in estimated durability of the low dose PIP at the lower dispersal proportion when the growth rate was varied. As the growth rate increased, density dependence in refuge compartments of contest competition simulations increased, and a differential between Bt and refuge compartments increased (discussed above). At the greater dispersal proportion, the durability decreased only with low diffusivity assumptions. In that particular case, the loss in durability at R_3 was 21% compared to the durability estimated at R_1. The same analyses for a low dose RIB and pest with contest competition dynamics shows that the durability of the low dose PIP was significantly greater with a low growth rate than with intermediate and high growth rates – as for block simulations (both dispersal proportions). The loss in durability at the lower dispersal proportion was approximately 17% and ranged from 7-19% at the higher dispersal proportion. For scramble competition with lower dispersal proportions, one significant difference was observed at the highest diffusivity tested; the lowest pest growth rate lead to the greatest durability of the PIP as well (Table 3.9). At the higher dispersal proportion, however, the lower pest growth rate value resulted in significantly greater durabilities at all three levels of diffusivity and extended the lifetime of the low dose PIP significantly.

A bootstrap analysis between results obtained with different IRM deployments and the same model of density dependence is presented in Tables 3.13 (AD_1) and 3.14 (AD_2). Holding all life-history parameters fixed but varying the IRM strategy, the BCa confidence limits (95%) for the ratio of the distributions at the 5% quantiles inform that at the lower dispersal proportion, blocks predicted greater life-times for the low dose PIP in six out of nine comparisons with contest competition and eight out of nine comparisons with scramble competition assumptions (Table 3.13). At the higher dispersal proportion, there were eight out of nine comparisons for both types of density dependent models where the durability for blocks was predicted to be higher than for RIBs. As was demonstrated in Table 3.2, when the refuge of the RIB was increased to 20% for deterministic low dose simulations, then the durability estimates between the two IRM strategies were similar with and without density dependence (but not so with a 50% refuge when blocks out-performed RIBs once more). Hence the differences observed between simulations obtained with a 10% RIB and 20% block refuge can be mostly attributed to the reduction in refuge (equivalent to a reduction of growth rate).

Table 3.9 Effects of growth rates on durability of LD PIPs using different refuge strategies, intra-specific competition, redistribution, and low dispersal

Parameters	10% RIB – LD		20% Block – LD	
	CC	SC	CC	SC
R_1 D_1 AD_1	13.6 [b]	11.9	17.9 [b]	15.0
R_2 D_1 AD_1	11.4 [a]	11.8	14.1 [a]	14.0
R_3 D_1 AD_1	11.4 [a]	11.5	14.7 [a]	14.0
R_1 D_2 AD_1	13.3 [b]	11.8	20.0 [b]	14.2
R_2 D_2 AD_1	11.6 [a]	11.0	13.1 [a]	14.8
R_3 D_2 AD_1	11.1 [a]	11.2	13.9 [a]	13.7
R_1 D_3 AD_1	13.1 [b]	12.7 [b]	19.6 [b]	15.0
R_2 D_3 AD_1	11.1 [a]	11.8 [ab]	13.4 [a]	14.8
R_3 D_3 AD_1	11.2 [a]	11.3 [a]	13.8 [a]	14.0
R_1 D_1 AD_2	14.1 [b]	12.7 [b]	18.6 [b]	15.6 [b]
R_2 D_1 AD_2	11.5 [a]	11.4 [a]	14.5 [a]	14.5 [ab]
R_3 D_1 AD_2	11.5 [a]	11.5 [a]	14.3 [a]	13.7 [a]
R_1 D_2 AD_2	13.0 [b]	12.3 [b]	19.0 [b]	16.0
R_2 D_2 AD_2	12.0 [ab]	11.0 [a]	13.8 [a]	14.7
R_3 D_2 AD_2	11.4 [a]	11.1 [a]	13.9 [a]	14.7
R_1 D_3 AD_2	13.4 [b]	12.9 [b]	19.8 [b]	15.3
R_2 D_3 AD_2	11.5 [a]	12.0 [ab]	13.6 [a]	14.0
R_3 D_3 AD_2	11.9 [a]	11.6 [a]	14.0 [a]	14.1

Note: intrinsic mean growth rate $R_1 = 10$, $R_2 = 20$, and $R_3 = 30$; mean adult dispersal frequency $AD_1 = 0.3$ and $AD_2 = 0.5$. Redistribution constants $D_1 = 3$, $D_2 = 10$, and $D_3 = 15$; CC = contest competition; SC = scramble competition. Shaded fields denote statistically significant interactions between CC and SC holding R and D fixed.

Table 3.10 Multiple comparisons of means for interactions in LD block simulations, DD x D x R

Density Dependence, DD	Mean Diffusivity - D	Mean Intrinsic Growth Rate - R_0		
		Low (10)	Medium (20)	High (30)
CC	Low	18.3 [a]	14.3 [b]	14.5 [b]
	Medium	19.5 [a]	13.4 [b]	13.9 [b]
	High	19.7 [a]	13.5 [b]	13.9 [b]
SC	Low	15.3 [b]	14.3 [b]	13.8 [b]
	Medium	15.1 [b]	14.7 [b]	14.2 [b]
	High	15.1 [b]	14.4 [b]	14.0 [b]

Note: standard deviations contained in Appendix 2.1.1

Table 3.11 Multiple comparisons of means for interactions in LD RIB simulations, R x DD

Density Dependence, DD	Mean Intrinsic Growth Rate - R_0		
	Low (10)	Medium (20)	High (30)
CC	13.4 [a]	11.5 [c]	11.4 [c]
SC	12.4 [b]	11.5 [c]	11.3 [c]

Note: standard deviations contained in Appendix 2.2.1

Table 3.12 Multiple comparisons of means for interactions in LD RIB simulations, DD x D

Density Dependence, DD	Mean Diffusivity - D		
	Low (3)	Medium (10)	High (15)
CC	12.2 [a]	12.0 [a]	12.0 [a]
SC	11.8 [ab]	11.4 [b]	12.0 [a]

Note: standard deviations contained in Appendix 2.2.1

Table 3.13 Bootstrap comparison between low dose simulations for RIBs and blocks and low dispersal

Parameters	Comparison of Distributions (BCa^2)			
	RIB : Block (LD)		RIB : Block (LD)	
	Level of Concern	CC	Level of Concern	SC
$R_1 D_1 AD_1$	5%	(0.8182, 1.0000)n.s.	5%	(0.6154, 0.7273)^
$R_2 D_1 AD_1$		(0.6923, 0.9000)^		(0.75, 0.90)^
$R_3 D_1 AD_1$		(0.7273, 0.9091)^		(0.6154, 0.7500)^
$R_1 D_2 AD_1$		(0.6250, 0.8333)^		(0.6154, 0.8182)^
$R_2 D_2 AD_1$		(0.818, 1.125)n.s.		(0.6667, 0.8182)^
$R_3 D_2 AD_1$		(0.6923, 1.0000)n.s.		(0.75, 1.00)n.s.
$R_1 D_3 AD_1$		(0.6667, 0.9091)^		(0.6429, 0.8182)^
$R_2 D_3 AD_1$		(0.7273, 0.9091)^		(0.6154, 0.7273)^
$R_3 D_3 AD_1$		(0.6667, 0.7273)^		(0.6154, 0.7273)^

Table 3.14 Bootstrap comparison between low dose simulations for RIBs and blocks and high dispersal

Parameters	Comparison of Distributions (BCa^2)			
	RIB : Block (LD)		RIB : Block (LD)	
	Level of Concern	CC	Level of Concern	SC
$R_1 D_1 AD_2$	5%	(0.6667, 0.8333)^	5%	(0.7143, 0.8333)^
$R_2 D_1 AD_2$		(0.6154, 0.7273)^		(0.7273, 0.9091)^
$R_3 D_1 AD_2$		(0.75, 0.90)^		(0.6667, 0.8182)^
$R_1 D_2 AD_2$		(0.6250, 0.6667)^		(0.7143, 0.8333)^
$R_2 D_2 AD_2$		(0.7500, 0.9000)^		(0.8000, 1.0000)n.s.
$R_3 D_2 AD_2$		(0.7500, 1.1111)n.s.		(0.6667, 0.7273)^
$R_1 D_3 AD_2$		(0.6250, 0.7692)^		(0.7692, 0.7692)^
$R_2 D_3 AD_2$		(0.6923, 0.6923)^		(0.90, 1.10)n.s.
$R_3 D_3 AD_2$		(0.6923, 0.9000)^		(0.6661, 0.8182)^

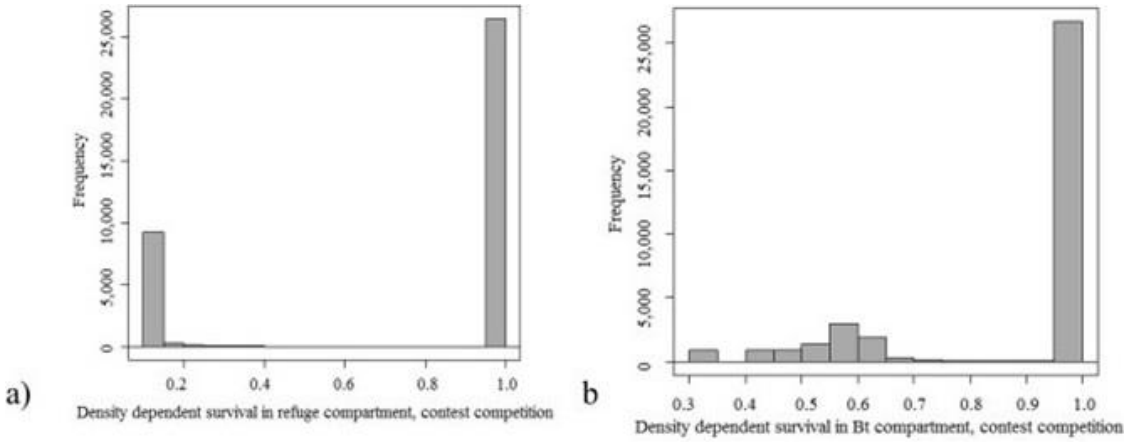

Figure 3.5 Range of density dependent survival in a) refuge and b) Bt for block simulations with contest competition and low growth rates

Notes: random subsample of original 30 simulations; growth rate = R_1, redistribution constant = D_2

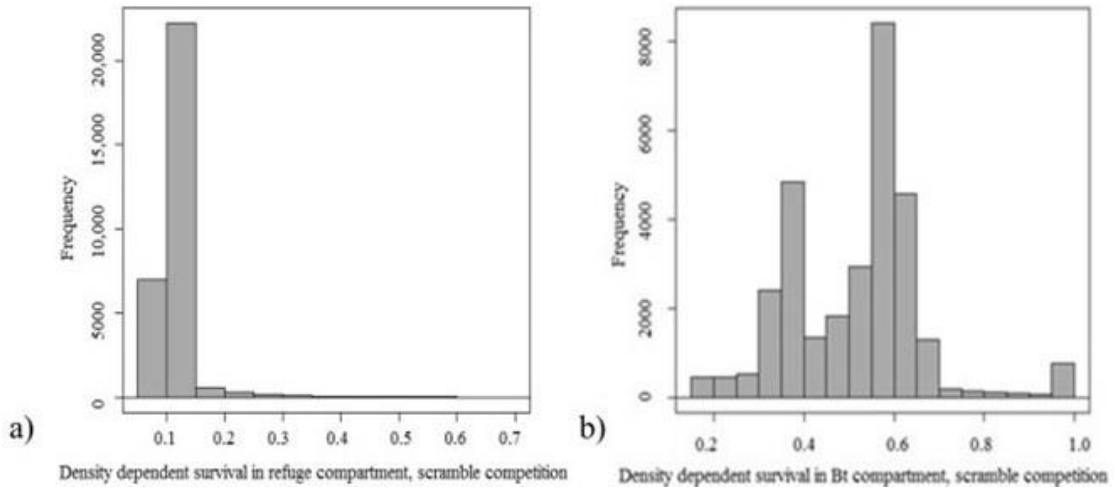

Figure 3.6 Density dependent survival ranges in a) refuge and b) Bt for block simulations with scramble competition and low growth rates

Notes: random subsample of original 30 simulations; growth rate = R_1, redistribution constant = D_2

3.6.2 Comparison of high dose and low dose results

In the prior section, I reported that the durability of a single high dose PIP deployed with a 20% block refuge was greatly reduced at the higher growth rates because

of greater density dependent effects that took place in the refuge. These effects of density dependence lead to greater generational increases in resistance allele frequencies than selection alone. Figures 3.7 and 3.8 place the durability of high and low dose PIPs deployed with a block and the same pest growth rate assumptions next to each other. In Figure 3.7, scramble competition dynamic was modeled, while in Figure 3.8 contest competition was assumed to take place. The graphs show that a single HD PIP deployed with a 20% block was more durable (statistically significant) than a single LD PIP at a lower pest growth rate but that at an intermediate and higher growth rate (R_2 and R_3) the time to pest resistance was not statistically different for either PIP. Figures 3.9 and 3.10 display the average durability results for single HD and LD PIPs deployed with a 10% RIB and both scramble and contest competition dynamics (respectively) with various growth rates. Here it can be observed that with a RIB deployment, the durability of the HD and LD single PIP did not differ at the low and intermediate pest growth rates (R_1 and R_2) but that at the highest pest growth rate (R_3), the LD PIP performed better (statistically significant) than the HD PIP. As discussed in the previous sections, the lower growth rates represent declining population in RIBs where density dependent interactions were much weaker. I also previously discussed that a reduction in growth rate was analogous to reducing the refuge further; hence, at the lower pest growth rates and with a 10% RIB, the high dose PIP was actually deployed with very little 'effective' refuge. This reduced the average durability for the HD PIP and made it similar to the estimate obtained for a LD PIP under the same conditions. Like for the block simulations, when the growth rate increased, density dependent effects became more unequal between Bt and non-Bt plants, and the durability of both HD and LD PIP decreased. But this

differential was more pronounced for the HD situation. Density dependent effects at R_3 were dampened for the LD PIP because of inter-plant movement mortality and population regulation was more similar between Bt and refuge plants. This lead to significant difference between durability estimates at the highest growth rate for LD and HD PIPs deployed with a 10% RIB.

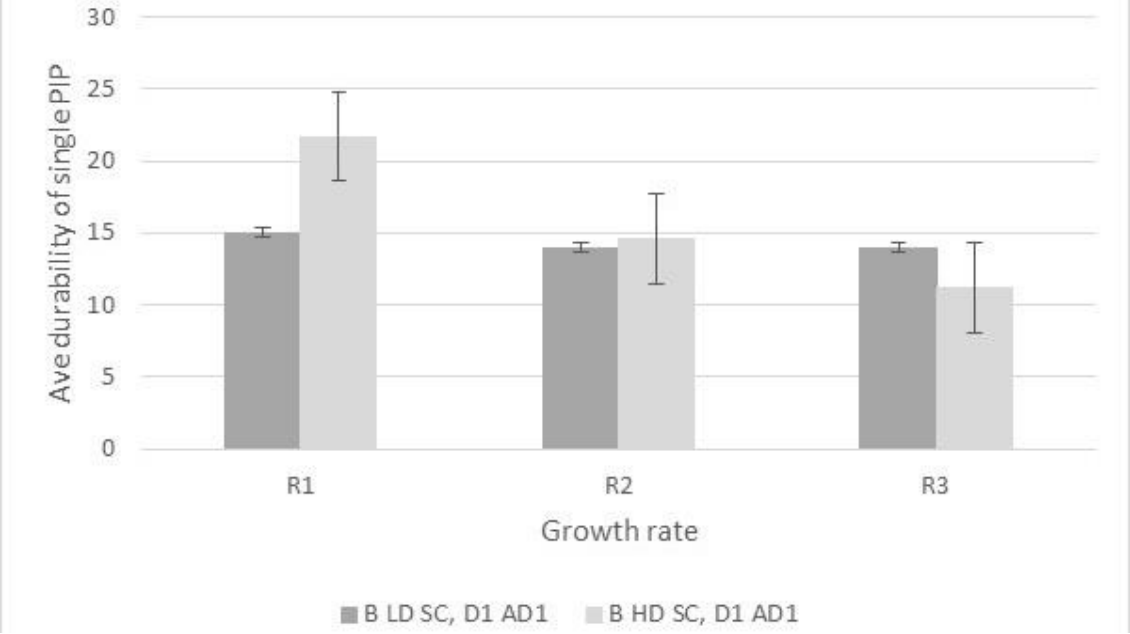

Figure 3.7 Durability of single PIPs deployed with block refuges, different pest growth rates, scramble competition, and low dispersal

Notes: Growth rates graphed were R_1=10, R_2=20, and R_3=30; the graph shows that the durability of the HD and LD PIP differed at R_1 but not at R_2 and R_3.

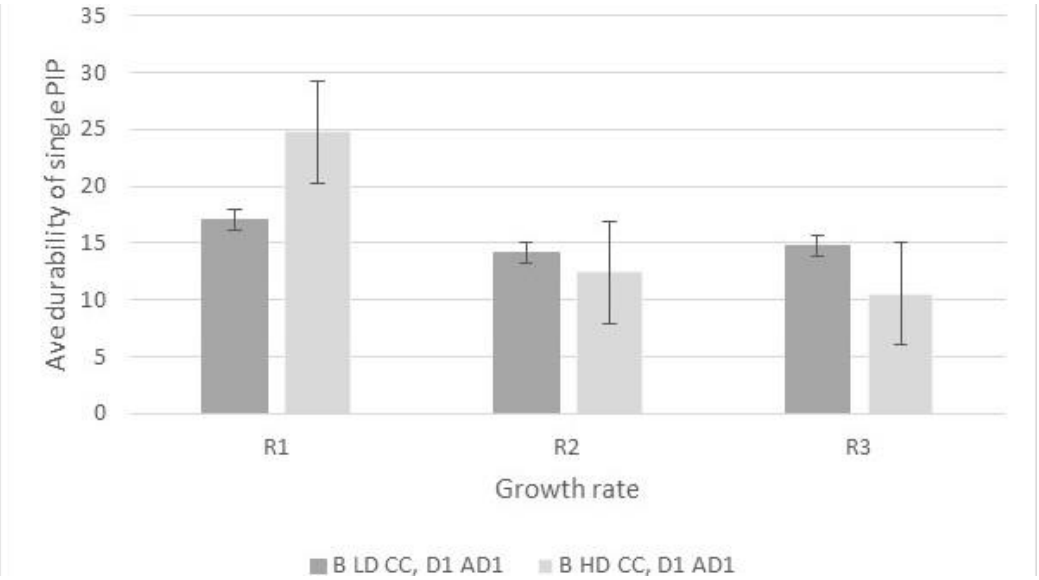

Figure 3.8 Durability of single PIPs deployed with a block, different pest growth rates, contest competition, and low dispersal

Notes: Growth rates graphed were R_1=10, R_2=20, and R_3=30; the graph shows that the durability of the HD and LD PIP differed at R_1 but not at R_2 and R_3.

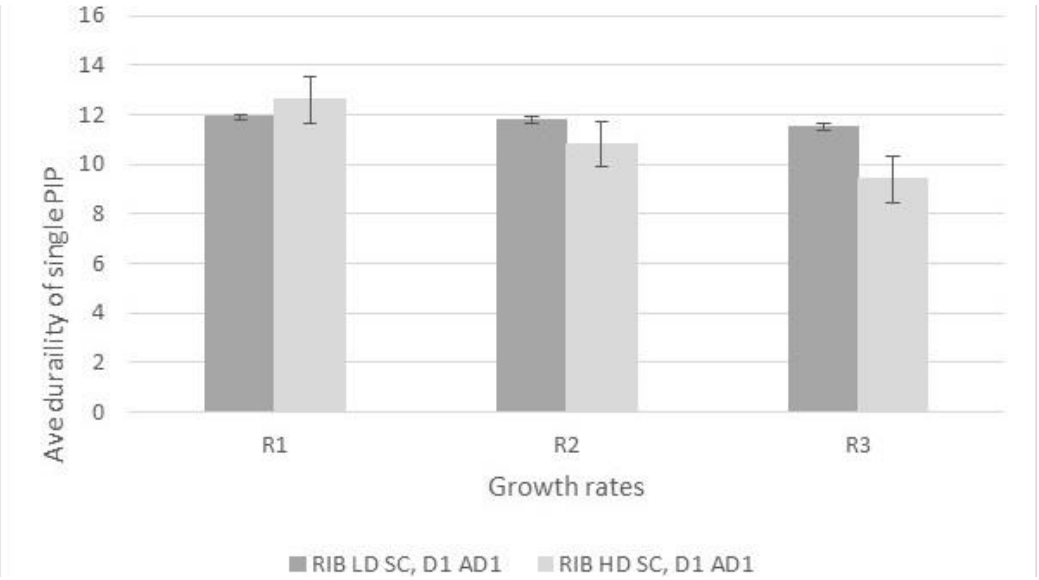

Figure 3.9 Durability of single PIPs deployed with RIB, different pest growth rates, scramble competition, and low dispersal

Notes: Growth rates graphed were R_1=10, R_2=20, and R_3=30; the graph shows that the durability of the HD and LD PIP did not differ at R_1 and R_2, but the durability of the LD PIP exceeded that of the HD PIP at R_3.

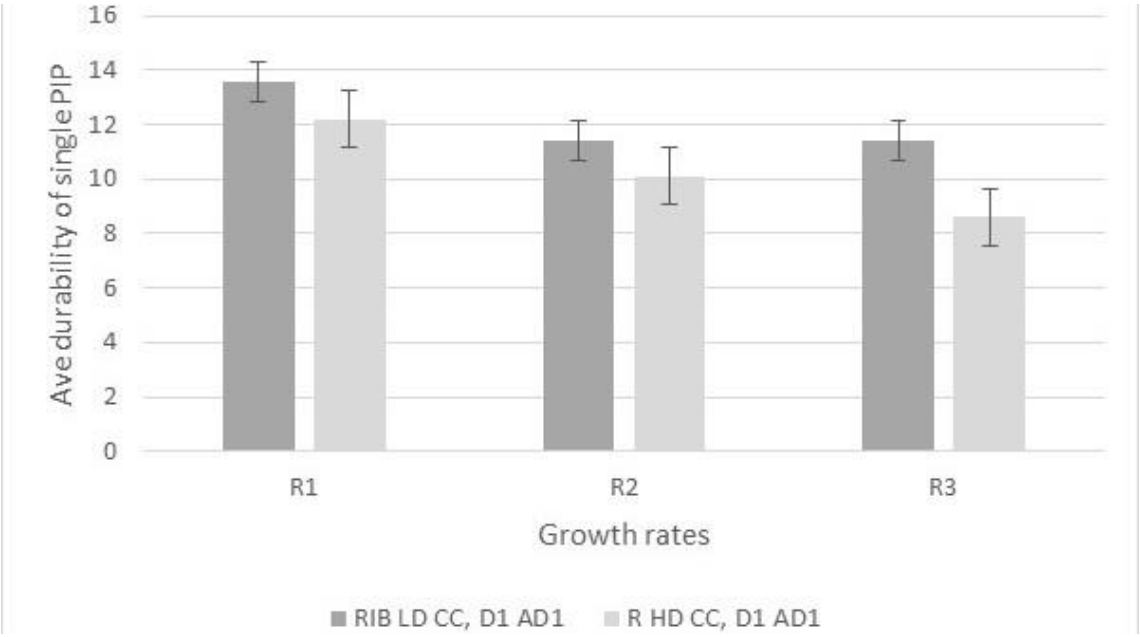

Figure 3.10 Durability of single PIPs deployed with RIB, different pest growth rates, contest competition, and low dispersal

Notes: Growth rates graphed were R1=10, R_2=20, and R_3=30; the graph shows that the durability of the HD and LD PIP did not differ at R_1 and R_2, but the durability of the LD PIP exceeded that of the HD PIP at R_3.

3.6.3 Effects of life-history and IRM parameters on dispersal of resistance genes

3.6.3.1 High dose results

In absence of resistance in the landscape, the r-frequencies in the square sections slowly and uniformly increased with each passing generation for block and RIB strategies when pest growth rates were low or high (Tables 3.15 through 3.18). A difference in average landscape resistance allele frequency was observed due to different assumptions of intra-specific competition. For low pest growth rates and block simulations with contest competition the frequency in the landscape was 0.0066 and for scramble competition 0.0104 (Table 3.15). For RIBs with low pest growth rate, the r-frequencies were 0.0096 and 0.0139 (CC and SC, respectively) after three generations from the start

of simulations (Table 3.17) and support the earlier results that a 10% RIB was less durable than a 20% block refuge. Contest competition in combination with a low growth rate has previously shown to increase the durability for high dose PIPs compared to scramble competition; the lower average r-frequency in the matrix support the earlier discussed results.

At the higher growth rates, average landscape r-frequencies were 0.0345 and 0.0249 for blocks and 0.0553 and 0.0317 for RIBs with contest and scramble competition in absence of resistance three generations after the start of simulations (Tables 3.16 and 3.18). The average landscape frequencies were higher when the pest exhibited contest rather than scramble competition, and all frequencies were greater with high compared to low growth rates, as previously discussed.

3.6.3.1.1 Block simulations

When the generic pest exhibited 10% resistance at locus 1 in a hypothetical hotspot [25][25], had a low growth rate and diffusivity, and was exposed to a 20% block refuge and 80% Bt crop, then the final average landscape resistance allele frequencies with contest and scramble competition after three dispersal events were approximately 110% and 53% higher compared to the 'no resistance' case at generation three (Table 3.15). I observed that with contest competition the r-frequencies in the square sections 1-2 increased between approximately 70-90% and in sections 3-4 by approximately 53-80% between each generation run. In sections 5-13, the increase in resistance from one generation to the next was approximately 25-30%. A resistance phenomenon was visible in sections 1-4 (generation 1), sections 1-5 (generation 2), and sections 1-7 (generation 3) and expanded away from the hotspot with each passing generation (and dispersal event).

Thereafter, the resistance allele frequencies were mostly uniform across the remainder of the landscape. I loosely refer to 'resistance phenomenon' when the r-frequency difference between adjacent sections was greater than 10%.

With a higher growth rate and diffusivity, the increase in average resistance in the landscape after three generations compared to the 'no resistance case' at R_3 was approximately 31% (CC) and 19% (SC) higher, but approximately 230% (CC) and 86% (SC) higher compared to the resistance levels obtained with a low growth rate (Tables 3.16 vs. 3.15). It becomes evident that resistance spread faster through the landscape with the higher growth rate value, and the sections farther removed from the hotspot reported greater average increases. The differences in farther removed sections with a high growth rate were approximately 213% (CC) and 137% (SC) higher after three generations. For block simulations with contest competition assumptions, a local resistance phenomenon was visible in sections 1-6 (generation 1) and sections 1-7 (generations 2 and 3). The increase in frequency between the respective sections after the successive generations ranged approximately from 89-180% (section 1-6) and 88-123% (section 7-13). With scramble competition assumptions, the resistance phenomenon was visible in sections 1-2 (generation 1) and sections 1-7 (generations 2 and 3). The increase in frequency between the respective sections after the successive generations ranged approximately from 80-200% (section 1-6) and 70-90% (section 7-13). Resistance was approximately uniform throughout the landscape starting at section 9 (CC) and section 8 (SC) after generation 3. To test whether there was an edge effect on the resistance allele frequency spread in the 51 x 51 matrix, I also ran a simulation for blocks with R3 D3 AD1 with a 61 x 61 matrix. I observed that the difference in resistance allele frequencies in the respective sections

during the time observed were minimal and did not affect the overall results (data not shown).

In conclusion, the resistance allele frequencies in the landscape were always highest around the hotspot (square section 1), then greatly dropped off in section 2 (between 415-2500%), and gradually decreased as the distance increased from the resistant site until there was a uniform distribution of frequencies in the last 4-5 sections. Higher pest growth rates spread resistance faster (distance and magnitude) through the landscape than simulations with low growth rates (effects of density dependent differentials with higher growth rates) because density dependent differentials were greatest between refuge and Bt compartments at the higher R-value (previously discussed).

Table 3.15 Resistance allele frequencies in matrix with different density dependence, low growth rate and dispersal, and block refuge (HD)

Location	**Generation**	**20% Block, HD**	
		CC – R_1 D_1 AD_1	**SC – R_1 D_1 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0066	0.0104
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1860	0.3583
Section 2		0.0106	0.0168
Section 3		0.0095	0.0130
Section 4		0.0085	0.0103
Section 5		0.0079	0.0086
Section 6		0.0076	0.0077
Section 7		0.0072	0.0073
Section 8		0.0074	0.0072
Section 9		0.0072	0.0069
Section 10		0.0074	0.0070
Section 11		0.0073	0.0069
Section 12		0.0072	0.0070

Table 3.15 (Continued)

Section 13		0.0073	0.0070
Section 1	2	0.3173	0.6204
Section 2		0.0181	0.0372
Section 3		0.0156	0.0284
Section 4		0.0130	0.0202
Section 5		0.0112	0.0146
Section 6		0.0102	0.0112
Section 7		0.0095	0.0096
Section 8		0.0095	0.0090
Section 9		0.0092	0.0085
Section 10		0.0094	0.0086
Section 11		0.0093	0.0086
Section 12		0.0092	0.0085
Section 13		0.0093	0.0086
Section 1	3	0.5480	0.8175
Section 2		0.0344	0.0798
Section 3		0.0282	0.0605
Section 4		0.0217	0.0409
Section 5		0.0169	0.0266
Section 6		0.0141	0.0179
Section 7		0.0125	0.0137
Section 8		0.0122	0.0119
Section 9		0.0117	0.0109
Section 10		0.0118	0.0107
Section 11		0.0116	0.0105
Section 12		0.0116	0.0105
Section 13		0.0117	0.0104
Matrix, with resistance	3	0.0138	0.0159

Note: growth rate = 10, redistribution constant = 3; adult dispersal = 30%; CC = contest competition; SC = scramble competition

Table 3.16 Resistance allele frequencies in matrix with different density dependence, high growth rate and dispersal, and block refuge (HD)

Location	**Generation**	**20% Block, HD**	
		CC – R_3 D_3 AD_1	**SC – R_3 D_3 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0345	0.0249

Table 3.16 (Continued)

10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.4015	0.3102
Section 2		0.0218	0.0118
Section 3		0.0177	0.0107
Section 4		0.0143	0.0100
Section 5		0.0123	0.0094
Section 6		0.0108	0.0089
Section 7		0.0102	0.0085
Section 8		0.0100	0.0083
Section 9		0.0098	0.0086
Section 10		0.0098	0.0084
Section 11		0.0095	0.0086
Section 12		0.0100	0.0086
Section 13		0.0100	0.0083
Section 1	2	0.7586	0.6616
Section 2		0.0614	0.0357
Section 3		0.0490	0.0291
Section 4		0.0366	0.0233
Section 5		0.0281	0.0189
Section 6		0.0225	0.0161
Section 7		0.0202	0.0145
Section 8		0.0191	0.0138
Section 9		0.0184	0.0141
Section 10		0.0182	0.0139
Section 11		0.0176	0.0140
Section 12		0.0187	0.0139
Section 13		0.0188	0.0137
Section 1	3	0.8526	0.7488
Section 2		0.1654	0.0822
Section 3		0.1289	0.0655
Section 4		0.0920	0.0502
Section 5		0.0667	0.0388
Section 6		0.0505	0.0317
Section 7		0.0427	0.0276
Section 8		0.0389	0.0257
Section 9		0.0389	0.0257
Section 10		0.0368	0.0252
Section 11		0.0359	0.0253
Section 12		0.0346	0.0252
Section 13		0.0367	0.0247
Matrix, with resistance	3	0.0453	0.0296

3.6.3.1.2 RIB simulations

When the generic pest had a 10% resistance at locus 1 in the hypothetical hotspot [25][25], a low growth rate and diffusivity and was exposed to a 10% RIB, then the final average landscape resistance allele frequencies with contest and scramble competition after three generations were approximately 73% and 115% higher compared to the 'no resistance' case at generation three (Table 3.17). I observed that with contest competition the r-frequencies in the square section 1 increased by 109% (generation 1-2) and 90% (generation 2-3). Then in section 2 and 3, the increase in resistance ranged from 110-200% with each passing generation and was lower and uniform after section 6 (generation 1), section 7 (generation 2), and section 10 (generation 3). A similar pattern of spread through the landscape could be observed when the pest engaged in scramble competition in a RIB environment though most frequencies in the sections as well as final matrix frequencies were higher. For example, the resistance allele frequency in the section immediately surrounding the hypothetical hotspot was 154%, 119%, and 29% higher in successive generations. The landscape resistance allele frequency at the end of three generations was 80% higher with scramble than with contest competition.

With a high growth rate and diffusivity, the increase in resistance after three generations compared to the 'no resistance case' at R_3 was 50% and 45% greater with contest and scramble competition and 400% and 54% greater compared to matrix r-frequencies obtained at R_1 (Tables 3.18 vs. 3.17). As for block simulations, it can be observed that resistance spread faster through the landscape with the higher growth rate value as measured by the average landscape resistance allele frequencies after each generation. Sections farther removed from the UXD site typically had resistance allele

frequencies with R_3 that were about two to six times the values reported for those obtained with R_1 in the same sections. Figures 3.11 and 3.12 show how the resistance allele frequencies in sections 1-3 of the landscape were higher with scramble competition and R_1 compared to R_3. Farther removed from the hotspot, however, the r-frequencies with R_3 had greater values with a higher growth rate. The opposite was observed for contest competition; resistance frequencies in sections 1-3 were higher with R_3 than with R_1 and remained that way throughout the matrix (effects of unequal density dependence in refuge and Bt compartment with resistance). It has previously been shown that increasing the diffusivity did not affect the resistance outcomes, hence the observations support that it was the higher growth rate and effects of population regulation (discussed earlier) that were responsible for the faster spread resistance through the matrix.

Table 3.17 Resistance allele frequencies in matrix with different density dependence, low growth rate and dispersal, and RIB (HD)

Location	**Generation**	**10% RIB, HD**	
		CC – R_1 D_1 AD_1	**SC – R_1 D_1 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0096	0.0139
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1856	0.4731
Section 2		0.0146	0.0234
Section 3		0.0143	0.0191
Section 4		0.0101	0.0143
Section 5		0.0082	0.0113
Section 6		0.0069	0.0096
Section 7		0.0064	0.0088
Section 8		0.0062	0.0081
Section 9		0.0062	0.0082
Section 10		0.0061	0.0083
Section 11		0.0061	0.0082
Section 12		0.0061	0.0081

Table 3.17 (Continued)

Section 13		0.0061	0.0081
Section 1	2	0.3885	0.8539
Section 2		0.0332	0.0903
Section 3		0.0301	0.0694
Section 4		0.0196	0.0455
Section 5		0.0141	0.0288
Section 6		0.0102	0.0184
Section 7		0.0086	0.0137
Section 8		0.0080	0.0115
Section 9		0.0078	0.0110
Section 10		0.0077	0.0109
Section 11		0.0076	0.0107
Section 12		0.0076	0.0107
Section 13		0.0076	0.0106
Section 1	3	0.7390	0.9553
Section 2		0.0990	0.2396
Section 3		0.0841	0.1833
Section 4		0.0520	0.1180
Section 5		0.0325	0.0698
Section 6		0.0192	0.0395
Section 7		0.0134	0.0246
Section 8		0.0111	0.0180
Section 9		0.0101	0.0155
Section 10		0.0098	0.0147
Section 11		0.0097	0.0141
Section 12		0.0096	0.0140
Section 13		0.0096	0.0138
Matrix, with resistance	3	0.0166	0.0299

Table 3.18 Resistance allele frequencies in matrix with different density dependence, high growth rate and dispersal, and RIB (HD)

Location	**Generation**	**10% RIB, HD**	
		CC – R_3 D_3 AD_1	**SC – R_3 D_3 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0553	0.0317
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.5056	0.4122

Table 3.18 (Continued)

Section 2		0.0333	0.0174
Section 3		0.0267	0.0151
Section 4		0.0209	0.0134
Section 5		0.0163	0.0119
Section 6		0.0136	0.0110
Section 7		0.0125	0.0104
Section 8		0.0119	0.0108
Section 9		0.0119	0.0106
Section 10		0.0120	0.0101
Section 11		0.0120	0.0106
Section 12		0.0117	0.0101
Section 13		0.0117	0.0101
Section 1	2	0.8779	0.8435
Section 2		0.1256	0.0810
Section 3		0.0977	0.0637
Section 4		0.0695	0.0458
Section 5		0.0484	0.0319
Section 6		0.0352	0.0237
Section 7		0.0293	0.0196
Section 8		0.0265	0.0189
Section 9		0.0258	0.0181
Section 10		0.0258	0.0173
Section 11		0.0257	0.0178
Section 12		0.0249	0.0178
Section 13		0.0247	0.0171
Section 1	3	0.9422	0.8389
Section 2		0.4203	0.2230
Section 3		0.3310	0.1708
Section 4		0.2301	0.1173
Section 5		0.1530	0.0780
Section 6		0.1031	0.0548
Section 7		0.0783	0.0424
Section 8		0.0657	0.0379
Section 9		0.0607	0.0349
Section 10		0.0589	0.0329
Section 11		0.0580	0.0332
Section 12		0.0558	0.0319
Section 13		0.0553	0.0318
Matrix, with resistance	3	0.0830	0.0459

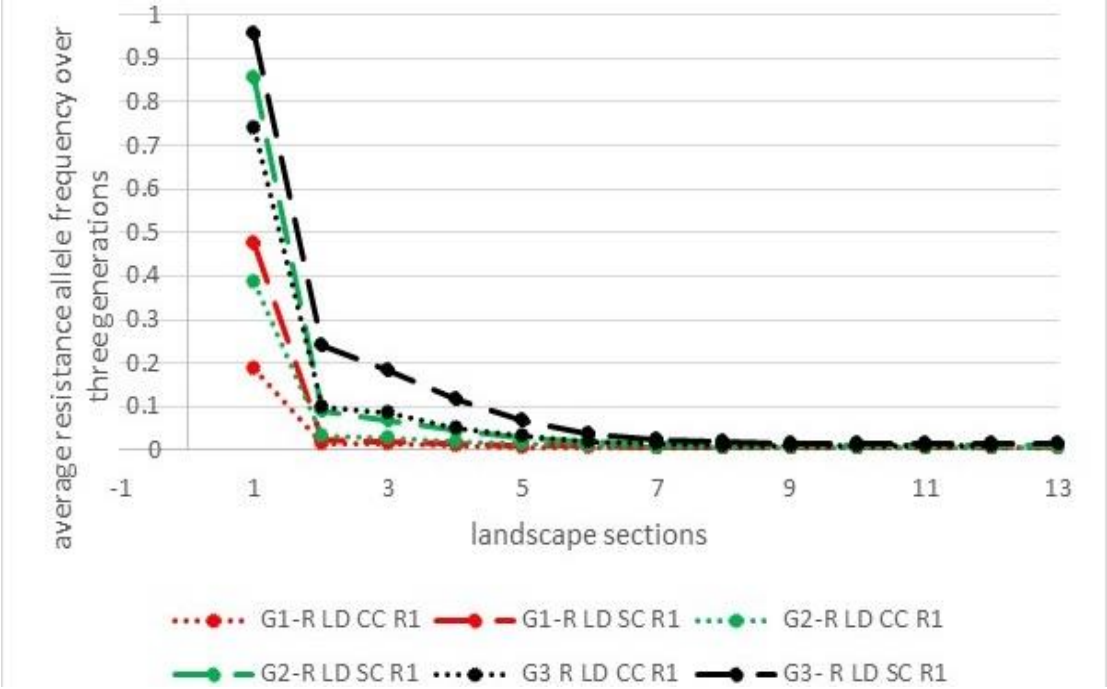

Figure 3.11 Resistance allele frequency across the landscape and over three generations when growth rate was low (HD RIB)

Note: G-1, G-2, and G-3 refer to generations. Contest competition is represented with dotted lines; scramble competition is represented with broken lines.

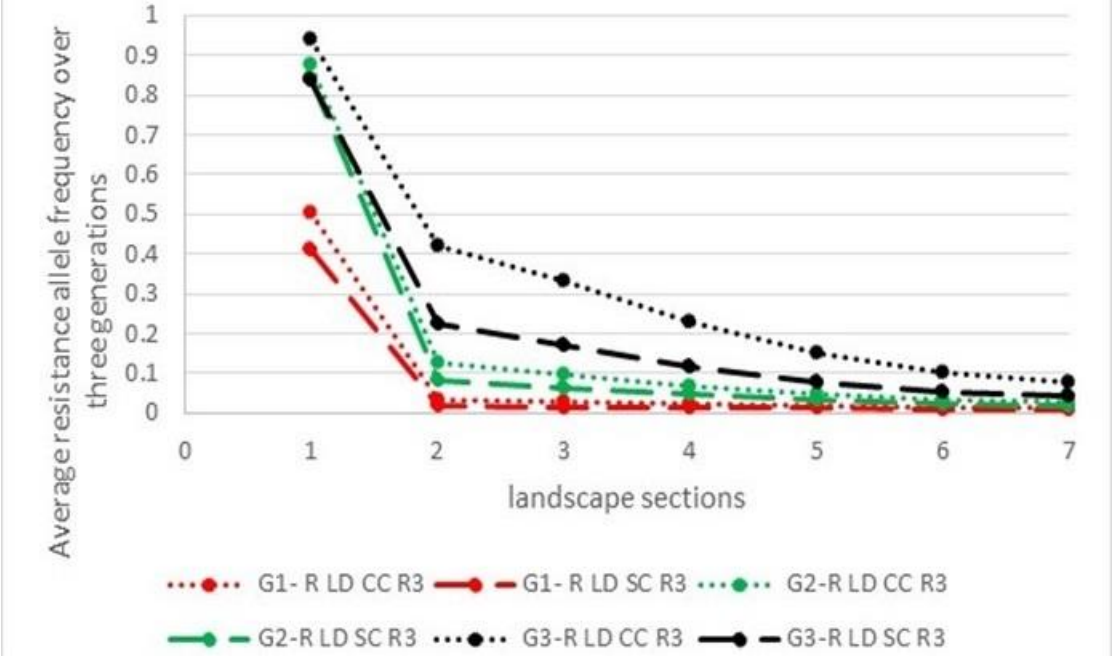

Figure 3.12 Resistance allele frequencies across the landscape over three generations when growth rate was high (HD RIB)

Note: G-1, G-2. G-3 refer to generations. Contest competition is represented with dotted lines; scramble competition is represented with broken lines

3.6.3.1.3 Mitigation of resistance by increasing refuge for high dose single PIPs

When RIB and block simulations with different assumptions of density dependence incorporated various values for available refuge for a single, high dose PIP with low and high growth rates and redistribution constants, then the average r-frequency in the landscape was always reduced (non-linear decrease) after three generations from the start of a simulation as the refuge proportion increased from 10-50% (Figures 3.13 and 3.14). When the pest had a low growth rate, the resistance allele frequencies were similar across the refuge range for contest and scramble competition. When the growth rate was increased, the resistance allele frequencies were much higher (>2 times for CC and 1.6 times for SC) compared to the results obtained with a low growth rate –

irrespective of the IRM strategy. The benefits of increasing the refuge were greatest between 10-20% for a pest with scramble competition and low growth rate and between 10-30% for the other growth rate and types of competition. After 30%, benefits of increasing the refuge diminished due to the non-linear decrease in the durability line. "Benefits", as referred to here, consider extending the life-time of the PIP, while the burden incurred on the growers increase when having to plant greater amounts of non-Bt crop, which could result in potentially yield loss. The lower r-frequencies with low growth rate were a function of there being less density dependent mortality in the refuge (less differential between refuge and Bt compartment), which reduced the increase in resistance allele frequency due to density dependence in a selection environment. With R_3, contest competition resulted in greater r-frequencies, while the reverse was true at R_1. As a reference, the projected average landscape frequencies were included when density dependence was turned off. It is evident that excluding population regulation from IRM models would lead to grossly overestimating the life-time of the PIPs.

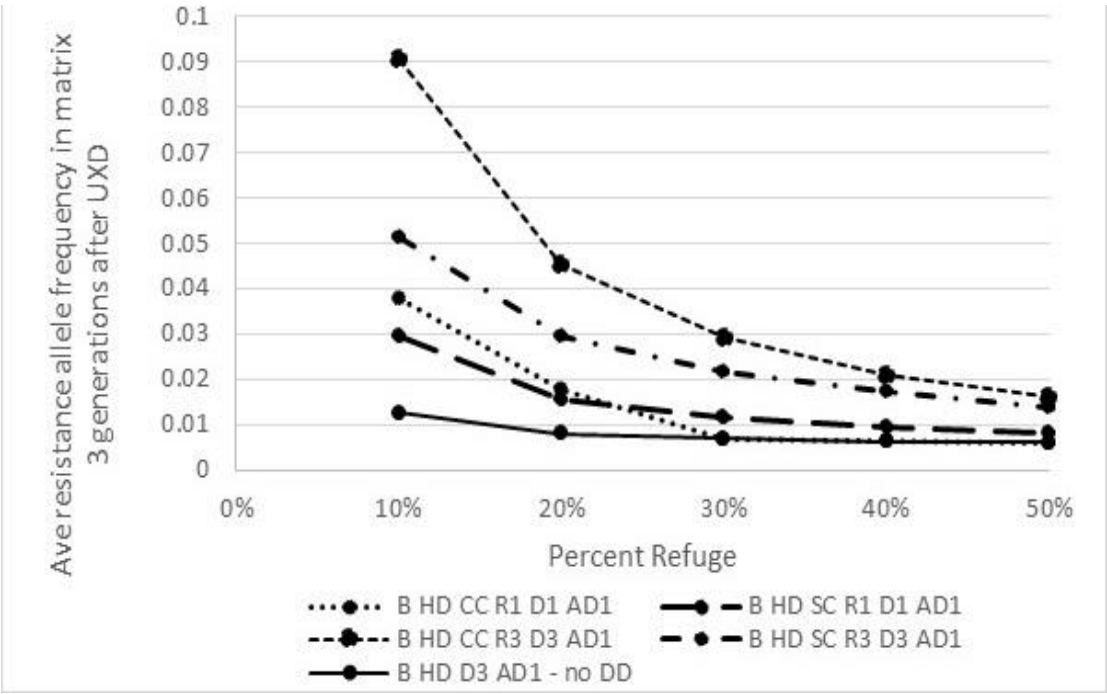

Figure 3.13 Average resistance allele frequency in matrix three generations after resistance was first detected (HD PIP block refuge)

Notes: different density dependent interactions (SC vs. CC), fecundity (R_1 vs. R_3), and dispersal (D_1 vs. D_3); the dark solid line is the projected resistance allele frequency at different refuge proportions in absence of density dependence.

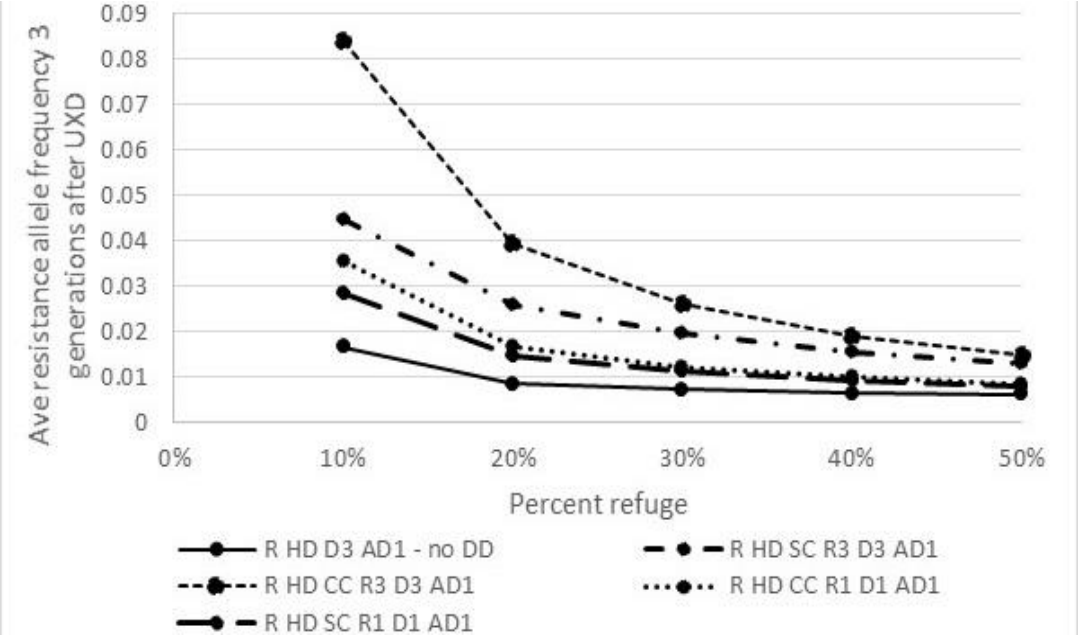

Figure 3.14 Average resistance allele frequency in matrix three generations after resistance was first detected (HD PIP RIB)

Notes: different density dependent interactions (SC vs. CC), fecundity (R_1 vs. R_3), and dispersal (D_1 vs. D_3); the dark solid line is the projected resistance allele frequency at different refuge proportions in absence of density dependence.

3.6.3.2 Low dose results

In absence of resistance in the UXD site, the r-frequencies in the square sections slowly and uniformly increased with each passing generation for block and RIB strategies when pest growth rates were low or high (Tables 3.19 through 3.22). A difference in average landscape resistance allele frequencies was observed due to different assumptions of intra-specific competition. For low pest growth rates and block simulations with contest competition the final frequency in the landscape after three generations from the start of a simulation was 0.0083 and for scramble competition 0.0116 (Table 3.19). For RIBs with low pest growth rate, the r-frequencies were 0.0112 and 0.0139 (CC and SC, respectively) (Table 3.21) and support the earlier results that a

10% RIB was less durable than a 20% block refuge most of the time when the pest had a low growth rate. Contest competition in combination with a low growth rate has shown to increase the durability for a low dose PIPs compared to scramble competition; the lower average r-frequency in the matrix support the earlier discussed results.

With higher growth rate values, average landscape r-frequencies were approximately equal for scramble and contest competition deployed with blocks (0.0118 and 0.0117) and RIBs (0.0143 and 0.0142) and in absence of resistance (Tables 3.20 and 3.22). The unchanged average landscape frequencies for scramble competition simulations reflect the previous results that the adaptation rate was not affected by increasing the pest growth rate for a low dose PIP. For contest competition simulations, pest adaptation rates as reflected by the resistance allele frequency increased with higher growth rate.

3.6.3.2.1 Block simulations

When the generic pest exhibited 10% resistance at locus 1 in the hotspot, had a low growth rate and diffusivity, and was exposed to a 20% block refuge and 80% Bt low dose crop, then the final average landscape resistance allele frequencies were approximately 13% (CC) and 3% higher (SC) compared to the 'no resistance' case at generation three (Table 3.19). Contest competition assumptions resulted in lower average resistance in the matrix compared to scramble competition with a low dose single PIP. It is important to remember that the final average frequency was affected by the size of the matrix (number of fields = 2601) and that with a larger landscape, the reported averages will decrease since the sections farther out (with lower frequencies) contribute more to the overall average. Increasing the matrix to a 51 x 51 was needed, however, to avoid

edge effects for the resistance allele frequency in the landscape. The increases reported in final landscape resistance allele frequency should be viewed in context of matrix size and the focus should remain on patterns of spread especially in sections closer to the UXD site. For both intra-specific competition assumptions, resistance was highest in the first square section during each of the three generations. In generation 1, a resistance phenomenon was visible in the first two and first section around the hotspot for contest and scramble competition, respectively. In generation 2, the phenomenon spread to section 4 for contest competition but did not spread greatly past section 1 for scramble competition. In generation 3, the resistance wave reached section 5 for contest and scramble competition and was less pronounced for the latter. Although overall landscape frequencies were lower with contest than with scramble competition after 3 generations, the r-frequencies in section 1 around the hotspot were always between 32 – 53% greater with contest competition. The drop in r-frequency from section 1-2 for both types of population regulation models dropped between 570-1100% over the time explored.

With a high growth rate and diffusivity, the increase in resistance after three generations compared to the 'no resistance case' at R_3 was approximately 5% for contest and 2% for scramble competition. Contest competition at R_3 predicted average resistance allele frequencies that were approximately 32% higher than those obtained at R_1. No difference was observed between average matrix frequencies with scramble competition (Tables 3.19 vs. 3.20). Resistance allele frequencies for both models of population regulation were lower in the section immediately surrounding the hypothetical hotspot when the pest had a high growth rate. For a low dose PIP, there was an indication that resistance also spread faster through the landscape when the pest had a high growth rate

and engaged in contest competition. The resistance allele frequencies remained mainly uniform throughout the landscape (sections 2-13) but were typically higher around the hotspot with contest competition assumptions.

In conclusion, resistance allele frequencies in the landscape were always highest around the hotspot (square section 1) and much lower thereafter. The resistance phenomenon was much more contained around the hotspot with low dose than with high dose exposure because there was less density dependent differential observed with low dose than high dose single PIPs. Hence resistance spread more slowly. Resistance allele frequencies slightly decreased in sections 2-13 and remained mostly uniform in the remainder of the landscape.

Table 3.19 Resistance allele frequencies in matrix with different density dependence, low growth rate and dispersal, and block refuge (LD)

Location	**Generation**	**20% Block, LD**	
		CC – R_1 D_1 AD_1	**SC – R_1 D_1 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0083	0.0116
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1171	0.0887
Section 2		0.0143	0.0072
Section 3		0.0096	0.0070
Section 4		0.0081	0.0068
Section 5		0.0069	0.0067
Section 6		0.0063	0.0066
Section 7		0.0061	0.0066
Section 8		0.0060	0.0065
Section 9		0.0059	0.0065
Section 10		0.0059	0.0065
Section 11		0.0059	0.0065
Section 12		0.0059	0.0065
Section 13		0.0059	0.0065
Section 1	2	0.1353	0.0973

Table 3.19 (Continued)

Section 2		0.0188	0.0105
Section 3		0.0132	0.0100
Section 4		0.0110	0.0095
Section 5		0.0089	0.0091
Section 6		0.0079	0.0088
Section 7		0.0074	0.0087
Section 8		0.0071	0.0087
Section 9		0.0071	0.0087
Section 10		0.0070	0.0087
Section 11		0.0070	0.0087
Section 12		0.0070	0.0087
Section 13		0.0070	0.0087
Section 1	3	0.1544	0.1011
Section 2		0.0232	0.0146
Section 3		0.0172	0.0138
Section 4		0.0142	0.0130
Section 5		0.0114	0.0123
Section 6		0.0098	0.0119
Section 7		0.0090	0.0117
Section 8		0.0086	0.0116
Section 9		0.0085	0.0116
Section 10		0.0084	0.0116
Section 11		0.0083	0.0116
Section 12		0.0083	0.0116
Section 13		0.0083	0.0116
Matrix, with resistance	3	0.0094	0.0119

Table 3.20 Resistance allele frequencies in matrix with different density dependence, high growth rate and dispersal, and block refuge (LD)

Location	**Generation**	**20% Block, LD**	
		CC – R_3 D_3 AD_1	**SC – R_3 D_3 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0118	0.0117
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1061	0.0650
Section 2		0.0082	0.0069

Table 3.20 (Continued)

Section 3		0.0077	0.0067
Section 4		0.0072	0.0066
Section 5		0.0069	0.0066
Section 6		0.0067	0.0066
Section 7		0.0067	0.0066
Section 8		0.0066	0.0066
Section 9		0.0066	0.0066
Section 10		0.0066	0.0066
Section 11		0.0066	0.0066
Section 12		0.0066	0.0066
Section 13		0.0066	0.0066
Section 1	2	0.1087	0.0757
Section 2		0.0117	0.0102
Section 3		0.0109	0.0098
Section 4		0.0100	0.0094
Section 5		0.0095	0.0091
Section 6		0.0092	0.0089
Section 7		0.0091	0.0088
Section 8		0.0090	0.0088
Section 9		0.0089	0.0088
Section 10		0.0088	0.0087
Section 11		0.0088	0.0088
Section 12		0.0088	0.0088
Section 13		0.0088	0.0088
Section 1	3	0.1126	0.0707
Section 2		0.0162	0.0139
Section 3		0.0150	0.0133
Section 4		0.0138	0.0127
Section 5		0.0129	0.0123
Section 6		0.0124	0.0120
Section 7		0.0121	0.0118
Section 8		0.0119	0.0118
Section 9		0.0119	0.0117
Section 10		0.0118	0.0117
Section 11		0.0118	0.0117
Section 12		0.0118	0.0117
Section 13		0.0118	0.0117
Matrix, with resistance	3	0.0124	0.0119

3.6.3.2.2 RIB simulations

When the generic pest exhibited contest or scramble competition and had a low growth rate and redistribution in a 10% RIB environment (R_1 D_1 AD_1), then the resistance allele frequencies were approximately 18% and 4% higher three generations after resistance was first observed (Table 3.21) compared to the 'no resistance' case. As always, the highest observed frequencies occurred in section 1 and then greatly dropped off in section 2 (up to 10- and 11-fold for CC and SC, respectively). Thereafter, a gradual decrease was visible throughout the landscape with each successive generation while overall resistance increased. The increase in resistance allele frequency in sections 2 through 7 ranged from 34-63% (CC) and 41-53% (SC). Scramble competition appeared to facilitate the spread of resistance through the landscape as the frequencies in section 1 were lower (46 – 126%) than for contest competition but higher in the sections farther removed from the hotspot (4-16%). A resistance phenomenon was visible up to section 2 in generation 1 (CC and SC), section 5 and section 3 for contest and scramble competition, respectively, in generation 2, and section 7 and 5 (CC and SC, respectively) in generation 3. The resistance phenomenon spread faster with contest competition dynamics than with scramble competition, although overall landscape frequencies were lower.

When the pest growth rate and diffusivity were increased (R_3 D_3), there was approximately a 5% increase in average resistance in the landscape for contest competition and three generations after resistance was first detected. The resistance phenomenon was localized, and resistance spread minimally for low dose PIPs deployed with a RIB. Tables 3.21 and 3.22 show that the greatest increase in resistance occurred in

section 1, the area immediately surrounding the UXD site. For contest and scramble competition simulations, resistance allele frequency distributions in section 1 were 13-26% and 29-40% lower compared to when the pest had a low growth rate. Resistance tended to be slightly higher in respective sections farther removed from the hotspot (raising overall averages in the matrix in generation 3) suggesting that there might be a slightly faster spread of resistance as well with greater growth rates and contest competition dynamics with RIBs.

Table 3.21 Resistance allele frequencies in matrix with different density dependence, low growth rate and dispersal, and RIB (LD)

Location	**Generation**	**10% RIB, LD**	
		CC – R_1 D_1 AD_1	**SC – R_1 D_1 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0112	0.0139
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1380	0.1006
Section 2		0.0124	0.0081
Section 3		0.0119	0.0075
Section 4		0.0096	0.0073
Section 5		0.0075	0.0072
Section 6		0.0079	0.0071
Section 7		0.0070	0.0070
Section 8		0.0066	0.0070
Section 9		0.0066	0.0070
Section 10		0.0065	0.0069
Section 11		0.0065	0.0070
Section 12		0.0065	0.0069
Section 13		0.0065	0.0069
Section 1	2	0.1933	0.1194
Section 2		0.0216	0.0124
Section 3		0.0192	0.0114
Section 4		0.0150	0.0108
Section 5		0.0116	0.0103
Section 6		0.0099	0.0100
Section 7		0.0091	0.0099

Table 3.21 (Continued)

Section 8		0.0087	0.0098
Section 9		0.0086	0.0098
Section 10		0.0086	0.0098
Section 11		0.0085	0.0098
Section 12		0.0085	0.0098
Section 13		0.0085	0.0098
Section 1	3	0.2751	0.1407
Section 2		0.0345	0.0185
Section 3		0.0296	0.0171
Section 4		0.0228	0.0160
Section 5		0.0171	0.0151
Section 6		0.0140	0.0145
Section 7		0.0125	0.0142
Section 8		0.0117	0.0141
Section 9		0.0114	0.0140
Section 10		0.0113	0.0140
Section 11		0.0113	0.0140
Section 12		0.0112	0.0140
Section 13		0.0112	0.0140
Matrix, with resistance	3	0.0132	0.0144

Table 3.22 Resistance allele frequencies in matrix with different density dependence, high growth rate and dispersal, and RIB (LD)

Location	**Generation**	**10% RIB, LD**	
		CC – R_3 D_3 AD_1	**SC – R_3 D_3 AD_1**
		Average resistance allele frequency, 3 generations after UXD	
Matrix – no resistance	3	0.0143	0.0142
10% Resistance in hypothetical hotspot field [25][25]			
Section 1	1	0.1205	0.0663
Section 2		0.0083	0.0074
Section 3		0.0080	0.0073
Section 4		0.0076	0.0072
Section 5		0.0073	0.0071
Section 6		0.0072	0.0070
Section 7		0.0071	0.0070
Section 8		0.0071	0.0070

Table 3.22 (Continued)

Section 9		0.0071	0.0070
Section 10		0.0070	0.0070
Section 11		0.0071	0.0070
Section 12		0.0070	0.0070
Section 13		0.0070	0.0070
Section 1	2	0.1439	0.0848
Section 2		0.0129	0.0118
Section 3		0.0122	0.0112
Section 4		0.0114	0.0107
Section 5		0.0107	0.0103
Section 6		0.0103	0.0101
Section 7		0.0101	0.0100
Section 8		0.0101	0.0099
Section 9		0.0100	0.0099
Section 10		0.0100	0.0099
Section 11		0.0100	0.0099
Section 12		0.0100	0.0099
Section 13		0.0100	0.0099
Section 1	3	0.1753	0.0858
Section 2		0.0196	0.0171
Section 3		0.0183	0.0148
Section 4		0.0168	0.0163
Section 5		0.0157	0.0155
Section 6		0.0150	0.0145
Section 7		0.0146	0.0143
Section 8		0.0144	0.0142
Section 9		0.0143	0.0142
Section 10		0.0143	0.0142
Section 11		0.0143	0.0142
Section 12		0.0143	0.0142
Section 13		0.0143	0.0142
Matrix, with resistance	3	0.0150	0.00146

3.6.3.2.3 Mitigation of resistance by increasing refuge for single PIPs

When RIB and block simulations with different assumptions of density dependence incorporated variable refuge values (10-50%) for a single, low dose PIP with low and high growth rates and various redistribution constants, then the average r-

frequency in the landscape was reduced (approximately linearly – exception was with CC R_1 where durability results followed those of density independent projections) three generations after a resistance occurrence in the hotspot (Figures 3.15 and 3.16). When the pest had a lower growth rate, the resistance allele frequencies were lower for contest than for scramble competition across the refuge range tested. When the growth rate was increased, the opposite phenomenon could be observed, and resistance allele frequencies were somewhat lower with scramble than for contest competition. When the pest exhibited scramble competition, there was no difference in projected durability for the low dose single PIP with different growth rates, however. For contest competition, greater growth rates resulted in somewhat higher resistance allele frequencies across the refuge range explored. This pattern held for both IRM strategies, though resistance allele frequencies typically were lower for block simulations than for RIBs. Unlike for HD simulations, low growth rates did not generate resistance allele frequencies in the landscape that were drastically different from those obtained with high growth rate assumptions – although visible differences were present (Figures 3.15 and 3.16).

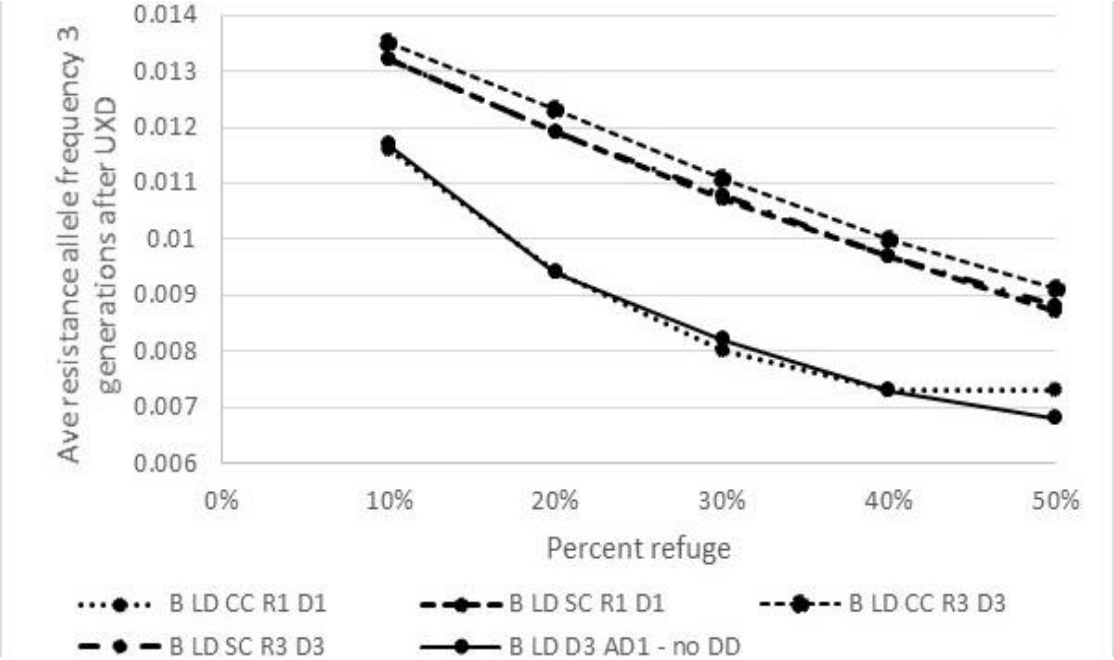

Figure 3.15 Average resistance allele frequency in matrix three generations after resistance was first detected (LD PIP block refuge).

Notes: different density dependent interactions (SC vs. CC), fecundity (R_1 vs. R_3), and dispersal (D_1 vs. D_3); the dark solid line is the projected resistance allele frequency at different refuge proportions in absence of density dependence.

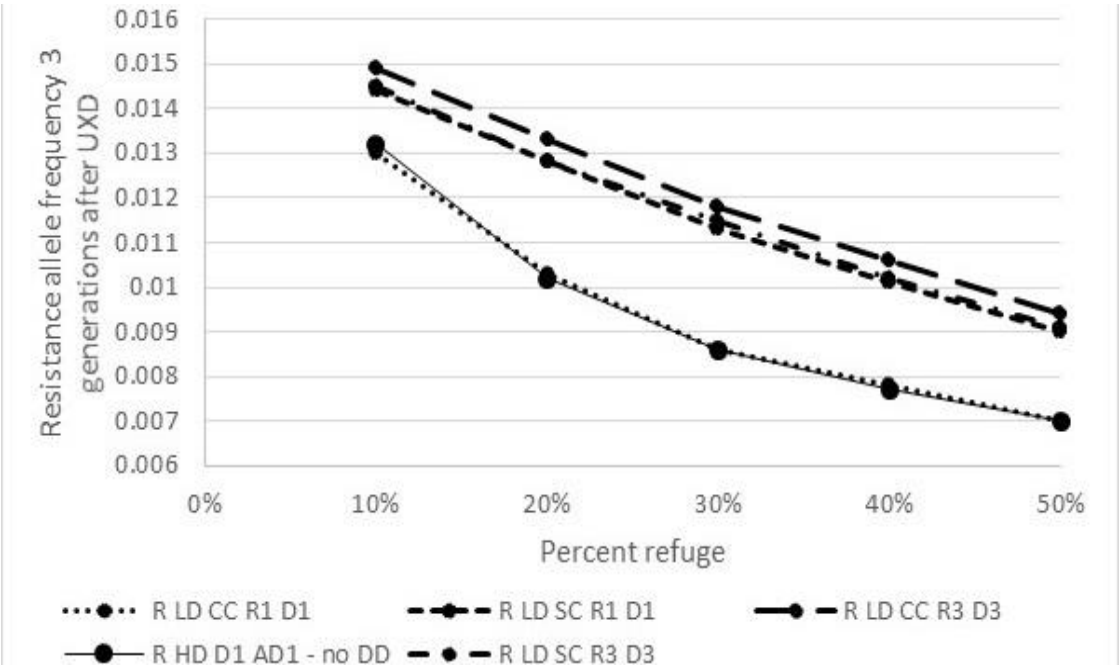

Figure 3.16 Average resistance allele frequency in matrix three generations after resistance was first detected (LD PIP RIB).

Notes: different density dependent interactions (SC vs. CC), fecundity (R_1 vs. R_3), and dispersal (D_1 vs. D_3); the dark solid line is the projected resistance allele frequency at different refuge proportions in absence of density dependence.

3.6.3.2.4 Mitigation of resistance by increasing refuge for pyramided PIPs

Likewise, resistance simulations were conducted for a low and high dose pyramid with different refuge percentages to observe whether the average resistance allele frequencies in the landscape were similarly affected with dual gene PIPs by such a mitigation strategy. The analysis identified that for a low dose pyramid deployed with a block refuge, the average landscape resistance allele frequencies also decreased approximately linearly as the percent refuge increased from 5 – 50% (Figure 3.17). The resistance allele frequency with a 50% compared to a 5% refuge was reduced by 104% (R_1) and 56% (R_3) for contest competition and 61% and 56% for scramble competition simulations. Effects of different assumptions for density dependence were visible as well,

and contest competition dynamics resulted in slightly greater resistance allele frequencies in the landscape than when the pest exhibited scramble competition (low and high pest growth rates). Furthermore, with a high growth rate, the resistance allele frequencies were once again greater than for a pest with a low growth rate (56% for CC and SC). Though the observed differences between different growth rate assumptions (holding everything else fixed) were not as great as for the single PIP analyses. All simulations including density dependence resulted in greater resistance allele frequencies compared to simulations without population regulations. This further strengthens the argument that including density dependent mechanisms in IRM models is a necessity.

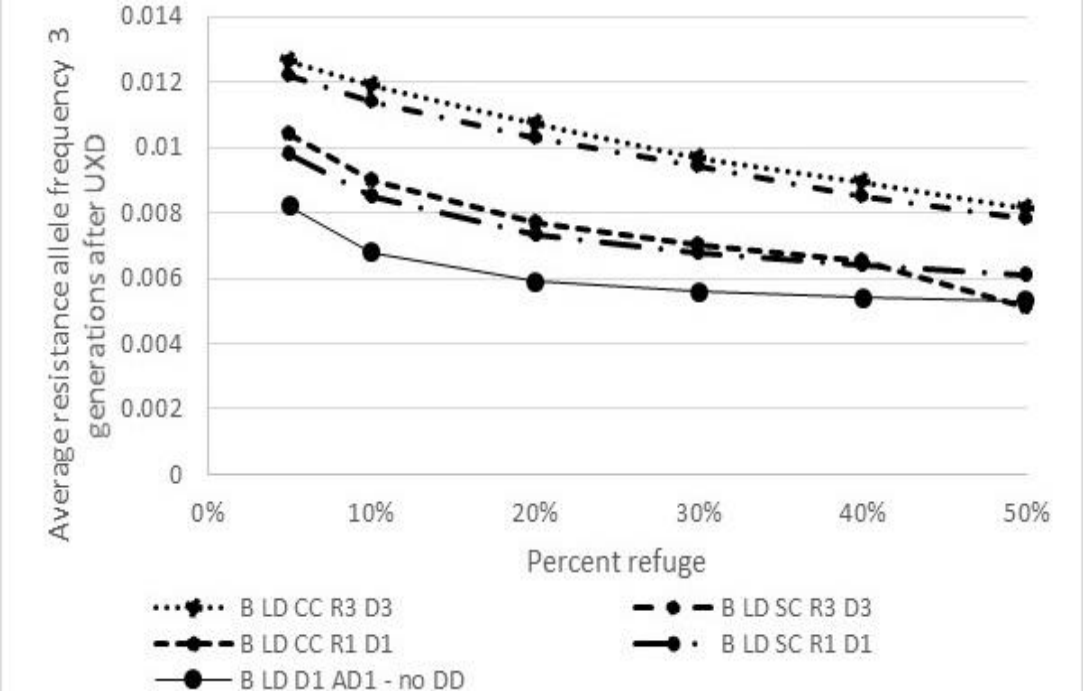

Figure 3.17 Average matrix resistance allele frequency three generations after resistance was first detected (LD pyramid block refuge).

For a high dose pyramid, the average landscape resistance allele frequencies remained unchanged with increasing refuge proportions and over the first three generations after resistance was detected (data not shown). There was no difference between IRM strategies, growth rates, and types of density dependence during this time interval. Density dependent mortality in the refuge still occurred as in previous simulations for single PIPs. Since rarely any individuals emerged from the simulated Bt fields with high dose assumptions, the effect of density dependence in the refuge had close to no effect on the resistance allele frequency over this initial time period.

3.7 Sensitivity analysis

Since the simulations in section 3.5.2 excluded variability sampling (except for initializing of pop size) and the results were reported for two extreme cases only, a brief sensitivity analysis was conducted over a broader range of intrinsic growth rate (R = 5-50) and redistribution constant (D = 0.5-112) for each type of density dependent model and refuge strategy.

The sensitivity analysis for diffusivity D informed that this parameter did not result in r-frequency changes for RIB and block simulations with low and high dose assumptions. The extreme values translated into average dispersal distances of one and 15 fields, respectively. This analysis supports the statistical findings discussed in section 3.5.1 that varying the redistribution kernel did not affect simulated adaptation to the PIP.

Varying the proportion of individuals dispersing increases from 0 to <1.0 greatly reduced the resistance allele frequency for a high dose. For a low dose PIP, there was no effect on r-frequency until the dispersal proportions reached values greater than 0.50. In

those cases, the resistance allele frequencies were lowered and extended the life-time of the PIP.

The sensitivity analysis for the intrinsic growth rate *R* revealed that increasing its value, while holding all other conditions fixed, resulted in increased resistance allele frequencies in the simulated landscape for a low dose PIP with contest competition and high dose PIP for both types of density dependent models.

3.8 Discussion

These research results indicate support that various life-history characteristics, refuge strategies, and dose of Bt PIP can affect the time to resistance in a generic (diploid) arthropod pest of Bt with sexual reproduction, often with significant interactions and surprising evolutionary outcomes. The intrinsic growth rate had the greatest effect on the simulated adaptation rate to a low and especially a high dose Bt PIP. Density dependence was the second most important parameter, and statistically significant differences were observed between contest and scramble competition simulations. Interactions between growth rate, type of density dependence, and the proportion dispersing were present for block simulations. For RIBs, interactions between growth rate and density dependence affected the time to resistance. The results suggest that a RIB for a single PIP might be more durable when the pest has a low growth rate. The magnitude of diffusivity had no significant effect on the rate of pest adaptation, and durabilities of the PIPs were not affected by varying the average dispersal distance in the distribution kernel function. Interesting it was only for high dose single PIP simulations that a greater dispersal proportion from the natal field resulted in increased durability. A pest with scramble competition dynamics and high growth rate may also help extend the lifetime of

a high dose single PIP. Furthermore, scramble competition dynamics resulted in higher durability estimates with higher growth rates than simulations with contest competition. This strengthens the importance to accurately model the type of intra-specific density dependence in simulation models because of different effects on the life-time of the PIPs and possible interactions with other parameters affecting the pest's adaptation rate. Dispersal as tested here seemed to be less important for extending the lifetime of low dose single PIPs. Only if the proportion was greatly increased (> 0.5) was the projected durability increased. Block simulations with a 20% refuge typically predicted higher durabilities for single PIPs than 10% RIBs using the same model of density dependence and various growth rates. This was mainly a function of the reduced available refuge with RIBs but also the added mortality of susceptible genotypes from inter-plant movement. These two factors together reduced the pest's 'effective' intrinsic growth rate compared to what the same pest would have experience in a block refuge/Bt environment. Overall, these results suggest that the interactions between life-history parameters can complicate resistance management. A generic approach should be avoided, and simulation modeling including a major target pest's specific life-history characteristic could be employed to determine resistance management strategies that most effective at prolonging resistance evolution.

That the life-time of the high dose single PIP was reduced to similar levels of a low dose single PIP if the pest had a higher intrinsic growth rates was very unexpected. The loss in durability from increasing the growth rate R_0 reached levels as high as 245% for blocks and 83% for RIBs. These results support preliminary findings reported by Caprio and Martinez (2012) where the durability of a high dose PIP was also greatly

reduced with a high pest growth rate. The main reason behind these results was the disproportionately occurring population regulation in refuge and Bt environments with high growth rates coupled with simultaneously occurring selection. Greater population regulation in the refuge decreased the susceptible pool of insects and sped up the rate of adaptation in a cell. The increase in resistance allele frequency caused by population regulation could reach levels that were six times greater than from selection alone. When the growth rate was lower, then population regulation and selection had similar effects on resistance allele frequency increases until a few generations before resistance was declared. At that point, selection became the main evolutionary force. For low dose single PIPs, this effect was also visible but somewhat muted by the fact that density dependent mortality typically occurred more equally in Bt and refuge fields. For resistance management purposes this suggests that low dose single PIPs may be more robust to withstand differences in life-history characteristics of different target pests than high dose PIPs.

Differences between simulations with scramble and contest competition were evident at all levels of growth rates in the high dose simulations. The lifetime of the PIP was estimated to last longer with contest competition and lower growth rates. When the pest growth rate and dispersal proportion were increased, it was scramble competition simulations that typically predicted greater durabilities. These results support that there may be considerable variability in the projected lifetimes of a high dose single PIP with different life-history characteristics of various arthropod pests, which suggests that considerable amount of analysis needs to go into the development of a resistance management plan for these types of toxins.

The results of this theoretical research suggest that the current understanding of the high dose + refuge paradigm may be incorrect for single PIPs when a pest has a high growth rate. Especially under these circumstances, it may be important to carefully evaluate the resistance management options and to consider alternate strategies, preferably pyramids, to extend the lifetime of each individual PIP. Resistance management plans should, therefore, always be based on analyses including pest-specific density dependent interactions and empirically estimated intrinsic growth rates to evaluate the life-time of single and pyramided PIPs. EPA and Bt technology providers may need to consider different refuge strategies that are based on major target pest characteristics and the type of PIPs deployed in various growing regions. Such a paradigm shift would add layers of complexity to resistance management that may not be viewed as practical or desirable by stakeholders. From a resistance evolution perspective and based on the results of these simulated studies, this would be needed to extend the life-time of the Bt technology.

The analysis for the spread of the resistance gene showed that with higher growth rates, the r-alleles spread faster through the landscape than with low pest growth rates. This was more pronounced for high dose PIPs than for low dose PIPs. I observed that with a low growth rate, resistance was high near the hotspot and after a rapid drop-off, gradually decreased throughout the remainder of the landscape. With a high growth rate, resistance was lower in the section immediately surrounding the UXD site, but resistance allele frequencies were higher in fields farther removed from the hotspot leading to greater overall matrix resistance allele frequencies and, hence, faster spread of resistance. The difference in resistance was 86% (scramble competition) and 228% (contest

competition) higher in the landscape three generations after resistance was first detected if the pest had a high rather than a low growth rate and was exposed to a high dose Bt PIP (block refuge). This difference was 54% and 400% if the pest was exposed to a 10% RIB and exhibited scramble and contest competition dynamics, respectively. This increase was not as drastic when the PIP expressed a low dose for a pest with contest or scramble competition dynamics. These results seem to suggest that mitigation in response to hotspot resistance needs to utilize effective tools that are deployed without delay to maintain the durability of a high dose single PIP in the remainder of the landscape.

As a mitigation strategy, the percent refuge was increased up to 50%, and a non-linear decrease in resistance allele frequency was observed for the high dose single PIP scenario where the greatest gain occurred between 10-30% for RIBs and blocks. After a 30% refuge, the allele frequencies decreased more slowly, and there may not be a great economic incentive for growers to consider planting greater refuges for such PIPs because of increased damage or potential yield loss that could occur with greater amounts of non-Bt protected plants per area. For low dose single PIPs and low dose pyramided PIPs, mitigating resistance in the landscape by increasing the percent refuge from 10-50% lead to an almost linear decrease in average landscape resistance allele frequency. Based on my simulated studies, increasing the refuge to 50% for such Bt PIPs would greatly improve their life-time. This implies that increasing the refuge may be an effective mitigatory strategy for such PIPs irrespective of the target pest's life-history characteristics. For a high dose pyramid, no decrease was observed in the average matrix resistance allele frequency with increased refuge over the short time period explored. Though density dependent mortality occurred in the refuge, a generational increase in

resistance during the first three generations was not observed. This suggests that mitigation of hotspot resistance at one locus may be effectively mitigated in the surrounding areas with a pyramid. The mechanisms behind these results need to be further investigated.

3.9 References

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CHAPTER IV

THEORETICALLY TESTED REMEDIATION IN RESPONSE TO INSECT RESISTANCE TO Bt CORN AND Bt COTTON

4.1 Abstract

Integrated Pest Management (IPM) participation increased the durability of a less than high dose pyramid (consisting of two Bt toxins) targeting corn rootworm and bollworm if implemented at the time of commercialization of a new PIP assuming no prior selection or cross-resistance. IPM + IRM participation at 40, 50 and 70% (as measured on a per field basis) delayed resistance between 9-50% and 22-45% for rootworm and bollworm, respectively, compared to using IRM alone. As IPM participation increased, the durability for the pyramid was extended. Results of this research show the importance of including non-selective periods for corn rootworm to reduce the selection pressure from continuous Bt use. For bollworm, the inclusion of non-selective periods did not significantly change the life-time of the PIP. This suggests that IPM + IRM programs should not be generic but pest specific and consider life-history characteristics.

Based on the IPM and/or IRM strategies chosen, the life-time of a pyramid was affected differently. For corn rootworm, simulating crop rotation to a non-host plant was preferable and the most effective strategy. Soil applied insecticide use with Bt deployment did not increase the durability of the pyramid based on reasonable

survivorship assumptions used in the model. Based on efficacy assumptions reported in the southern U.S., the use of larvacides in Bt corn and cotton to control bollworm extended the durability of the pyramid equivalent to 70% IPM + IRM participation as measured on a per field basis. Increasing the refuge for a low dose pyramid to 20 – 30 % for corn rootworm and to 30% for bollworm extended the life-time of the pyramid and should be another management option made available to growers. Based on these simulated results, development of an incentive program may need to be considered that rewards growers who voluntarily increase the percent refuge for less-than-high dose pyramids.

A local resistance phenomenon for corn rootworm was apparent in the landscape surrounding the resistance hotspot and spread slowly through the landscape. This was a function of the pyramided Bt PIP, which kept the resistance phenomenon from spreading quickly across the landscape. Local remediation one generation after detection of field failure had no effect on the rate of adaptation if remedial action strategies were applied randomly. Under these circumstances, regional mitigation was always superior to local remediation and reduced the overall resistance to levels equivalent to Bt selection with IPM upfront. If more effective tools were applied immediately during the remediation process (e.g. non-random application of crop rotation), then local remediation was also effective at reducing landscape resistance allele frequencies. It should be expected that resistance genes will always escape a resistance hotspot, and the results imply that mitigation of resistance for a pest that engages in dispersal will be more effective on a larger geographic scale. In the case of widespread (10 and 50%) resistance at one locus,

regional mitigation on 70 or 100% of the fields only minimally increased the life-time of the pyramid.

For bollworm, when resistance was widespread at one locus (10 and 50%), the lifetime of the pyramid was compromised in 7.6 and 3.9 years (respectively) with an IRM approach only. When 70% of the fields were mitigated the year after resistance was first visually detected, the pyramid lasted at best two to three years longer. These results show that mitigation can only minimally extend the durability of the pyramid, but proactive IPM+IRM implemented across most of the landscape at the beginning of a new PIP deployment is superior to IRM alone.

4.2 Introduction

Western corn rootworm (*Diabrotica virgifera virgifera* LeConte) resistance to Cry3Bb1 was first documented in Iowa (Gassmann et al. 2011, 2012; Gassmann et al. 2012) and later identified in multiple locations in Illinois (Gray 2012). In light of these reports, scientists at the US EPA echoed academia's' concern over the current ineffective resistance detection tools for corn rootworm (EPA 2011, 2013). In response, the US EPA held a Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) meeting to obtain expert recommendations for improvements.

The FIFRA SAP (1998) had concluded that IRM alone was not sufficient for low dose Bt toxins and should be used together with IPM to manage pests. The SAP (2014) more explicitly expressed that IRM for low dose toxins aimed at controlling corn rootworm were insufficient and needed to be combined with an IPM approach to prolong the lifetime of the current Bt technology. The Panel concurred with EPA that generic remedial action plans for corn rootworm needed to be replaced with specific and

theoretically tested remediation proposals. In addition, any remedial action plan should undergo scientific scrutiny, much like required insect resistance management (IRM) plans at the time of the registration submission to the Agency, and that theoretical models should be employed to explore the potential success of different mitigation strategies. These plans needed to be in place before field resistance developed so that pest-specific remediation would be triggered with little delay and when resistance was confirmed (US EPA 2013). Many of the weaknesses identified in the corn rootworm resistance management program apply also for *H. zea*. For example, there are no single or pyramided Bt PIPs commercialized that express a high dose in Bt corn and Bt cotton, and variability in susceptibility measured with diet bioassays is great (Ali et al. 2006, Ali and Luttrell 2007, Tabashnik et al. 2008) as for corn rootworm. Diet bioassays may also not provide a diagnostic tool for detecting *H. zea* resistance to Bt in the field.

Under the current regulatory process for resistance management, remediation for corn rootworm and bollworm resistance would likely never be triggered. This is partly because of challenges with LC_{50}/EC_{50} diet bioassays, the diapausing egg stage of corn rootworm, and the stepwise regulatory process leading to confirming resistance (US EPA 2013). Previous analyses (Chapter 3) predicted that delaying remediation for a generic pest of Bt allows the resistance allele frequency to increase across the landscape within just one generation after resistance has been detected and that a gradient becomes apparent during the first few generations with the frequencies being highest near the resistant site. This rapid spread occurred irrespective of the magnitude of diffusivity explored (D = 0.5 - 112) and was driven by higher pest growth rates and nature of single PIP.

My research investigated whether remediation strategies for corn rootworm had the potential to slow the adaptation rate in the landscape one and three generations after resistance was first visually detected through field failure (assumption that resistance was at 10% in the population – see Roush & Miller (1986)). Furthermore, I evaluated whether IPM practices upfront with the use of IRM for corn rootworm and bollworm increased the durability of the Bt PIP compared to IRM alone. The term IPM here means to 1) use diverse methods of pest controls (cultural, physical, biological, chemical, etc.) but also implies that 2) these techniques be implemented together with monitoring for pest abundances to reduce unnecessary pesticide use and minimize risk to people, other non-target species, and the environment (latter part not tested or addressed here). The analyses also estimated by how much the lifetime for the second gene in the pyramid could be extended with mitigatory strategies once the first gene was compromised. The research provided a theoretical approach for testing remedial action plans in case of resistance development in corn rootworm and bollworm. Ultimately, this research will provide simulated model results to assist the regulatory agency and others as to how current remedial action plans and resistance monitoring programs for corn rootworm and bollworm could be improved. This type of theoretical work differs from the current approach that relies on no theoretical testing of proposed remedial actions and represents a possible paradigm shift in approaches to development of resistance management strategies.

4.3 Materials and methods

4.3.1 Models structure

Two species-specific, stochastic, spatially explicit, and frequency-based probabilistic models were developed (developed in Java using NetBeans IDE 7.4) to explore the effects of various mitigatory strategies (e.g. local (field) vs. regional mitigation (matrix)) to delay corn rootworm and bollworm resistance from spreading in the landscape after resistance was first visually detected as field failure (unexpected damage site = UXD) with 10% resistance. The probability sampling was excluded to investigate the spread of the resistance allele in the deterministic mode. The models allowed me to investigate effects of IPM with IRM use on time to resistance in the two pests at the onset of commercialization of a hypothetical two-gene Bt PIP. Finally, I explored options to manage a compromised pyramid when resistance was localized or geographically widespread and resistance was at 10 or 50% at locus 1.

The landscape consisted of a 51 x 51 field matrix (one field represents 50 ha of corn with 4 million plants; matrix size = 36.1 km x 36.1 km) and was designed as a torus so that all fields were identical, and each could be viewed as the center of the torus. Since a torus assumes that the simulated region is surrounded by similar systems, I essentially placed a recurring resistance hotspot into the landscape every 36.1 km. This assumption may represent a worst-case scenario for corn rootworm in the Corn Belt and bollworm in the cotton growing states. Because the distance between hotspots was relatively short, a preliminary investigation had to be conducted to investigate the presence of edge effects on the resistance allele frequency from successive dispersal over the time period investigated (6 generations). The results showed that the observed differences in r-

frequency in the outer sections of the matrix between a 51 x 51 field matrix compared to a 61 x 61 field matrix were marginal; hence, the smaller size matrix sufficed for the purpose of the resistance allele spread analysis (described later), and a hypothetical hotspot was placed into field [25][25].

The models used a redistribution process of dispersal to simulate adult movement (described in Chapter 3). Average displacement distance ($\sqrt{2D}$) is one characteristic of dispersal; the proportion of a population engaging in redistribution is another parameter and will be discussed in the pest specific sections. The theory states that the motion of each individual is random in addition to being independent of the motion of all other individuals. This type of movement is also referred to as 'random walk' dispersal where no other external factors affect the path an individual takes. The Gaussian diffusion equation (solution for Fick's equation for two-dimensional movement) was used and provided a probability distribution for a fraction (gamma) of individuals leaving the natal site (Okubo 1980) (previously discussed in Chapter 3):

$$y(x,y) = y_0 \frac{e^{-(x^2+y^2)/2(2D)}}{2\pi 2D} \tag{4.1}$$

The fraction remaining in the natal habitat (1-gamma) engaged in trivial motion; these two types of distinct dispersal behaviors of a population result in a leptokurtic distribution in the landscape (Okubo 1980).

The models were discrete time step models with one generation per year for *D. virgifera* and six generations/year of *H. zea.* Typically, it has been assumed for the purpose of simulations that resistance is complete and governed by one major gene. These same assumptions were made in these models here, although the assumption may

be incorrect for corn rootworm (SAP 2009, Gassmann et al. 2011) as well as for bollworm (Burd et al. 2003, Li et al. 2004, Jackson et al. 2006). None of the currently commercialized PIPs express a high dose for these two pests, and two hypothetical low dose, pyramids were, therefore, modeled. At each of the two loci, there were two alleles resulting in three possible genotypes: susceptible (*SS*), heterozygous resistant (*RS*), and homozygous resistant (*RR*). The mean fitness components were $W_{SS} = 0.20$, mean $W_{RS} = 0.28$ ($h = 0.10$), and $W_{RR} = 1.0$, mean. Total genotypic survival from exposure to the two gene Bt PIP was determined by multiplying the two fitness components at both loci. No fitness costs were assumed since this was the most conservative assumption. In absence of fitness costs, resistance can be expected to evolve faster, and simulations should predict lower durability estimates. I was mostly interested in relative durability comparisons between different IPM or remedial action approaches rather than absolute time estimates and, therefore, excluding potential cost to resistance seemed reasonable.

Stochasticity was added into the system at the beginning of a simulation by initializing the corn rootworm population size in each 50 ha field with an egg density randomly drawn from an interval between 0 and 60,000,000, which translated into ≤0.75 adult beetles per corn plant; in the resistant site this population size was increased to ≥440,000,000 eggs, which translated into ≥22 million beetles in a 50 hectare field (5% survivorship) or ≥5 beetles per plant. For bollworm, the simulation started out in the third generation with an initial egg load in whorl stage corn randomly selected between 0 and 6,000,000 (max = 1.2 larvae/plant). When there was a resistance hotspot, the initial egg density in a field was higher and started out at 18,000,000 as to increase the larval abundance and to increase visible damage (between 14.4 E^{+6} before and 6.9 E^{+6} after

natural larval mortality occurs). This translated into 3.6 larvae per corn plant (before natural mortality) in the affected field. The random initialization of population densities allowed for potential source sink dynamics in the landscape. Further variability in the system was introduced by sampling life-history and other parameter values from a PERT-Beta (mode, minimum, and maximum), Poisson (variance = mean), and uniform distribution with predetermined ranges (minimum and maximum) (see Tables 4.1 and 4.2).

4.3.2 Western corn rootworm specifics

Dispersal for adult Western corn rootworm was simulated in two steps in this model: general pre-mating dispersal followed by another event for female post-mating dispersal. For pre-mating dispersal, the diffusivity *D* was set to a mean of 3.0 (min 1.0, max 5.0) resulting in an average dispersal distance of 2.45 fields/generation (1732 x 1732 m^2/generation). Nowatzki et al. (2003) reported that adult daily dispersal distance was on average 14.5 m. Caprio and Glaser (2010) reevaluated their movement data using a Brownian motion model and reported that adult movement was underestimated and should have been around 41.8 m/day. The fields in this current corn rootworm model consisted of 50 ha (707 x 707 m^2). Considering that females and males have an average longevity of 78.2 days and 102.4 days, respectively, (Hill 1975) the possible range of adult average dispersal distance captured by the two researchers' proposed mean daily dispersal distance would be 1170 -3268.8 m for female and 1484.8 – 4280.3 m for male beetles. An average distance as calculated by a diffusivity of 3 appears reasonable using Caprio and Glasser's (2010) average daily dispersal distance but would be a slight over-estimate using Nowatzki's estimate (2003).

Movement proportion was set to a mean value of 10% with a minimum and maximum value of 5% and 30%, respectively though estimates of mean proportion dispersing seem to be closer to 20% (Dr. Spencer, U IL, *pers. com*.). The mean was set below Spencer's estimate to try and create a local resistance phenomenon, which is typically assumed for corn rootworm – though empirical data to support this assumption are currently lacking. The above dispersal assumptions were used here to describe the proportion that left the natal field but did not address trivial motion of adults in the field, Naranjo (1991) calculated based on a comparison between trap successes and theoretical simulations that (61.9%) Western corn rootworm were primarily emigrants when originating from early planted corn plots, although it is not clear what the physical separation was between the plots under investigation. In a mark-release-recapture study conducted in Europe (1% recapture rate), it was calculated that 50% of corn rootworm dispersed between 117 to 425 m per day, and one percent of the adults travelled between 775 m and > 8 km (Carrasco et al. 2010). It was further calculated that between nine to 45% of adults and 0.6 to 21% adults would escape a 1,000 m and 5,000 m buffer zone, respectively, within one generation. This zone represents 1.4 and 7.0 linear field distance in this model. In absence of more precise empirical data for the U.S., this European study can further inform the present understanding of the possible dispersal proportions and distances of Western corn rootworm in North America. The proposed average displacement and dispersal proportions for Western corn rootworm in this model were a reasonable first assumption based on limited available information.

The proportion of females dispersing post-mating has been reported as low as 15% (Coats et al. 1986) and as high as 25% (Naranjo 1990); the mean value in this model

was set to 15% (min 5%, max 25%). The diffusivity for post mating dispersal was set to a mean value of 8.0 (min 6.0, max 10.0) resulting in a mean displacement of four fields/generation (2828 x 2828 m^2). From flight mill studies, it is known that sustained flights can last up to four hours, and a maximum distance of 24 km (equal to 33.9 linear fields as described in this model) was covered during one flight event (Coats et al. 1986). Considering that insects do typically not travel in a straight line (unless carried by a storm system or an equivalent mechanism) but have shown to engage in random walk (Kareiva 1983), these estimates by Coates should not be interpreted as linear average (post mating) dispersal distances. My assumption of four fields (2.8 km^2) could, however, be an underestimate.

Density dependence was modeled as scramble competition for corn rootworm using the Hassell equation (1975) to regulate population densities in each field (method previously described in Chapter 2). The IRM strategies employed for the dual gene pyramid reflected the EPA requirement for a 5% structured block refuge or seed blend (RIB, Refuge-in-the-Bag). The value for *b* in the modified Hassel equation was 2.999, and the value for *a* was calculated using the modified equation described in Chapter 2.

The same process of movement was used as described by Mallet and Porter (1992) with the exception that adult movement occurred twice per generation as opposed to once. Larval movement occurred once only as in Mallet and Porter. There was a probability ($M = 0.30$) that a larva left or remained (1-M) on a plant. If a larva dispersed, there was a probability that it could land on a Bt plant (1-V) or refuge plant (V). V took on the same value as the parameter for percent refuge plants per field (*PropRef* = 0.05), while 1-V was equal to the proportion of Bt plants in the seed blend (*PropBt* = 0.95). Bt

mortality was incurred before movement took place, and only survivors moved. If the movement was from Bt-to-Bt plant, additional selection occurred after inter-plant movement was completed. Unlike Mallet and Porter, however, an additional 20% movement penalty was incorporated into the RIB simulations. This mortality could be greater in the field when movement occurs between rows rather than within rows where roots of one corn plant are more likely to touch those of neighboring plants. In absence of any empirical data and as a first approximation, the value for this parameter remained fixed at 20%. This particular section of code was validated without the additional movement mortality and density dependence excluded, and results of the RIB demonstrated (as in Mallet and Porter, 1992) that the durability of the PIP was reduced compared to the estimated durability of the block refuge with the same refuge proportion (*ceteris* paribus). Life-history parameters that are included in the variability sampling and their values and/or ranges are listed in Table 4.1.

Table 4.1 *D. virgifera* parameters and values for PERT-Beta, Poisson, and uniform distributions

Parameter	Min	Mode	Max	Comments
Natural larval survival	0.85	0.90	0.95	Mode derived with information provided by Hibbard et al. (2010)
Natural Fecundity	1087			Hill (1975); Poisson distribution
Egg viability	0.029	0.084	0.10	Hibbard et al. (2010)
Overwintering survival	0.4	0.5	0.6	Mode is value reported by Godfrey & Turpin (1983); Onstad et al. (2006)
[1]IRAF	0.001	0.005	0.01	Same for both loci
IRAF-2	0.01	0.015	0.02	Includes prior years of commercialization
Adult redistribution kernel	1.0	3.0	5.0	Estimate based on Spencer (U IL, *pers. com*.)
Adult dispersal proportion	0.05	0.10	0.30	Spencer (U IL, *pers. com*)
Female post-mating redistribution kernel	6.0	8.0	10.0	Estimate based on Coats et al. (1986)
Female post-mating dispersal proportion	0.05	0.15	0.25	Coats et al. (1986); Naranjo (1990)
W*ss*	0.1	0.2	0.3	Same for both loci
Dominance	0.05	0.10	0.15	Same for both loci
Base larval movement in RIB	0.10	0.30	0.40	Similar to Caprio & Glaser (2010)
[2]SAI survivorship	0.5-1.0			Uniform distribution; assumes mode of actions (MOA) are rotated every year.
Adulticide survivorship	0.10-0.30			Uniform distribution; range allows for early and late emerging beetles to escape control; assumes MOA are rotated every year.

Note: [1]IRAF = initial resistance allele frequency; [2]SAI = soil applied insecticides; PERT-Beta distribution reported minimum, maximum, and mean values; Poisson distribution reported mean and standard deviation; uniform distribution reported minimum and maximum values of range.

4.3.3 Bollworm specifics

Movement for adult bollworm in these simulations included one dispersal event per generation, which occurred before mating took place. Bollworm have a high propensity to disperse and engage in long-distance migration (Gould et al. 2002, Sparks

et al. 1986). On a smaller geographic scale, it has been documented that ovipositing females left their natal and neighboring fields to lay eggs in more distant plots (Isley 1935). In a rubidium study to track movement of bollworm, it was determined that males flew between 0.5 and 2.5 km from their point of origin (Graham et al. 1978); in my system, this would be approximately an average displacement of 3.53 fields/generation. As a first assumption, the diffusivity was set to a mean of 10.0 (min 8.0, max 12.0), which translated into 4.89 fields and slightly exceed the reported distance by Graham et al. (1978). The dispersal proportion was set to 0.5 based on anecdotal evidence and in absence of empirical data. Larval movement was not simulated here because only block refuges were modeled. The *H. zea* model analyzes resistance evolution in a regional population (as reflected by the torus), and the pest propensity to migrate was excluded for this purpose.

Density dependence was modeled as contest competition for bollworm using the modified Hassell equation. The values of *b* were calculated based on R_0 assumptions (see Chapter 2) and set to 1.05 in the early natural host, 1.1 in corn, and 1.05 in cotton and 1.15 in late natural host. The values for parameter *a* were based on carrying capacity, R_0, and values for *b* and were calculated using the respective equation and parameter values previously discussed (Chapter 2). The IRM strategies employed was a 20% block refuge for Bt corn controlling bollworm in the southern US, and a 20% cultivated/natural host refuge was assumed for Bt cotton. For the first two generations of bollworm, 20% of the landscape was dedicated to an early season, non-cultivated host onto which all overwintering survivors later oviposited their eggs. This was a modification from the approach described in chapter 2, where only 20% of the overwintering survivors were

allowed to colonize the 20% natural host space in the matrix. Life-history parameters that were included in the variability sampling and their values and ranges are listed in Table 4.2.

Table 4.2 *H. zea* parameters and values for PERT-Beta, Poisson, and uniform distribution

Parameter	Min	Mode	Max	Comments
Larv surv on natural host	0.25	0.35	0.45	Caprio et al. (2009)
Larv surv on corn	0.38	0.48	0.58	
Larv surv on cotton	0.10	0.15	0.20	
Pupa surv	0.70	0.80	0.90	Kring et al. (1993)
Adult surv	0.70	0.80	0.90	
Fecundity on natural host	[1]600			Poisson distribution
Fecundity on corn	[1]350			Poisson distribution
Fecundity on cotton	[1]500			Poisson distribution
Egg viability on natural host	0.40	0.50	0.60	Caprio et al. (2009)
Egg viability on corn	0.70	0.80	0.90	
Egg viability on cotton	0.50	0.60	0.70	
Overwintering survival	0.05	0.0625	0.075	Caprio et al. (2009), Stadelbacher & Pfrimmer (1972)
IRAF	0.001	0.005	0.01	Assumes no pre-commercialization release, no cross-resistance with existing commercialized toxins.
IRAF-2	0.01	0.015	0.02	Includes prior years of commercialization
Adult redistribution kernel	8	10	12	
Adult dispersal proportion	0.40	0.5	0.75	
Susceptible fitness - W_{SS}	0.25	0.20	0.15	Hypothetical low dose
Resistant fitness - W_{RR}	1.0			Complete resistance
Dominance *h*	0.05	0.1	0.2	Hypothetical values
Larvacide survivorship	0.35-0.45			Uniform distribution range based on Mississippi State Control Guide (2014)

Note: [1] fecundity values were adjusted to achieve R_0 values reported by Caprio et al. (2009); PERT-Beta distribution reported minimum, maximum, and mean values; Poisson distribution reported mean and standard deviation; uniform distribution reported minimum and maximum values of range.

4.3.4 IPM and remediation modeling

4.3.4.1 *D. virgifera* modeling scenarios

The corn rootworm modeling scenarios explored are listed in Table 4.3, and included IPM+IRM approach at the outset of the PIP commercialization, as recommended by the SAP (2014) to prolong the life-time of less durable, low dose PIPs. An IRM baseline scenario (no insecticides) was established to later determine the relative durability gain from the combined use of the four proposed corn rootworm IPM strategies. For corn rootworm IPM, non-Bt corn was made available as a strategy (but withheld in later remediation scenarios) to observe impacts of non-selective periods on the rate of adaptation of corn rootworm. Other IPM and IRM tools modeled were crop rotation, soil applied insecticides (SAI) with Bt deployment, and increased refuge (20%). When the economic threshold for corn rootworm adults was exceeded, an additional control tool was used (adulticide spraying). According to the Illinois Field Crop Scouting Manual (2012), the economic threshold for corn rootworm adults is exceeded when more than five individuals are present on a plant; control measures (e.g. adulticide spraying) are then recommended to protect pollination of ears and prevent silk clipping. This economic threshold was used for non-resistance simulations. When resistance developed at locus 1, however, adulticides were applied in those fields when the beetle population size exceeded a threshold of 0.75 adults per plant as to prevent egg laying and protect against future root injury during the following season (Illinois Field Crop Scouting Manual, 2010).

Each IPM and IRM tool was evaluated alone to understand the relative benefit of choosing one over the other. In scenario (1), combined strategies were used in the IPM

participation simulations (explained below) at the on-set of a new product commercialization. The use of the term "IPM participation" throughout the remainder of the chapter refers to combining IPM and IRM tools. There was an equally likely chance that the four IPM and IRM tools explored (crop rotation, SAI with Bt, non-Bt corn, and increased refuge) were applied to a field during a particular year; the baseline IRM strategy as mandated by EPA (planting of 5% refuge with Bt) had the greatest probability of being applied to a field (explained more below). First, a random number was sampled from a predetermined range at the beginning of each year and for each field. When the landscape IPM participation rate (measured on a per-field basis) was set to 0.40, 0.50, or 0.70 and the sampled number fell within 40%, 50%, or 70% of the predetermined range, then IPM was applied to a field during a particular generation run. If the sampled number fell outside the cut-off, then 'IRM only' was applied to the field (refuge at 5%). If the first random number fell within the chosen IPM participation rate value, then a second random number was generated to determine which of the four IPM tools to apply to a particular field during a generation run. If the number fell in the first quarter of the range, crop rotation was implemented on that field. If the number fell within the second quarter of the range, then soil applied insecticides were applied together with Bt deployment. If the number fell into the next or last quarter of the range, then the refuge for the pyramid was increased from 5% to 20% or non-Bt corn was planted on that field (respectively). For instance, when IPM participation was set to 70%, then a field had a 30% chance to have the pyramid deployed with a 5% refuge (IRM only), and a 17.5% chance that crop rotation occurred, SAIs were applied with a 5% IRM plan, a 20% refuge was planted, or non-Bt corn was grown during that planting season. The same process was applied for

scenario (2), but now the non-Bt corn option was removed from the IPM tool kit. Hence, for a 70% IPM participation analysis, there was now a 23.3% chance that crop rotation occurred, SAIs were applied with Bt deployment, or the refuge for the pyramid was increased. A comparison between results of the two approaches determined whether there was an observed effect on the time to corn rootworm resistance when non-Bt corn was withheld as an IPM tool. Various IPM participation rates were chosen to reflect US EPA's inability to mandate that corn growers use IPM practice together with IRM and Bt technology providers cannot require that a certain IPM strategy be applied on corn growers' land.

In another analysis (scenarios 3 and 4), effects of local remediation were explored for different response times (one and three generations after field failure) when resistance was localized at the UXD site. In scenarios 5 and 6, resistance was localized at the UXD site, but effects of remediation were explored regionally, applied at to 70% of the fields in the matrix (2601 fields) one and three generations after the field failure was observed. Scenario (7) describes a situation where resistance was widespread in the landscape (10%) at locus 1, and remediation (using IPM and IRM) was applied to 70% of fields in the landscape. The model projected how many more years could be gained from the compromised gene with mandatory IPM and before the pyramid became a single PIP. Another analysis determined how many more years were gained for the second PIP when resistance at locus 1 was ≥ 0.50. For this purpose, the initial resistance allele frequency at locus 2 was increased to account for prior use of the PIP (see IRAF-2 in Table 4.1). The likelihood of remediation was also set to 100% to establish a relative maximum as a reference, and the non-Bt corn option was excluded from the remediation strategies. The

reason for removing the non-Bt corn option was that cost-to-resistance was absent in the model due to unresolved uncertainties (Meihls et al. 2012, Hoffman et al 2012, and Petzold-Maxwell et al. 2013).

In this corn rootworm model, SAIs were prophylactically used as insurance strategy to protect crop yield from potentially resistant corn rootworm populations and reflect current grower behavior in the Corn Belt. Gray et al. (1992) have expressed, however, that the use of soil applied insecticides should not be viewed as an IRM tool because they were not reliably effective, but rather they served a role in root and yield protection of corn. It was assumed in these simulations that there was a likelihood that growers would choose to prophylactically treat their Bt fields with SAIs. The upper range of survivorship to SAIs was increased to 100% to include results by Gray et al. (1992). Storer (2003) used a survivorship range of 0.2 to 0.80 for SAIs based on published information by Sutter et al. (1991). Considering all information, I decided to use a uniform distribution in corn rootworm simulations as in Storer (2003) with a minimum and maximum survivorship value of 0.5 to 1.0, respectively. At the beginning of each year, a random value was drawn from this range and applied to rootworm larvae on all fields where SAIs were used. Adulticide sprays were applied to insects in both block refuge and Bt compartments as well as RIBs – but only if economic thresholds were exceeded. The minimum and maximum survivorship from adulticide sprays was 10% and 30%, and values were sampled uniformly from this range. Adulticides are extremely efficacious contact poisons against susceptible corn rootworm and can kill them within a short time (Caprio et al. 2006). Because adults, however, emerge before and continue to emerge after an adulticide application has occurred (Ostlie, UMN, *pers. com.*), the model

used the above mentioned survivorship range to account for any beetles that temporally escaped control efforts and went on to disperse and reproduce. SAIs and adulticides were not modeled with resistance genes in the insects, and it was assumed here that there would be different modes of actions (MOA) available with equivalent efficacy range and between which a grower could rotate to avoid rapid selection.

Table 4.3 *D. virgifera* IPM/IRM and remediation scenarios in Bt corn explored with a stochastic, spatially explicit model

Scenario	**r-freq. in field[25][25]**	**r-freq. out-side UXD**	**Timing**	**IPM+IRM & remedial action likelihood**	**Strategies**
1) IPM+IRM in landscape using 5% RIB and 5% block refuge	0.005	0.005	Up-front	40%, 50%, & 70%	**Refuge corn**, Bt with refuge, rotation, increased refuge, SAI, and adulticides when economic threshold is exceeded
2) IPM+IRM in landscape using 5% RIB and 5% block refuge	0.005	0.005	Up-front	40%, 50%, & 70%	No refuge corn; otherwise equal to strategies in 1).
3) Remediation in UXD site using 5% RIB and 5% block refuge	0.10	0.005	After 1st generation	100%	Bt corn with refuge, crop rotation, increased refuge, SAI, adulticides when economic threshold is exceeded
4) Remediation in UXD site using 5% RIB and 5% block refuge	0.10	0.005	After 3rd generation	100%	

Table 4.3 (Continued)

5) Regional mitigation using 5% RIB and block	0.10	0.005	After 1st generation	70%	
6) Regional mitigation using 5% RIB and block	0.10	0.015	After 3rd generation	100% and 70%	
7) Regional mitigation using 5% RIB and block	≥0.50	0.015	At 1st generation	100% and 70%	

4.3.4.2 *H. zea* modeling scenarios

The IPM and IRM tools employed in this bollworm model were 1) planting of 100% isoline corn on a 50 ha field coupled with one larvacide spray sometime between emergence and mid-whorl stage of corn if the economic threshold was exceeded (≥1 larvae per plant; Mississippi Insect Control Guide 2014), 2) increasing the non-Bt corn refuge from 20% to 30 or 50% for the pyramid, and 3) using IRM for Bt corn with a standard 20% refuge only. If, and only if, the economic threshold was exceeded in refuge whorl stage corn, the Bt corn portion of that field was also sprayed as not to increase the resistance allele frequency in the population. Likewise, larvacide spraying in Bt cotton was initiated only when larval densities reached or exceeded the economic threshold for 'before bloom' and 'after cut out' (8 larvae/100 plant; Mississippi Insect Control Guide 2014). Previous analyses (Chapter 2) have shown that density dependence on cotton was minimal (lower population densities than in corn) and, therefore, this option should be

rarely triggered. Crop rotation for the purpose of suppressing bollworm was excluded as an IPM option because *H. zea* feeds on many different agricultural crops, ornamentals, and wild hosts (Stadelbacher et al. 1983).

The modeling scenarios explored for bollworm are listed in Table 4.4, and, like for corn rootworm, included a combined IPM+IRM approach at the outset of the new low dose pyramid commercialization to estimate by how many more years the lifetime of less-than high dose pyramided could be extended. The IRM strategies for increased refuge in corn were individually explored to evaluate the relative gain in durability compared to Bt deployment with the standard, mandated 20% refuge (IRM alone). Scenario 1 simulated a case where different IPM participation percentages (40%, 50%, and 70%) were evaluated to determine what participation level would provide the greatest lifetime for such a hypothetical dual gene product.

Scenario 2 was an attempt to describe a case where resistance was widespread at locus 1 and at 10% ($q = 0.10$, $q^2 = 0.010$). Local remediation of resistance in bollworm was considered a futile exercise because of the species high propensity to disperse and was not modelled. Fields in the entire landscape were mitigated with a 70% likelihood when corn was in the whorl stage one year after resistance was visually detected in Bt cotton during the previous growing season.

Scenario 3 described a case, where resistance at locus 1 was widespread (50%), and the mean resistance at locus 2 was elevated to a mean of 0.015 to simulate prior commercialization. Here, the objective was to determine how much more utility could be gained from the second gene by implementing remediation immediately. The participation rate was set at 70% again rather than 100% because refuge compliance for

corn PIPs has been variable over the years (depending on pest approximately 70-90% in the Corn Belt vs. 30-70% in the cotton growing regions, US EPA 2011 and 2014); based on this information, the assumption was made that 100% remediation in such an area would likely not be a realistic goal even if regulatory triggers for confirmed resistance were met. Isoline corn was excluded during area-wide remediation modeling.

The same process described for corn rootworm was used to determine whether IPM and/or IRM would be applied to a field in the bollworm simulations. When IPM was selected, then another random process in the model decided which IPM strategy was applied to a particular field during a particular generation run. Since both Bt corn and cotton expressed the same dual-gene Bt toxins in the model used, once resistance had developed in corn, further selection occurred in Bt cotton. No specific, alternate mitigation, aside from spraying with conventional insecticides was introduced in Bt cotton at such a time because the majority of the southern U.S. has adopted the natural refuge paradigm where wild hosts, weeds, and other cultivated crops provide the refuge for Bt cotton (US EPA 2007). In other states (California, Arizona and New Mexico) and several counties in Texas, growers are, however, required to plant a structured refuge.

Table 4.4 *H. zea* IPM/IRM and remediation scenarios in Bt corn explored with a stochastic, spatially explicit model

Scenario	r-freq. in UXD site	r-freq. in remaining fields	Timing	IPM participation	Strategies
1) IPM+IRM in landscape using block refuge	0.005	0.005	Up-front	40%, 50%, & 70%	Bt corn with 20%, 30%, and 50% refuge, isoline corn, and larvacides
2) Remediation in landscape when resistance is widespread	0.10	0.10	After 1st generation	70%	Bt corn with 20%, 30%, and 50% refuge, and larvacides when economic thresholds are exceeded
3) IPM in matrix for Locus 2 in pyramid when Locus 1 is compromised	0.50	0.015	After 1st generation	70%	

Note: Crop rotation was not a viable option for the polyphagous *H. zea* because the pest feeds on several hundreds of different vegetables, forage crops, ornamentals and wild hosts (Stadelbacher et al. 1986). 70% IPM participation was explored in the southern U.S. based on the current compliance with refuge requirements for Bt corn PIPs (US EPA 2011 and 2014).

4.4 Analysis

I analyzed my data using R software (R Core Team, 2013, package version 3.0.2). The effects of each single IPM strategy on the durability of the dual gene PIP, described in sections 4.3.1 and 4.3.2, were evaluated using a one-way ANOVA (20 simulations each); bootstrap analyses were conducted to estimate differences in the distributions of time to resistance between RIBs and blocks. The joint effects of all IPM tools at different participation percentages were evaluated in scenarios (1) and (2) for corn rootworm (with and without isoline corn option) and scenario (1) for bollworm (100 simulations each) using a separate one-way ANOVA for RIBs and blocks. Again a bootstrap analysis was

performed to identify whether the RIB and Block distributions for time to resistance differed significantly for corn rootworm simulations. The 95% confidence interval for the ratio of the distributions at the 5% quantile (R software, version 3.0.2) was calculated to report the significance level using the 'adjusted percentile method' (BCa) (Davison and Hinckley, 1997).

Effects of timing of mitigation on the spread and increase in resistance allele frequency in the landscape were assessed at different times after resistance in corn rootworm populations (1 and 3 generations) was first detected in the UXD site. Average resistance allele frequencies were reported in square sections 1 -13 (see Figure 4.1, shaded sections). The variability sampling for life-history characteristics was switched off, and all parameters were set to the mean values as to reduce the noise and to detect patterns in the landscape. The remaining source of variability in the simulations stemmed from stochastically initializing the egg load in the fields at the beginning of the simulation and the random selection of IPM and/or IRM tools for each field at the beginning of a generation run (between field variation).

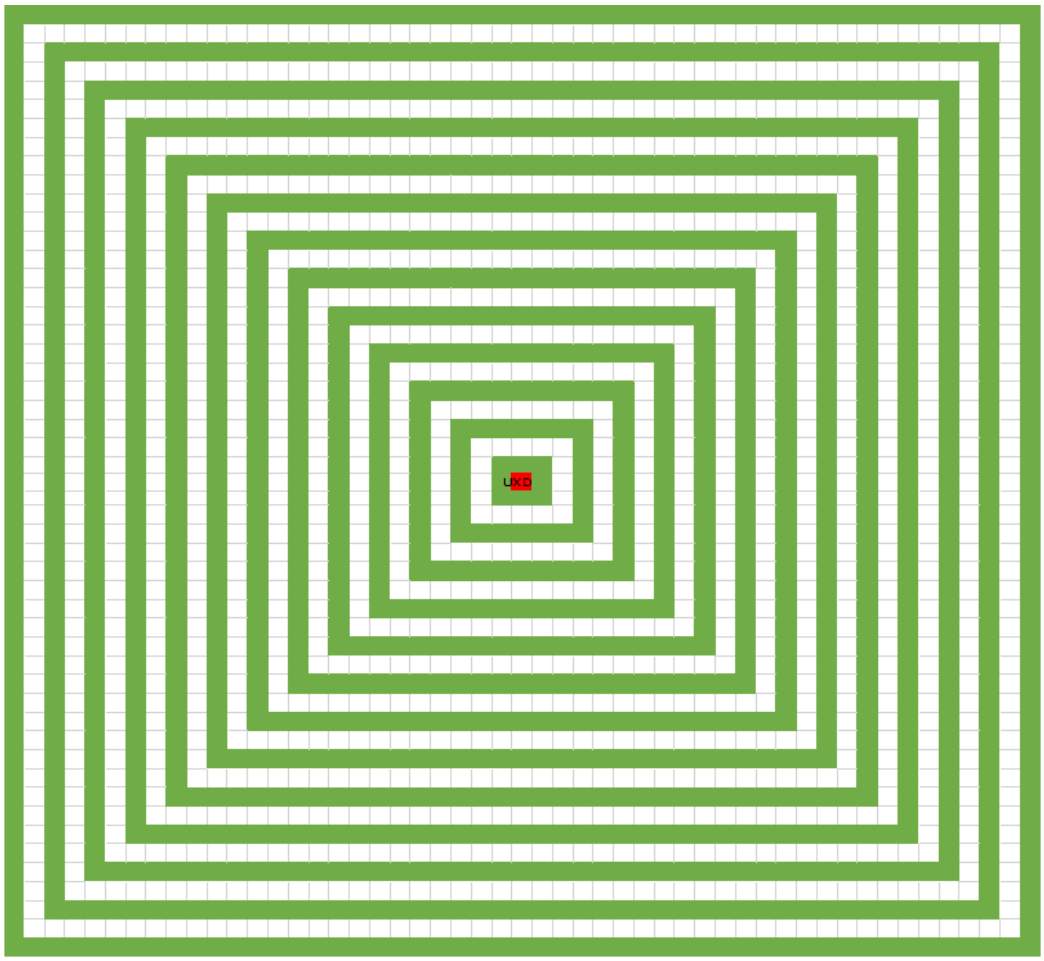

Figure 4.1 Matrix and square sections around resistant site where resistance allele frequencies were recorded

Notes: 51 x 51 fields 50 ha each; resistance was visibly detected in the field and assumed to have been the product of 10% resistance (Roush and Miller 1986); population density was modeled as very high in a resistant site. Resistance allele frequencies were reported for the first 13 rectangular sections.

4.5 Results

4.5.1 IPM and IRM strategies and resistance evolution in *D. virgifera* to a two-gene pyramided Bt PIP

Block refuges and RIBs alone without any further IPM and IRM approaches were estimated to provide a mean durability of 23.4 and 22.7 generations, respectively, for the low dose pyramid (Table 4.5). These simulated results do not differ significantly; both

IRM strategies estimate similar durabilities with IRM alone (BCa [0.8182, 1.0526]) (Table 4.6). Crop rotation to a non-host on all fields resulted in extirpation of corn rootworm within five to six generations for both RIBs and block strategies assuming that there was a possibility of 2% volunteer corn each year but ignoring other potentially cultivated crops in the agricultural landscape (e.g., cucurbits, Howe et al. 1976). Rotation was the best of all IPM strategies, followed by increased refuge, and both IRM and SAI with IRM (Blocks: p-value $<2e^{-16}$, Df = 3, 76, F = 509.9) (RIBs: p-value $<2e^{-16}$, Df = 3, 76, F = 763.3) (Table 4.5). SAI applications with IRM on all fields did not extend the durability of blocks or RIBs in these simulations compared to when only IRM was implemented. A bootstrap analysis informs that there was no statistically significant difference between block and RIB results with this strategy (BCa [1.000, 1.050]). Increasing the refuge proportion from 5% to 20% on all fields lead to the greatest gain in durability for blocks and RIBs. Likewise, the results for blocks and RIBs with the increased refuge strategy did not differ significantly (BCa [0.933, 1.049]). The percent increase in durability compared to 'no action' (IRM only) or IRM + SAI was 98% and 103% for blocks and approximately 115% compared to both strategies with RIBs. Figures 4.2 and 4.3 visualize this comparison for IRM strategies and shows that the distribution for time to resistance with increased refuge was significantly greater than results obtained with other strategies (SAI+Bt or IRM only). Crop rotation was not included in the figures because those results represented extinction cases not cases of resistance. From a practical perspective, crop rotation appears to be the answer to managing resistance in this insect.

Table 4.5 Time resistance or extermination for a LD pyramid targeting *D. virgifera* with a 5% refuge using IPM or RM strategies

Strategy	SAI	Crop Rotation	Increased Refuge	No Action
5% Block	22.9[b](20-27)	5.1*[a]	46.5[c] (40-63)	23.4[b] (18-29)
5% RIB	22.6[b] (18-26)	5.2*[a]	48.8[c] (42-58)	22.7[b] (18-27)

Note: Letters show level of significance for different IPM/IRM strategies within the same group (refuge strategy). Numbers in parenthesis report the range of durability. Results were based on 20 simulations; * indicates 'time to extirpation; 2% volunteer corn allowed with crop rotation; SAI use assumes rotation between different modes of action and no resistance to insecticides; refuge was increased from 5% to 20%.

Multiple Comparisons of Means (block simulations) -Tukey contrasts

	Estimate	Std. Error	t value	Pr(>|t|)
IRM-only - Rotate == 0	18.300	1.063	17.218	<1e-05 ***
IRM+SAI - Rotate == 0	17.850	1.063	16.794	<1e-05 ***
Incr. Ref - Rotate == 0	41.400	1.063	38.952	<1e-05 ***
IRM+SAI - IRM-only == 0	-0.450	1.063	-0.423	0.974
Incr. Ref - IRM-only == 0	23.100	1.063	21.734	<1e-05 ***
Incr. Ref - IRM+SAI == 0	23.550	1.063	22.157	<1e-05 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple comparisons of Means (RIB simulations)-Tukey contrasts:

	Estimate	Std. Error	t value	Pr(>|t|)
IRM only - Rotate == 0	17.5500	0.9212	19.052	<1e-06 ***
IRM+SAI - Rotate == 0	17.4000	0.9212	18.889	<1e-06 ***
Incr.Ref - Rotate == 0	43.6500	0.9212	47.385	<1e-06 ***
IRM+SAI - IRM only == 0	-0.1500	0.9212	-0.163	0.998
Incr.Ref - IRM only == 0	26.1000	0.9212	28.334	<1e-06 ***
Incr.Ref - IRM+SAI == 0	26.2500	0.9212	28.496	<1e-06 ***

Table 4.6 Results for 95% BCa confidence limits for RIB and block distributions and individual IPM strategies

Bootstrap 95% CI	SAI	Increased Refuge	No Action
RIB:Block	(1.000, 1.050)n.s.	(0.933, 1.049)n.s.	(0.8182, 1.0526)n.s.

Note: Bootstrap confidence interval computations were based on 10,000 bootstrap replicates; 95% BCa confidence limits of the ratios of 5% quantiles of distributions**;** ^ = ratio of distributions differed significantly for the 95% C.I. for the ratios of the two distributions at the 5% quantile; n.s. = no statistically significant difference observed.

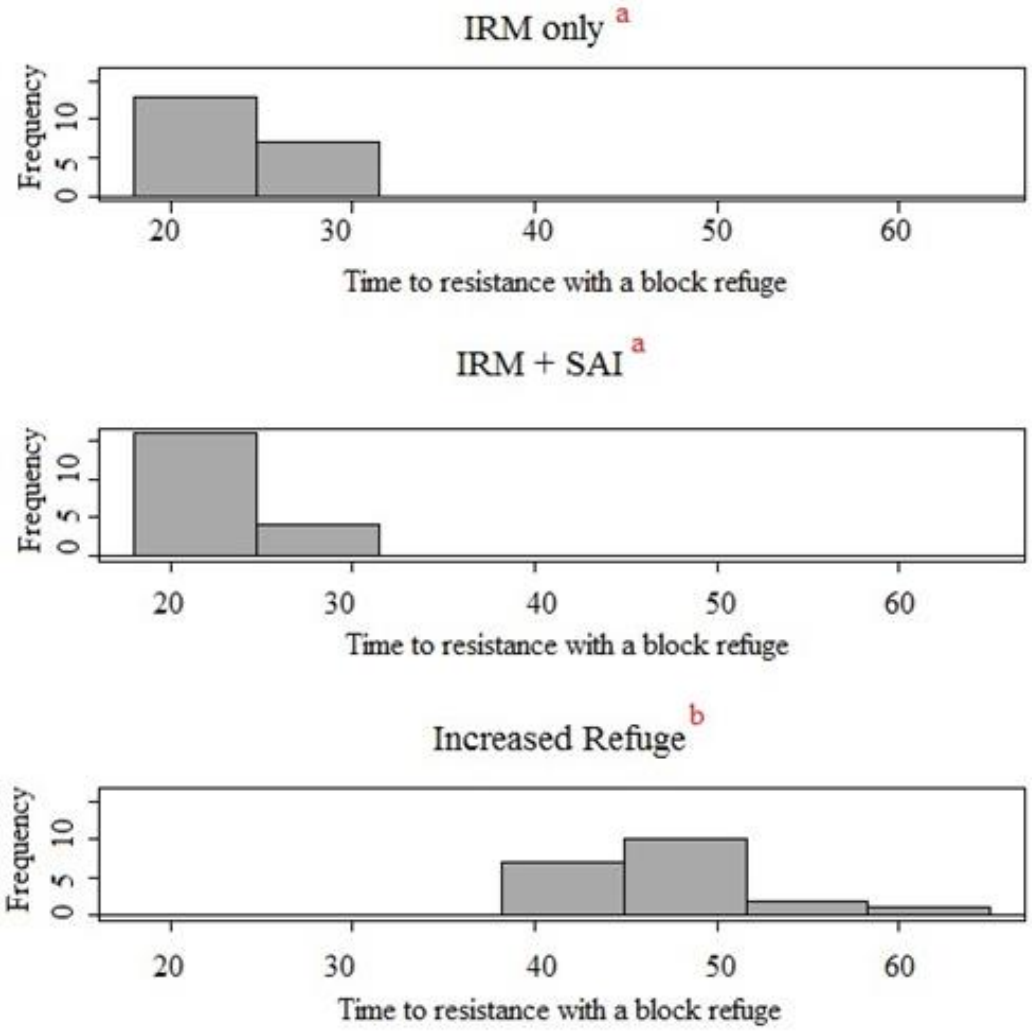

Figure 4.2 Distributions of durability for a LD Bt pyramid targeting *D. virgifera* using IPM +IRM for block refuges

Notes: ANOVA determined a significant difference between increased refuge (b), and the Bt pyramid deployed with SAI+Bt (a) or IRM only (a) strategies.

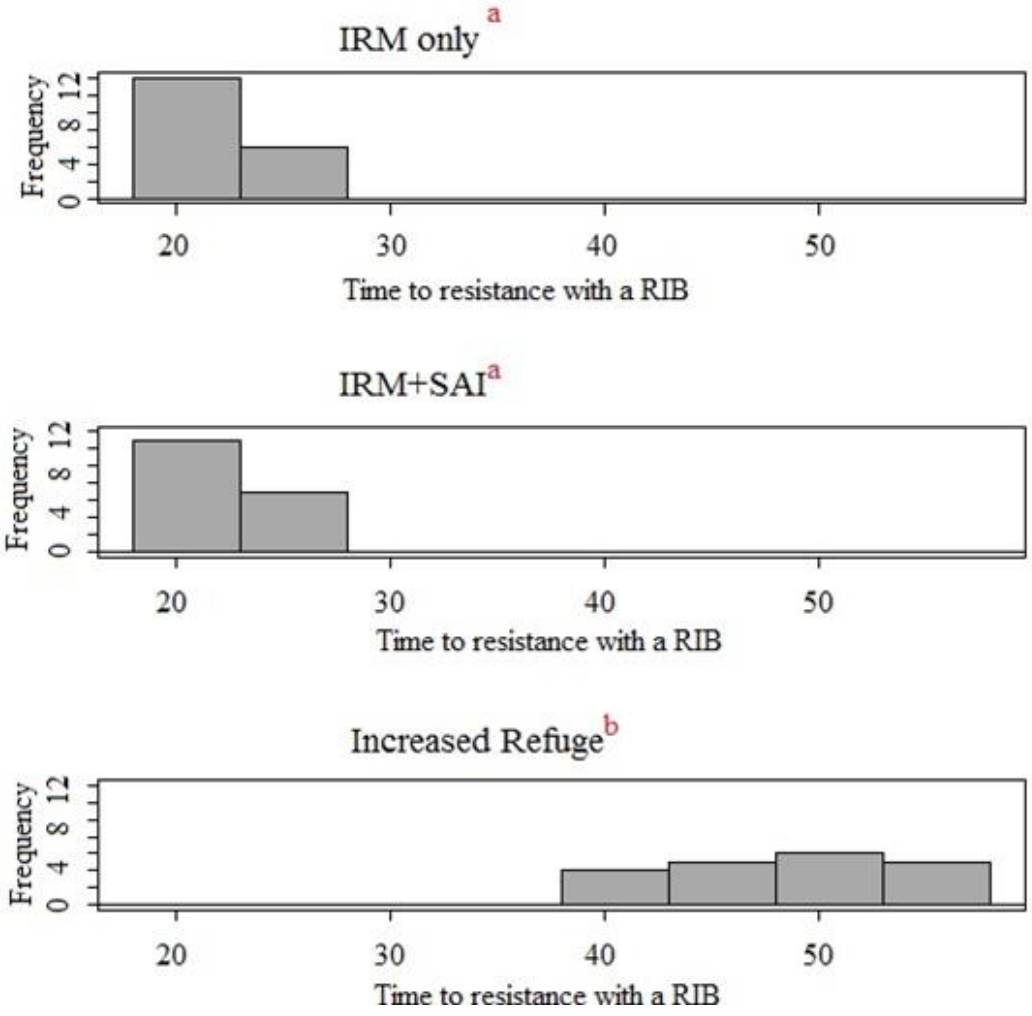

Figure 4.3 Distributions of durability for a LD Bt pyramid targeting *D. virgifera* using IPM +IRM for RIBs

Notes: ANOVA determined a significant difference between increased refuge (b), and the Bt pyramid deployed with SAI+Bt (a) or IRM only (a) strategies.

Table 4.7 lists the results for the IPM implementations with different participation rates and including/excluding the planting of non-Bt corn as an IPM option. The ANOVA results comparing all strategy combinations for blocks and RIBs are listed in Tables 4.8 and 4.9, respectively. ANOVA results for block and RIB block simulations including and excluding non-selective periods show that there was always a significant difference in durability between simulations at each IPM participation percentage tested (see Figure

4.4, data in Tables 4.8 and 4.9). The percent increase in durability at 40, 50, and 70% IPM participation were approximately 13%, 18%, and 23% for blocks and 10%, 20%, and 24% for RIBs, respectively (Table 4.7). As IPM participation increased, the impact of having non-Bt expressing corn amplified, this was more pronounced for RIB than block strategies. Figure 4.5 visualizes the significant increase in durability for blocks with IRM only and increasing IPM participation with isoline corn (Blocks: $p = 2e^{-16}$, Df = 3, 316, F = 56.6) (RIBs: $p = 2e^{-16}$, Df = 3, 316, F = 66.4). Out of 15 ANOVAs comparing durability estimates, 13 were statistically significant indicating that when IPM participation with or without non-Bt corn increased, the durability increased as well. When IPM participation was, however, at 70% without non-selective periods interspersed, then durability was not statistically different from results obtained at 40% participation with and 50% participation excluding isoline corn. This suggests that if Biotechnology providers and US EPA efforts fail to have a large grower IPM participation that having non-selective periods interwoven with Bt selection can make up for the lack of participation.

A between-refuge strategy comparison (RIB vs. blocks) showed that the durability distributions were not different at low IPM participation without isoline corn (40 and 50%). If IPM participation was 70%, however, block simulations predicted greater durabilities for the pyramid than the RIB. Blocks were always more durable than RIBs when non-selective periods were included in the mix of IPM options irrespective of percent participation (see Table 4.10).

Table 4.7 Average years to resistance for a LD pyramid with 5% refuge targeting *D. virgifera* using IPM strategies with different participation rates

IPM participation	Non-Bt corn option	Block	RIB	Comments
40%	No isoline corn	26.2 (20-36)[a]	24.8 (18-32)[a]	IPM/IRM strategies: SAI, crop rotation, Bt corn w/refuge, increased refuge (20%), and adulticide spraying at economic threshold exceeded.
	With isoline corn	29.5 (22-39)[b]	27.6 (21-38)[a]	
50%	No isoline corn	27.7 (22-40)[a]	26.5 (20-34)[a]	
	With isoline corn	32.6 (24-52)[b]	31.9 (22-46)[a]	
70%	No isoline corn	28.7 (22-38)[b]	27.0 (21-35)[a]	
	With isoline corn	35.2 (26-50)[b]	33.6 (23-43)[a]	

Note: IPM was implemented at the on-set of PIP commercialization; letters denote significant differences between block and RIB simulations using the same IPM participation rate (results in Table 4.10); results were based on 100 simulations; shaded fields denote significant differences between block and RIB results.

Table 4.8 ANOVA results for different IPM participation rates and block simulations

Model comp	40% IPM w/isoline	50% IPM no isoline	50% IPM w/ isoline	70% IPM no isoline	70% IPM w/ isoline
40% IPM no isoline corn	p =2.0e^{-10}, Df=1, 198 F = 45.0	p =0.00174, Df=1,198 F = 10.1	p=2e^{-16}, Df=1,198 F = 126.9	p =3.3e^{-07} Df=1, 198 F = 30.0	p =2e^{-16}, Df=1, 198 F = 265.7
40% IPM with isoline corn		p =7.3e^{-4}, Df=1, 198 F = 11.8	p=6.3^{-07}, Df=1, 198 F = 6.3	p=0.122, Df=1,198 F = 2.4	p =2e^{-16}, Df=1, 198 F = 93.2
50% IPM no isoline corn			p=2.2e^{-14}, Df=1,198 F = 68.14	p=0.0514, Df=1,198 F = 3.8	p =2e^{-16}, Df=1,195 F = 167.9
50% IPM with isoline corn				p=3.0e^{-10}, Df=1,198 F = 44.1	p=1.3^{-04}, Df =1,198 F = 15.3
70% IPM no isoline corn					p=2.0e^{-16}, Df=1,198 F = 128.0

Note: Isoline corn = non-Bt corn; Shaded fields denote significant differences between IPM strategy comparisons.

Table 4.9 ANOVA results for different IPM participation rates and RIB simulations

Model comp	40% IPM w/isoline	50% IPM no isoline	50% IPM w/ isoline	70% IPM no isoline	70% IPM w/ isoline
40% IPM no isoline corn	p=4.7e^{-09}, Df =1,198 F = 37.6	p=4.53e^{-05}, Df =1,198 F = 17.4	p=2e^{-16}, Df =1,198 F = 188.3	p=3.3e^{-07}, Df = 1, 198 F 28.0	p=2e^{-16}, Df =1,198 F = 298.5
40% IPM with isoline corn		p=7.3e^{-04}, Df =1,198 F = 11.8	p=6.3e^{-07}, ,Df =1,198 F = 26.5	p=0.122 Df =1,198 F = 2.4	p=2e-16, Df =1,198 F = 93.2
50% IPM no isoline corn			p=2e^{-16}, Df =1,192 F = 24.4	p=0.287, Df =1,198 F = 1.1	p=2e^{-16}, Df =1,198 F = 174.2
50% IPM with isoline corn				p=2e^{-16}, Df =1,198 F = 86.5	p=0.00663, Df =1,198 F = 7.5
70% IPM no isoline corn					p=2e-^{16}, Df =1,198 F = 156.9

Note: Isoline corn = non-Bt corn; Shaded fields denote significant differences between IPM strategy comparisons.

Table 4.10 Results for 95% BCa confidence limits for RIB and block distributions with various IPM participation

IPM Strategy	Isoline option	95% BCa C.I.: RIB:Block
40%	No isoline	(0.8571, 1.0500) n.s.
	With isoline	(0.8400, 0.9545) ^
50%	No isoline	(0.9091, 1.0000) n.s.
	With isoline	(0.8462, 0.9200) ^
70%	No isoline	(0.8750, 0.9565) ^
	With isoline	(0.7930, 0.9259) ^

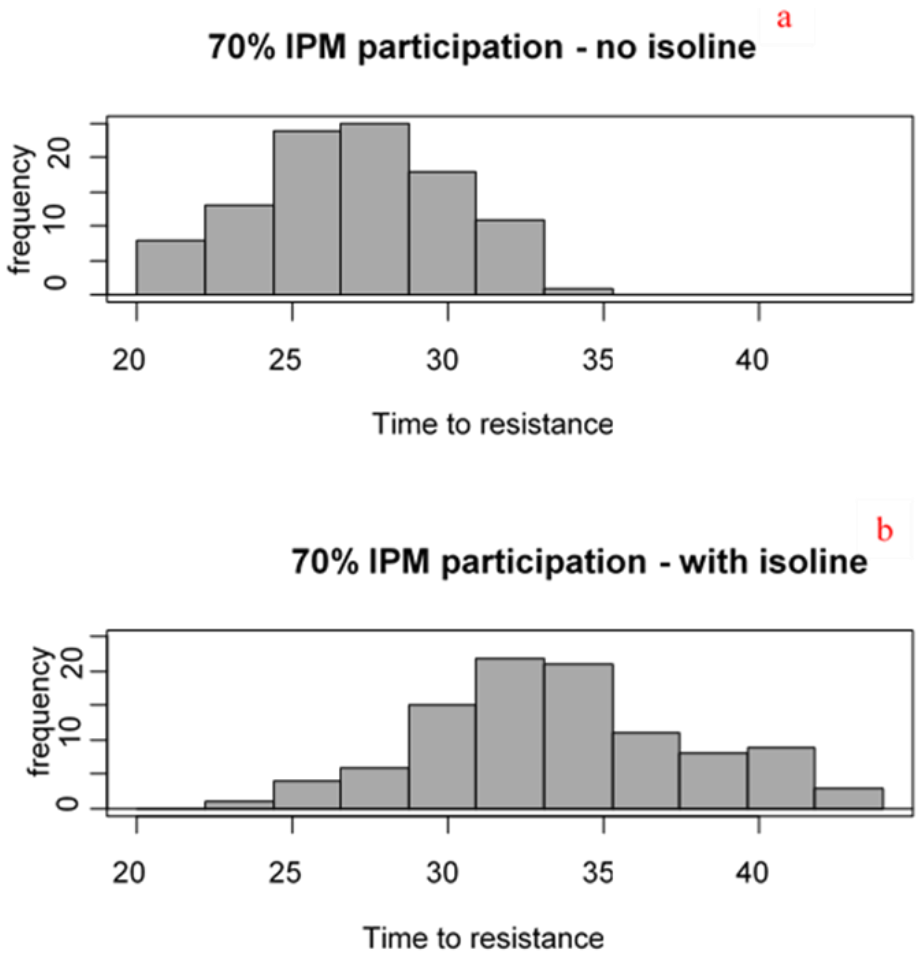

Figure 4.4 Percent distributions of durability for a LD pyramid deployed with RIBs, 70% IPM participation, and no isoline corn option

Notes: a = lower mean durability; b = greater mean durability, p =$2e^{-16}$, Df =1, 198, F = 156.9

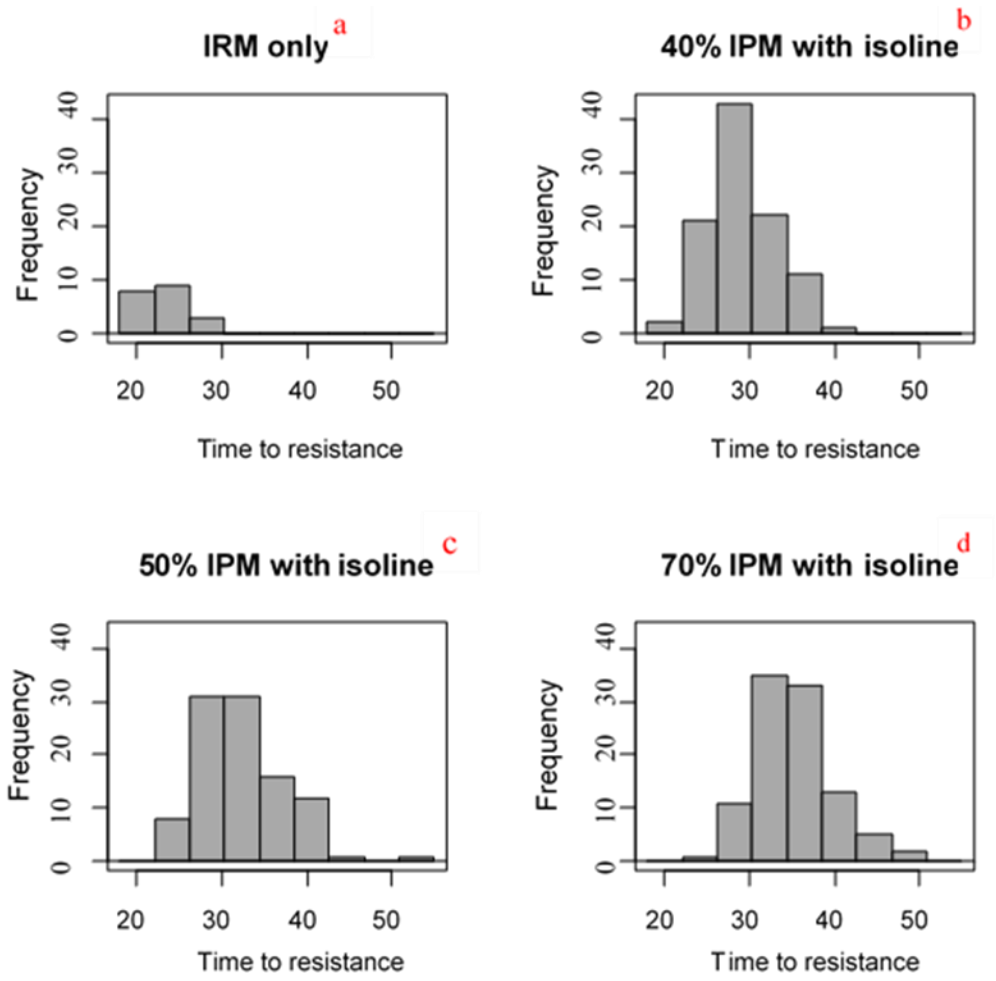

Figure 4.5 Percent distributions of durability for LD pyramid with a block refuge and different or no IPM strategies

Notes: a = lowest mean durability; b = intermediate mean durability; c = second highest mean durability; and c =greatest mean durability.

4.5.2 Mitigation of *D. virgifera* resistance one generation after field failure

Block and RIB simulations gave similar numerical results over the first six generations reported and when mitigation was initiated one generation after resistance first became visible; hence, no distinction was made when results were reported with different IRM deployment. Second, although simulations were run in the deterministic mode, numerical fluctuations were observed at each section in the landscape between

simulations because different mitigation strategies had different effects on the resistance allele frequency (stochastic noise). For example, crop rotation applied to a resistant field or surrounding areas was a much better remediation strategy than increasing the refuge (as implied by results in Table 4.5) and had a decreasing effect on the resistance allele frequency the following year (data not shown). Although the model was designed to select each strategy with equal likelihood, since I only run 10 simulations with 6 generations each for this particular analysis (60 data points for each field), it was unlikely that all fields were mitigated with the same frequency for each strategy (explaining some of the observed variation). A preliminary analysis also showed that the order in which a tool was applied to a field over the six generations had an effect on the final resistance allele frequency after six generations. If crop rotation occurred in the first year of mitigation, then the overall resistance in the landscape and around the hotspot was much lower the following generation than if other strategies were employed first (another source of variation) (data not shown). The r-frequency results at generations 4-6 in Table 4.11 (and Table 4.12) were, therefore, reported as ranges.

In the first (no-resistance) case (1a) without mitigation and no isoline corn option, the average section and landscape r-frequencies increased uniformly with each generation (gen 1-6 from 0.0066 to 0.0334), and all were equal during successive generation runs (Table 4.11). This was to be expected since all fields started out with the same initial resistance allele frequency and values for each life-history parameter. In simulated case (1b), the resistance allele frequencies after six generations with mitigation in absence of resistance were approximately 19-38% lower than in absence of mitigation. This was

essentially an “IPM up-front” approach, and its benefits were already discussed in a previous section (see 4.5.1).

Case (2) simulated resistance in the UXD site and the spread of resistance allele through the landscape in absence of mitigation. In all six generations, resistance allele frequencies were highest in the section immediately surrounding the UXD site (section 1). In section 2, the resistance allele frequency was approximately seven or eight fold lower in all six generations and a resistance phenomenon was visible. With each increasing generation, this phenomenon spread more across the landscape. The final landscape resistance allele frequency in generation 6 was approximately 5% higher than compared to case (1a) (no resistance, no mitigation).

In case (3) (scenario (3) in Table 4.3), mitigation occurred in the resistant site only and began one generation after resistance was detected (in generation or year 2, F_2-larvae faced mitigation). Given the assumptions for mitigation, there was no benefit to local mitigation in the UXD site one generation after field failure or not mitigating at all as measured by the final matrix resistance allele frequency or judging from the r-frequencies in section 1. Again, a resistance phenomenon was clearly visible in the landscape section immediately surrounding the hotspot for all six generations. If, however, crop rotation was implemented in the UXD site for two consecutive years, then the final resistance allele frequency after six generations was similar to the ‘no resistance – no mitigation’ case (data not shown). This implies that even with some time delay, hotspot resistance could be effectively mitigated locally with successive years of crop rotation.

In case 4, mitigation occurred on 70% of the fields in an area of 36.1 km^2 in response to visibly detected damage at the UXD site (scenario (5) in Table 4.3). The resistance allele frequencies in the matrix at generation 6 were similar to the IPM up-front scenario and lower than in the 'no resistance – no mitigation' case. However, a resistance phenomenon was always visible around the hotspot and over the time investigated because mitigation tools were randomly selected in the UXD site as well as the remaining landscape. Regional mitigation preserved the life-time of the technology in non-resistant areas, while resistance could persist at a local scale. The resistance allele frequencies in section 7-13 were also lower with regional mitigation than when remediation occurred locally. The final resistance allele frequency range after generation 6 bolster the previous comment that not all mitigation strategies are equally effective at slowing the pest's adaptation rate (also see Figure 4.3).

The results support that global mitigation in response to 10% resistance in a hotspot effectively maintained the life-time of a low dose pyramid in other regions of the landscape with lower resistance levels. Regional mitigation was always superior to local mitigation, even if crop rotation was the preferred strategy in the UXD site in two successive generations. Local mitigation was non-effective if remediation tools were applied at random. If crop rotation was used the first year during remediation, a preliminary analysis suggests that resistance could be managed effectively even with one year delay time based on conservative dispersal assumptions made here. A future analysis should explore whether this result holds when adult pre-mating dispersal proportion is increased.

Table 4.11 Resistance allele frequencies at locus 1 with local and regional mitigation one generation after field failure

Mitigation Scenarios	Location Freq.	Gen 1	Gen 2	Gen 3	Gen 4	Gen 5	Gen 6
1a) no resistance, no mitigation	Matrix freq.	0.0066	0.0088	0.0119	0.0165	0.0233	0.0334
1b) no resistance with 70% regional mitigation	Matrix freq.	0.0066	0.0086-0.0088	0.0109-0.0115	0.0150-0.0154	0.0203-0.0211	0.0210-0.0271
2) UXD, 10% resistance, no mitigation	Sect. 1	0.1470	0.2362	0.3649	0.5328	0.6445	0.7521
	Sect. 2	0.0176	0.0326	0.0441	0.0621	0.0922	0.1388
	Sect. 3	0.0178	0.0300	0.0392	0.0539	0.0793	0.1187
	Sect. 4	0.0120	0.0202	0.0269	0.0373	0.0546	0.0812
	Sect. 5	0.0089	0.0138	0.0187	0.0262	0.0385	0.0570
	Sect. 6	0.0073	0.0106	0.0144	0.0203	0.0298	0.0439
	Sect. 7	0.0070	0.0096	0.0130	0.0182	0.0263	0.0385
	Sect. 8	0.0067	0.0091	0.0123	0.0171	0.0245	0.0355
	Sect. 9	0.0067	0.0089	0.0120	0.0166	0.0236	0.0340
	Sect. 10	0.0066	0.0088	0.0119	0.0164	0.0233	0.0335
	Sect. 11	0.0066	0.0088	0.0118	0.0163	0.0231	0.0332
	Sect. 12	0.0066	0.0088	0.0118	0.0163	0.0231	0.0331
	Sect. 13	0.0066	0.0088	0.0118	0.0163	0.0230	0.0330
Ave Freq	**Matrix**	**0.0069**	**0.0093**	**0.0125**	**0.0173**	**0.0245**	**0.0352**

Table 4.11 (Continued)

3) UXD Site with 10% resistance & *local* mitigation	Sect. 1	0.1460	0.2267	0.3526	0.5017-5130	0.6428-0.6511	0.7605-0.7670
	Sect. 2	0.0109	0.0200	0.0282	0.0408-0.0489	0.0600-0.0719	0.0900-0.1071
	Sect. 3	0.0122	0.0213	0.0287	0.0401	0.0586-0.0589	0.0867-0.871
	Sect. 4	0.0101	0.0164	0.0220	0.0321-0.0307	0.0451-0.0465	0.0660-0.0685
	Sect. 5	0.0082	0.0125	0.0169	0.0234-0.0238	0.0339-0.0348	0.0499-0.0509
	Sect. 6	0.0073	0.0103	0.0141	0.0196-0.0198	0.0282-0.0288	0.0413-0.0420
	Sect. 7	0.0069	0.0094	0.0127	0.0175-0.0178	0.0251-0.0258	0.0364-0.0375
	Sect. 8	0.0067	0.0090	0.0122	0.0166-0.0171	0.0237-0.0245	0.0343-0.0354
	Sect. 9	0.0066	0.0089	0.0120	0.0164-0.0168	0.0232-0.0239	0.0334-0.0345
	Sect. 10	0.0066	0.0088	0.0119	0.0163-0.0166	0.0230-0.0236	0.0330-0.0340
	Sect. 11	0.0066	0.0088	0.0119	0.0162-0.0166	0.0229-0.0236	0.0227-0.0338
	Sect. 12	0.0066	0.0088	0.0119	0.0162-0.0166	0.0229-0.0236	0.0227-0.0337
	Sect. 13	0.0066	0.0088	0.0119	0.0161-0.0166	0.0228-0.0236	0.0226-0.0337
Ave Freq	**Matrix**	**0.0068**	**0.0092**	**0.0124**	**0.0169-0.0173**	**0.0240-0.0246**	**0.0343-0.0352**
4) UXD Site with 10% resistance & 70% *regional* mitigation	Sect. 1	0.1460	0.2179	0.2846	0.3734-0.5046	0.5708-0.7052	0.2187-0.8256
	Sect. 2	0.0110	0.0200	0.0310	0.0471-0.0791	0.0752-0.1526	0.1018-0.2602
	Sect. 3	0.0104	0.0182	0.0275	0.0408-0.0611	0.0640-0.1161	0.0850-0.1995
	Sect. 4	0.0090	0.0147	0.0211	0.0302-0.0382	0.0463-0.0712	0.0625-0.1239
	Sect. 5	0.0078	0.0116	0.0156	0.0213-0.0297	0.0317-0.0520	0.0442-0.0864
	Sect. 6	0.0071	0.0100	0.0128	0.0165-0.0157	0.0237-0.0344	0.0321-0.0523
	Sect. 7	0.0068	0.0093	0.0114	0.0142-0.0217	0.0197-0.0276	0.0268-0.0385
	Sect. 8	0.0067	0.0090	0.0108	0.0131-0.0186	0.0178-0.0234	0.0240-0.0301
	Sect. 9	0.0066	0.0089	0.0106	0.0127-0.0167	0.0172-0.0220	0.0227-0.0272

Table 4.11 (Continued)

	Sect. 10	0.0066	0.0088	0.0105	0.0126-0.0161	0.0170-0.0215	0.0219-0.0261
	Sect. 11	0.0066	0.0088	0.0105	0.0126-0.0159	0.0168-0.0213	0.0220-0.0258
	Sect. 12	0.0066	0.0088	0.0105	0.0126-0.0158	0.0168-0.0213	0.0220-0.0256
	Sect. 13	0.0066	0.0088	0.0105	0.0125-0.0158	0.0168-0.0212	0.0218-0.0256
Ave Freq	**Matrix**	**0.0068**	**0.0091**	**0.0124**	**0.0130-0.0170**	**0.0181-0.0237**	**0.0235-0.0301**

Note: deterministic runs although population size was randomly initiated in each field; shaded sections indicate the generation when mitigation was initiated. UXD IRAF = 0.10; landscape IRAF = 0.005; each field was sampled individual for mitigation approaches. F1 frequencies were reported in generation1 in rings around damaged site, F-2 frequencies during generation 2, etc.; no differences in results for block refuge and RIB simulations were observed during the few generations simulated, hence no distinction was being made when results for simulations were reported.

4.5.3 Mitigation three generations after field failure

Simulated cases (1a), (1b), and (2) in Table 4.12 are identical to those listed in Table 4.11 and results have been discussed in the previous section. Case (3) describes a scenario where mitigation occurred in the resistant site only, and remedial action was initiated with a delayed response time of three generations. The resistance allele frequency results in the landscape after six generation show that, given the assumptions for mitigation, there was no benefit of mitigating locally and resistance allele frequencies with local mitigation did not differ compared to 'resistance without mitigation'. This makes sense, since mitigation in the UXD site one generation after resistance did not slow the adaptation rate either. A preliminary investigation with crop rotation applied in the fourth generation (first year of remedial response) showed that a local resistance phenomenon was still visible around the hotspot because of the three years where resistance genes were allowed to escape from the UXD site (data not shown). This

suggests that remediation with a delay time of more than one generation has to focus on a larger region than the UXD site alone. Crop rotation in a well-established hotspot has, however, still value and can aid in reducing overall resistance frequencies in the landscape.

Results for regional mitigation three generations after resistance detection (case 4, RIBs and Blocks) suggest that with better mitigation strategies chosen early on, average resistance allele frequencies in the landscape could at best be as low as reported for worst-case levels with regional mitigation 1 generation after resistance detection (Tables 4.11 vs. 4.12) . If less effective strategies were chosen in the first few generations of remedial action, then 70% regional mitigation starting in generation 4 predicted levels of resistance that were similar to the case of 'resistance – no mitigation'.

Table 4.12 Resistance allele frequencies at locus 1 with local and regional mitigation three generations after field failure

Mitigation Scenarios	**Location Freq.**	**Gen 1**	**Gen 2**	**Gen 3**	**Gen 4**	**Gen 5**	**Gen 6**
1a) no resistance, no mitigation	Matrix freq.	0.0066	0.0088	0.0119	0.0165	0.0233	0.0334
1b) no resistance with 70% regional mitigation	Matrix freq.	0.0066	0.0088	0.0118	0.0163-0.0164	0.0223-0.0224	0.0225-0.0311
2) UXD, 10% resistance, no mitigation	Sect. 1	0.1470	0.2362	0.3649	0.5328	0.6445	0.7521
	Sect. 2	0.0176	0.0326	0.0441	0.0621	0.0922	0.1388
	Sect. 3	0.0178	0.0300	0.0392	0.0539	0.0793	0.1187
	Sect. 4	0.0120	0.0202	0.0269	0.0373	0.0546	0.0812
	Sect. 5	0.0089	0.0138	0.0187	0.0262	0.0385	0.0570

Table 4.12 (Continued)

	Sect. 6	0.0073	0.0106	0.0144	0.0203	0.0298	0.0439
	Sect. 7	0.0070	0.0096	0.0130	0.0182	0.0263	0.0385
	Sect. 8	0.0067	0.0091	0.0123	0.0171	0.0245	0.0355
	Sect. 9	0.0067	0.0089	0.0120	0.0166	0.0236	0.0340
	Sect. 10	0.0066	0.0088	0.0119	0.0164	0.0233	0.0335
	Sect. 11	0.0066	0.0088	0.0118	0.0163	0.0231	0.0332
	Sect. 12	0.0066	0.0088	0.0118	0.0163	0.0231	0.0331
	Sect. 13	0.0066	0.0088	0.0118	0.0163	0.0230	0.0330
Ave Freq	**Matrix**	**0.0069**	**0.0093**	**0.0125**	**0.0173**	**0.0245**	**0.0352**
3) UXD Site with 10% resistance & *local* mitigation	Sect. 1	0.1459	0.2281	0.3524	0.5012-5043	0.6424-6430	0.7596-7619
	Sect. 2	0.0142	0.0263	0.0359	0.0491-0.0497	0.0728-0.0730	0.1075-0.1077
	Sect. 3	0.0104	0.0183	0.0255	0.0361-0.0380	0.0531-0.0562	0.0784-0.0829
	Sect. 4	0.0090	0.0144	0.0198	0.0279-0.0314	0.0411-0.0459	0.0604-0.0673
	Sect. 5	0.0077	0.0117	0.0161	0.0227-0.0236	0.0331-0.0346	0.0484-0.0506
	Sect. 6	0.0073	0.0104	0.0141	0.0197-0.0199	0.0254-0.0289	0.0415-0.0421
	Sect. 7	0.0068	0.0093	0.0127	0.0177-0.0178	0.0242-0.0257	0.0369-0.0373
	Sect. 8	0.0067	0.0090	0.0121	0.0169-0.0170	0.0243-0.0244	0.0349-0.0351
	Sect. 9	0.0066	0.0089	0.0119	0.0166-0.0170	0.0236-0.0237	0.0340-0.0341
	Sect. 10	0.0066	0.0088	0.0119	0.0165-0.0167	0.0233-0.0234	0.0335-0.0337
	Sect. 11	0.0066	0.0088	0.0118	0.0165-0.0166	0.0233-0.0234	0.0335-0.0336
	Sect. 12	0.0066	0.0088	0.0118	0.0164-0.0165	0.0232-0.0233	0.0333-0.0334
	Sect. 13	0.0066	0.0088	0.0118	0.0164-0.0165	0.0232-0.0233	0.0332-0.0334
Ave Freq	**Matrix**	**0.0068**	**0.0091**	**0.0123**	**0.0171-0.0172**	**0.0242-0.0244**	**0.0340-0.0348**
4) UXD Site with 10% resistance & 70% *regional* mitigation	Sect. 1	0.1460	0.2283	0.3563	0.5119-0.5165	0.6465-0.7077	0.7668-0.7902
	Sect. 2	0.0154	0.0280	0.0382	0.0387-0.0526	0.0615-0.0782	0.0870-0.1158
	Sect. 3	0.0116	0.0209	0.0292	0.0382-0.0408	0.0593-0.0602	0.0827-0.0890

Table 4.12 (Continued)

	Sect. 4	0.0112	0.0183	0.0245	0.0295-0.0334	0.0447-0.0489	0.0621-0.0718
	Sect. 5	0.0087	0.0131	0.0177	0.0219-0.0245	0.0321-0.0359	0.0448-0.0526
	Sect. 6	0.0072	0.0102	0.0139	0.0192-0.0195	0.0273-0.0284	0.0379-0.0416
	Sect. 7	0.0068	0.0092	0.0125	0.0174-0.0176	0.01241-0.0254	0.0332-0.0370
	Sect. 8	0.0067	0.0090	0.0121	0.0163-0.0169	0.0223-0.0242	0.0306-0.0349
	Sect. 9	0.0066	0.0089	0.0119	0.0160-0.0166	0.0217-0.0236	0.0297-0.0339
	Sect. 10	0.0066	0.0088	0.0119	0.0159-0.0164	0.0215-0.0233	0.0293-0.0335
	Sect. 11	0.0066	0.0088	0.0118	0.0159-0.0164	0.01214-0.0232	0.0291-0.0332
	Sect. 12	0.0066	0.0088	0.0118	0.0159-0.0164	0.0214-0.0232	0.0290-0.0332
	Sect. 13	0.0066	0.0088	0.0118	0.0158-0.0164	0.0213-0.0232	0.0290-0.0332
Ave Freq	**Matrix**	**0.0068**	**0.0092**	**0.0123**	**0.0165-0.0172**	**0.0225-0.0244**	**0.0306-0.0350**

4.5.4 Mitigation when resistance is wide-spread at one locus

4.5.4.1 Extending the life-time of compromised gene

Table 4.13 shows the results of the spread of resistance through the landscape with variability sampling turned off and when no mitigation occurred (case (1)). When the initial frequency was 10% in all fields, resistance always evolved in just four generations at locus 1 (r-frequency ≥0.05) rendering the dual gene pyramid a single gene PIP. Simulated case (2) describes a situation where resistance again was 10% at locus 1 but now mitigation occurred on 70% of the fields. The first resistance occurrence again took place after four generations. At best, resistance was delayed by one generation compared to 'no mitigation' and occurred during generation five. Mitigation in response

to resistance at Locus 1 was not very successful at extending the lifetime of the compromised gene when resistance was wide-spread.

Table 4.13 Resistance allele frequencies in matrix with wide-spread resistance at locus 1 and regional remediation.

Mitigation Scenarios	Location Freq.	Gen 1	Gen 2	Gen 3	Gen 4	Gen 5	Gen 6
1) 10% wide-spread resistance at locus 1 & no mitigation	Sect. 1	0.1471	0.2221-0.2225	0.3448-0.3479	0.5371-0.5427*	0.7495-0.7548*	0.8965-0.8992*
	Sect. 2						
	Sect. 3						
	Sect. 4						
	Sect. 5						
	Sect. 6						
	Sect. 7						
	Sect. 8						
	Sect. 9						
	Sect. 10						
	Sect. 11						
	Sect. 12						
	Sect. 13						
2) 10% wide-spread resistance at locus 1 & 70% *regional* mitigation	Sect. 1	0.1283-0.1471	0.1664-0.2224	0.2611-0.3419	0.4018-0.5178*	0.5943-0.7203*	0.7875-0.8759*
	Sect. 2						
	Sect. 3						
	Sect. 4						
	Sect. 5						
	Sect. 6						
	Sect. 7						
	Sect. 8						
	Sect. 9						
	Sect. 10						
	Sect. 11						
	Sect. 12						
	Sect. 13						

4.5.4.2 Extending life-time of the low dose pyramid

I was interested in determining by how much the life-time of a low dose pyramid could be extend if there was 10% regional resistance for corn rootworm at one locus and 70% of the fields in the landscape received a randomly chosen remedial strategy as discussed previously. Variability sampling was turned back on, and 100 simulations were run for blocks and RIBs. The initial resistance allele frequency at locus 2 was increased to simulate commercialization of Bt prior to the simulation (min 0.01, mode 0.015, max 0.02). I found that Blocks outperformed RIBs by several generations when remedial action was implemented on a regional scale. Compared to no remediation for the same resistance scenario, 100% and 70% remedial action extended the lifetime of the pyramid by 24.2 and 15.6% with a block refuge and 23.9 and 17.1% with a RIB compared to no remediation (Table 4.14). When no remedial action was implemented (IRM only), then the pyramid failed in approximately twelve and ten generations (blocks and RIBs). Once the gene at locus 1 was compromised (four generations with no mitigation; four to five generations with mitigation), the pyramid became an effective single Bt PIP deployed with a 5% refuge. With no remediation and low protection still available at the first gene (until r-freq = 1.0), the second gene lasted another seven and five generations (blocks and RIBs, respectively). When regional mitigation occurred at 70 and 100%, significant differences were observed and the lifetime was extended by another two and three generations for blocks (70 & 100% mitigation) and two generations for RIBs (70% and 100%). Likewise, when the resistance allele frequency was 50% at locus 1, mitigation efforts applied to 70% and 100% of the fields in the matrix had minimal effects on extending the remaining life-time of the compromised pyramid. The simulations

incorporating 100% remediation on all fields serve as a reference for maximum obtainable benefits that remediation could provide given the assumptions made. Although these results demonstrate that remediation could extend the durability of a compromised pyramid, mitigation in response to resistance was minimally successful and less effective at extending the overall life-time of the dual gene (low dose) PIP than IPM upfront.

Table 4.14 Average years to complete failure of LD pyramid targeting *D. virgifera* with wide-spread resistance at locus 1

IRM strategy	**100% mitigation**	**70% mitigation**	**No mitigation**	**Average increase in durability with mitigation**	**Mitigatory tools**
Block, 10% resistance at L1	15.9[c] (13-22)	14.8[b] (12-18)	12.8[a] (10-17)	24.2% & 15.6%	SAI, increased Refuge, adulticides if ET > 0.75 beetles/plant, crop rotation
RIB, 10% resistance at L1	14.5 [c] (11-17)	13.7 [b] (11-16)	11.7 [a] (10-14)	23.9% & 17.1%	
Block, 50% resistance at L1	12.5 [c] (10-16)	12.0 [b] (9-15)	10.8 [a] (8-13)	15.7% & 11.1%	
RIB, 50% resistance at L1	11.3 [b] (9-17)	11.2 [b] (8-13)	9.6 [a] (8-12)	17.7% & 16.6%	

Note: Results based on 100 simulations; ET = economic threshold to reduce egg laying the following year. Increased r-frequency at second locus to (0.01, 0.015, 0.02) to simulate previous years of commercialization. ANOVA results for within IRM strategy comparison.

ANOVA: blocks 10% resistance:

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Remediation	2	509.6	254.82	118.5	<2e-16 ***
Residuals	297	638.7	2.15		

Multiple Comparisons of Means (block 10% resistance): Tukey Contrasts
Fit: aov(formula = TotalGen ~ Remediation, data = Dataset); Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
70% - NONE == 0	2.0700	0.2074	9.981	< 1e-06 ***
100% - NONE == 0	3.1400	0.2074	15.141	< 1e-06 ***
100% - 70% == 0	1.0700	0.2074	5.159	1.2e-06 ***

Table 4.14 (continued)

no M 70%M 100%M
"a" "b" "c"

ANOVA: RIB 10% resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Remediation	2	428.9	214.46	157	<2e-16 ***
Residuals	296	404.3	1.37		

Multiple Comparisons of Means (RIB 10% resistance): Tukey Contrasts
Fit: aov(formula = TotalGen ~ Remediation, data = Dataset); Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
70% - none == 0	2.0975	0.1657	12.66	< 1e-05 ***
100% - none == 0	2.8200	0.1653	17.06	< 1e-05 ***
100% - 70% == 0	0.7225	0.1657	4.36	5.28e-05 ***

no M 70%M 100%M
"a" "b" "c"

ANOVA: Block 50% resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Remediation	2	147.8	73.92	50.32	<2e-16 ***
Residuals	297	436.3	1.47		

Multiple Comparisons of Means (block 50% resistance): Tukey Contrasts
Fit: aov(formula = TotalGen ~ Remediation, data = Dataset); Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
70% - none == 0	1.1900	0.1714	6.942	<1e-04 ***
100% - none == 0	1.6700	0.1714	9.742	<1e-04 ***
100% - 70% == 0	0.4800	0.1714	2.800	0.015 *

no M 70%M 100%M
"a" "b" "b"

ANOVA: RIB 50% resistance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Remediation	2	176.0	88.02	68.7	<2e-16 ***
Residuals	297	380.6	1.28		

Multiple Comparisons of Means(RIB 50% resistance): Tukey Contrasts
Fit: aov(formula = TotalGen ~ Remediation, data = Dataset); Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
70% - none == 0	1.6200	0.1601	10.120	<1e-06 ***
100% - none == 0	1.6300	0.1601	10.182	<1e-06 ***
100% - 70% == 0	0.0100	0.1601	0.062	0.998

no M 70%M 100%M
"a" "b" "c"

4.5.5 IPM/IRM strategies and *H. zea* resistance evolution to a two-gene pyramided PIP

Three refuge baseline scenarios were evaluated individually (without larvacide) to simulate *H. zea* resistance to Bt where the refuge proportion was varied from 20% (standard requirement in the southern U.S. for a pyramided PIP), 30% to 50% for Bt corn, and the natural refuge for Bt cotton was held constant at 20% (Table 4.16). The results are included in Figure 4.6 and were based on 20 simulations for each refuge scenario. The one-way ANOVA indicates that durability increased with higher refuge percentages (using contest competition model of density dependence) ($p = 0.000142$, $Df=2, 57$, $F = 10.4$) (Table 4.11); a 20%, 30%, and 50% refuge was estimated to provide durabilities of 84, 95, and 102 generations, respectively. The multiple comparison of means (Tukey contrasts) showed that the 20% refuge differed from 30% or 50% refuge ($p= 0.0229$, $p < 0.0001$) but not between a 30% and 50% refuge strategy ($p = 0.18$). This was a function of increased density dependent effects that occurred with greater refuge proportions - equivalent to an increase in the intrinsic growth rate – which reduced the benefits of further increasing refuge proportions after some threshold value (see chapter 3 for discussion).

When IPM participation was varied between 40%, 50%, and 70% (with larvacide spraying in corn and Bt cotton when the economic threshold was exceeded), sampling for three refuge proportions in corn occurs with equal likelihood, and isoline corn as an IPM tool was either included or excluded, there were no observed significant differences between mean durability estimates for a within-IPM participation comparison (ANOVA results in Table 4.18). This lack of difference between mean simulation estimates with or excluding the isoline corn option can be attributed to greater overall density dependent

effects that occurred with contest competition in a 100% non-Bt field before the population reached the carrying capacity as opposed to scramble competition dynamics (see Figure 2.1) for corn rootworm. The benefits were minimal at best for including non-Bt corn in the IPM tool box for bollworm resistance management under the current simulated conditions and assumptions.

Table 4.15 Average generations to resistance for a LD pyramid targeting *H. zea* using different IRM strategies

Strategy	20% refuge	30% refuge	50% refuge	Comments
20% Block	84.1[a] (70-97)	95.4[b] (77-138)	102.9[b] (76-126)	Baseline scenarios, no larvacide sprays ≥ E.T.

Note: E.T. = economic threshold; Red letters denote significance levels obtained with one-way ANOVA. It was assumed that *H. zea* had generations per year.

Multiple comparison of means: Tukey contrasts:

	Estimate	Std. Error	t value	Pr(>|t|)
30% Ref - 20% Ref == 0	11.350	4.164	2.726	0.0229*
50% Ref - 20% Ref == 0	18.850	4.164	4.527	<0.001 ***
50% Ref - 30% Ref == 0	7.500	4.164	1.801	0.1783

---Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

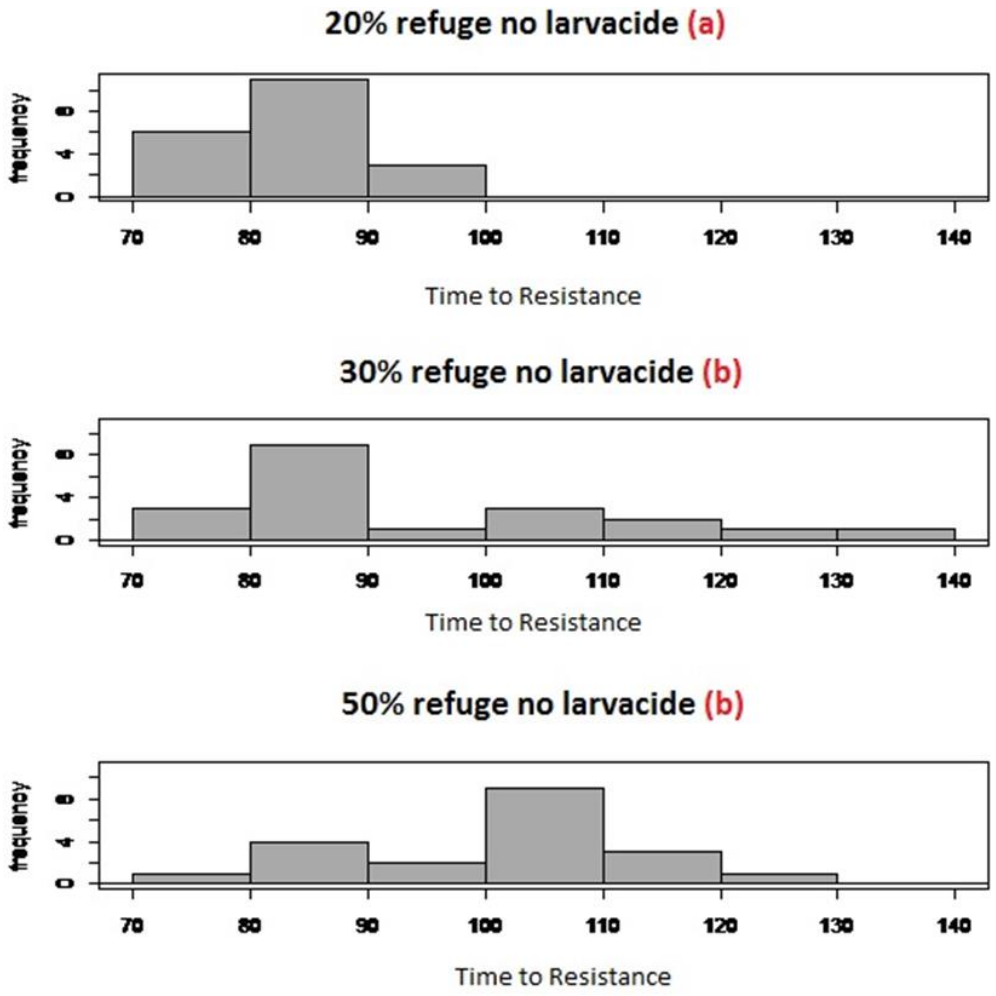

Figure 4.6 Distribution of predicted durability for a LD pyramided Bt PIP deployed with different refuge percentages targeting *H. zea*

Notes: Increasing the refuge proportions leads to significant durability increases for the pyramid with 50% and 30% refuges being the most durable and 20% refuge being the least durable IRM option.

Table 4.16 Predicted durability for a LD pyramid targeting *H. zea* with different IPM/ IRM participation

Various IPM participation	Non-Bt corn option	Block scenario	Comments
40% IPM	No isoline corn	103.3 (75-136)[1]	IPM for zea includes IRM only (20% refuge), and 30% and 50% increased refuge; all options use larvacide sprays in corn and cotton when E. T. is met or exceeded to protect crop yield.
	With isoline corn	100.3 (79-146)[1]	
50% IPM	No isoline corn	107.1 (78-157)[2]	
	With isoline corn	107.8 (78-153)[2]	
70% IPM	No isoline corn	119.3 (90-122)[3]	
	With isoline corn	121.5 (84-173)[3]	

Note: IPM participation refers to the combined use of applying IPM and IRM options to fields; each IPM participation was tested with and without the use of an isoline corn option. Red numbers refer to one-way ANOVA results for within a participation percentage comparison excluding/including an iosline corn option.

Table 4.17 ANOVA results for IPM/IRM participation comparisons simulating a LD pyramid targeting *H. zea*

Model Comp.	40% IPM w/isoline	50% IPM no isoline	50% IPM w/ isoline	70% IPM no isoline	70% IPM w/ isoline
40% IPM no isoline corn	p =0.0991, Df=1,198 F = 2.7	p =0.0456, Df=1,198 F = 4.0	p =0.0232, Df=1,198 F = 5.2	p =2.65e^{-13} Df=1, 198 F = 61.5	p =6.2e^{-16} Df=1, 198 F = 77.7
40% IPM with isoline corn		p=0.00064D f=1, 198 F = 12.0	p=0.00029D f=1,198 F = 13.6	p <2e^{-16} Df=1, 198 F = 81.3	p <2e^{-16} Df=1,197 F = 98.9
50% IPM no isoline corn			p =0.748, Df=1,198 F = 0.1	p = 6.46e^{-8} Df=1,198 F = 31.6	p =4.4e-10 Df=1,198 F = 143.1
50% IPM with isoline corn				p =5.61e^{-7} Df=1, 198 F = 26.8	p =5.3e^{-9} Df=1, 198 F = 37.3
70% IPM no isoline corn					p =0.335, Df=1, 198 F = 0.9

Note: IPM participation refers to the combined use of IPM and IRM options to fields; each IPM participation was tested with and without the use of an isoline corn option.

4.5.6 Mitigation when resistance is wide-spread

Without any kinds of grower intervention against a 10% widespread resistance for bollworm, 'IRM alone' simulations predicted an estimated time of 45.6 generations (approx. 7.5 years) before resistance evolved at the second locus (case 1a in Table 4.18) and the pyramid was compromised (r-frequency at locus 2 $\geq$0.5). This result is significantly different from using an IRM strategy with larvacides (case 2a, 67.6 generations or 11.3 years) when population densities reach or exceed the economic thresholds ($p < 2e^{-16}$, Df=1, 198, F=601.9). When 70% of the fields in the landscape receive mitigation in response to 10% regional resistance at locus 1, the durability was statistically lower than the durability obtained with IRM and larvacide only, although the gain in durability was only one year ($p = 3.63e^{-08}$, Df=1, 198, F = 32.9). This suggests that spraying (refuge and Bt) corn and (Bt) cotton for the purpose of protecting crop yield can also aid in resistance management and mitigation of resistance for Bt PIPs.

When the simulations were initialized with a resistance allele frequency of 0.5 at locus 1 and the IRAF was increased at locus 2 to simulated previous use of Bt (case 3a), then no mitigation in response to resistance rendered the pyramid ineffective in 23.6 generations (3.9 years). IRM with larvacides use if the economic threshold was exceeded extended the durability to 33 generations (10 generations or 1.6 years). With 70% of the fields being mitigated (not 100% as in IRM with larvacide use) (case 3b), the results predict a durability of 31 generations (5.2 years to resistance) (two generations less than IRM with larvacide use). Despite the biologically insignificant difference between these two approaches, a statistical difference was identified (p = 0.000159, Df =1, 198, F = 14.8). While the results predict that different mitigatory strategies or IRM with larvacide

use approach could extend the durability of a low dose pyramid, once resistance in bollworm has established at one locus (10% or 50%), the second trait may also compromised within a short period of time (5-7 years) in absence of cost to resistance.

Table 4.18 Average generations to complete failure of LD pyramid targeting *H. zea* with different mitigation strategies and resistance levels

Mitigation Strategy	Resistance at Locus 1	Mean Time to Resistance for Pyramid	% Increase w/IPM	Comments
1) IRM no larvacide	10%	45.6[b] (36-63)	N/A	Baseline scenarios
	50%	23.6[a] (19-30)		
2a) IRM w/ larvacide	10%	67.6[b] (52-88)	-9.7%	Increased refuge (30% and 50%), IRM; all strategies use larvacides when, ET is exceeded.
2b) 70% of fields mitigated		61.6[a] (46-82)		
3a) IRM w/ larvacide	50%	33.7[b] (25-49)	-5.0%	
3b) 70% fields mitigated		31.4[a] (23-42)		

Note: E.T. = economic threshold for bollworm as reported for corn and cotton in the Mississippi State University Cropping Guide (2014); IRM with larvacide treatments were applied when the E.T. was exceeded on whorl stage corn or Bt cotton; letters denote statistical significance obtained with one-way ANOVA for within-mitigation comparisons. Estimated times to resistance were based on 100 simulations each.

4.6 Discussion

The simulation results show that as IPM + IRM participation percentages increased in the landscape at the time of a new Bt commercialization, the lifetime of a low dose pyramid targeting corn rootworm and bollworm was extended sequentially. The durability gain was greater for corn rootworm, however. Part of that can be attributed to a more diverse IPM tool kit but also more effective control methods such as crop rotation. Crop rotation is not an available IPM option for bollworm population suppression, and less effective IPM tools were modeled for this pest. Another scenario to model would be

the use of a non-related pyramid allowing rotation of Bt modes of action. This could not be accomplished here because of model limitations (2-locus rather than a 4-locus model). The analysis also showed that the inclusion of conventional corn into the IPM approach did not elicit comparable results between the two pests. For example, interspersing Bt deployment with non-selective periods by using non-corn rootworm protected maize increased the durability of the pyramid compared to simulations excluding this tool. Yet for bollworm, including or excluding conventional corn with IPM participation simulations had no effect on the lifetime of the pyramid. One plausible hypothesis is that increased effects of intra-specific density dependence reduced simulated population densities and in turn reduced benefits of planting conventional corn on some of the fields in the landscape. Further investigations are needed, however, to explore and confirm the mechanisms behind the different results for bollworm and corn rootworm. It can be concluded though that IPM + IRM approaches should not be generic in nature but tailored towards each pest's life-history and ecology.

A separate analysis investigated the effectiveness of each individual IPM + IRM tool. The simulation results show that an increase from the mandated 5% refuge to 20% for a corn rootworm protected pyramid extended its lifetime, while an analogous approach for bollworm only increased the durability from the standard 20% refuge up to 30%. Further increases, thereafter, did not incur additional durability gains. This implies that increasing the refuge cannot be assumed to always extend the durability. Simulation models incorporating a target pest's specific life-history characteristics should be used to evaluate the refuge proportion to achieve the greatest durability for the Bt pyramid.

Remediation results for hotspot resistance in corn rootworm show that if mitigatory strategies are applied at random, that local (UXD site) actions are ineffective – given the assumptions made here. In such cases, regional remediation was superior in the simulations. When, however, crop rotation was implemented in the UXD site with a delay time, resistance was effectively mitigated and the spread of resistance slowed. This implies that remedial action plans in response to confirmed resistance or mitigatory response to suspected resistance should not be generic but specific in nature and rely on the most effective tools immediately (i.e. crop rotation for corn rootworm) to maintain the durability of the pyramid in the remaining landscape. A future analysis of hotspot remediation should address a diverse cropping system with different modes of actions (single and pyramided including cross-resistance between some toxins) to further explore the effectiveness of various remediation as well a geographic scope approaches. In reality, local population extirpation (e.g. via adulticide spraying) is not likely achieved because emergence of corn rootworm occurs over a period of time, and conventional pesticides cannot be reapplied until a certain number of days have passed in order to avoid harmful exposure to humans and the environment. Resistant corn rootworm should, therefore, always have an opportunity to escape a resistant field, and mitigation efforts may need to focus on a larger geographic scale rather than the failed field even if crop rotation is used.

Remediation in response to widespread resistance at one locus (10%) in bollworm and corn rootworm was not very effective at extending the lifetime of the low dose pyramid. The difference between implementing and excluding remedial action was only a few years. This result supports the SAP's (2014) recommendation to US EPA that IPM +

IRM should be used to extend the lifetime of less than high dose Bt toxins. A future analysis should explore whether effectiveness of remediation to widespread resistance increases when more effective tools are used more frequently (rather than random selection of available tools) in a diverse landscape consisting of multiple Bt products with different modes of action.

The resistance allele spread analysis for corn rootworm showed that a local resistance phenomenon became apparent in the landscape immediately around the UXD site and persisted and spread through the landscape slowly and over the six generations explored. Resistance alleles spread more slowly across the landscape with a pyramided product compared to a single PIP (see chapter 3). A significant resistance phenomenon persisted for single PIPs with low and high growth rates and variable dispersal, and resistance swept across the landscape much faster than witnessed for the pyramid. This implies that it was the nature of the Bt PIP (dual gene pyramid) that kept resistance phenomena more localized, plausibly because with a pyramid some control was still maintained and large populations could not build up that lead to large amounts of dispersal from the site with the resistant population. More work is needed to explore the exact mechanisms. Nonetheless, the results suggest that the use of a pyramid aids remediation efforts even if with a delayed response time.

4.7 References

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APPENDIX A

CHAPTER III ANOVAS FOR EFFECTS OF PARAMETERS OF LIFE-HISTORY, DOSE OF TOXIN, REFUGE CONFIGURATION, AND INTRA-SPECIFIC DENSITY DEPENDENCE ON THE LIFE-TIME OF SINGLE PIPs

A.1 High dose results

A.1.1 Block analyses

A.1.1.1 Multi-way ANOVAs

Table A.1 Four-way ANOVA for HD block simulations, DD x R x D x AD

Response: TotalGen

	Sum Sq	Df	F value	Pr(>F)
ADType	1241	1	55.9117	1.602e-13 ***
DD	224	1	10.0902	0.001534 **
kernel	3	2	0.0563	0.945246
RType	34158	2	769.2230	< 2.2e-16 ***
ADType:DD	192	1	8.6648	0.003316 **
ADType:kernel	36	2	0.8189	0.441186
DD:kernel	4	2	0.0856	0.917971
ADType:RType	58	2	1.3155	0.268796
DD:RType	1330	2	29.9499	2.249e-13 ***
kernel:RType	75	4	0.8401	0.499767
ADType:DD:kernel	5	2	0.1057	0.899681
ADType:DD:RType	138	2	3.1090	0.045061 *
ADType:kernel:RType	50	4	0.5683	0.685705
DD:kernel:RType	51	4	0.5774	0.679084
ADType:DD:kernel:RType	87	4	0.9779	0.418611
Residuals	23202	1045		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A.2 Multiple comparisons of means using Tukey contrasts, HD block simulations, DDx R x AD

DD:ADType:RType, means

	TotalGen	std	r	Min	Max
CC:AD1:R1	24.505495	10.018176	91	12	83
CC:AD1:R2	10.811111	2.316984	90	7	18
CC:AD1:R3	7.911111	1.533480	90	5	12
CC:AD2:R1	24.277778	6.240548	90	12	45
CC:AD2:R2	13.066667	4.104807	90	7	30
CC:AD2:R3	9.788889	2.696208	90	6	17
SC:AD1:R1	18.633333	5.172203	90	10	32
SC:AD1:R2	10.744444	2.221251	90	7	19

Table A.2 (Continued)

SC:AD1:R3	8.600000	1.512495	90	6	13
SC:AD2:R1	22.088889	5.999334	90	12	42
SC:AD2:R2	13.977778	3.650389	90	9	28
SC:AD2:R3	10.877778	2.936893	90	6	20

alpha: 0.05 ; Df Error: 1045
Critical Value of Studentized Range: 4.632238

Harmonic Mean of Cell Sizes 90.08249
Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
CC:AD1:R1 - CC:AD1:R2	13.69438339	0.000000	***	11.39992282	15.98884397
CC:AD1:R1 - CC:AD1:R3	16.59438339	0.000000	***	14.29992282	18.88884397
CC:AD1:R1 - CC:AD2:R1	0.22771673	1.000000		-2.06674385	2.52217730
CC:AD1:R1 - CC:AD2:R2	11.43882784	0.000000	***	9.14436726	13.73328841
CC:AD1:R1 - CC:AD2:R3	14.71660562	0.000000	***	12.42214504	17.01106619
CC:AD1:R1 - SC:AD1:R1	5.87216117	0.000000	***	3.57770060	8.16662175
CC:AD1:R1 - SC:AD1:R2	13.76105006	0.000000	***	11.46658949	16.05551064
CC:AD1:R1 - SC:AD1:R3	15.90549451	0.000000	***	13.61103393	18.19995508
CC:AD1:R1 - SC:AD2:R1	2.41660562	0.028751	*	0.12214504	4.71106619
CC:AD1:R1 - SC:AD2:R2	10.52771673	0.000000	***	8.23325615	12.82217730
CC:AD1:R1 - SC:AD2:R3	13.62771673	0.000000	***	11.33325615	15.92217730
CC:AD1:R2 - CC:AD1:R3	2.90000000	0.002315	**	0.59920987	5.20079013
CC:AD1:R2 - CC:AD2:R1	-13.46666667	0.000000	***	-15.76745680	-11.16587653
CC:AD1:R2 - CC:AD2:R2	-2.25555556	0.060695	.	-4.55634569	0.04523458
CC:AD1:R2 - CC:AD2:R3	1.02222222	0.951901		-1.27856791	3.32301236
CC:AD1:R2 - SC:AD1:R1	-7.82222222	0.000000	***	-10.12301236	-5.52143209
CC:AD1:R2 - SC:AD1:R2	0.06666667	1.000000		-2.23412347	2.36745680
CC:AD1:R2 - SC:AD1:R3	2.21111111	0.073011	.	-0.08967902	4.51190125
CC:AD1:R2 - SC:AD2:R1	-11.27777778	0.000000	***	-13.57856791	-8.97698764
CC:AD1:R2 - SC:AD2:R2	-3.16666667	0.000449	***	-5.46745680	-0.86587653
CC:AD1:R2 - SC:AD2:R3	-0.06666667	1.000000		-2.36745680	2.23412347
CC:AD1:R3 - CC:AD2:R1	-16.36666667	0.000000	***	-18.66745680	-14.06587653
CC:AD1:R3 - CC:AD2:R2	-5.15555556	0.000000	***	-7.45634569	-2.85476542
CC:AD1:R3 - CC:AD2:R3	-1.87777778	0.241120		-4.17856791	0.42301236
CC:AD1:R3 - SC:AD1:R1	-10.72222222	0.000000	***	-13.02301236	-8.42143209
CC:AD1:R3 - SC:AD1:R2	-2.83333333	0.003403	**	-5.13412347	-0.53254320
CC:AD1:R3 - SC:AD1:R3	-0.68888889	0.998071		-2.98967902	1.61190125
CC:AD1:R3 - SC:AD2:R1	-14.17777778	0.000000	***	-16.47856791	-11.87698764
CC:AD1:R3 - SC:AD2:R2	-6.06666667	0.000000	***	-8.36745680	-3.76587653
CC:AD1:R3 - SC:AD2:R3	-2.96666667	0.001560	**	-5.26745680	-0.66587653
CC:AD2:R1 - CC:AD2:R2	11.21111111	0.000000	***	8.91032098	13.51190125
CC:AD2:R1 - CC:AD2:R3	14.48888889	0.000000	***	12.18809875	16.78967902

Table A.2 (Continued)

CC:AD2:R1 - SC:AD1:R1	5.64444444	0.000000	***	3.34365431	7.94523458
CC:AD2:R1 - SC:AD1:R2	13.53333333	0.000000	***	11.23254320	15.83412347
CC:AD2:R1 - SC:AD1:R3	15.67777778	0.000000	***	13.37698764	17.97856791
CC:AD2:R1 - SC:AD2:R1	2.18888889	0.079906	.	-0.11190125	4.48967902
CC:AD2:R1 - SC:AD2:R2	10.30000000	0.000000	***	7.99920987	12.60079013
CC:AD2:R1 - SC:AD2:R3	13.40000000	0.000000	***	11.09920987	15.70079013
CC:AD2:R2 - CC:AD2:R3	3.27777778	0.000217	***	0.97698764	5.57856791
CC:AD2:R2 - SC:AD1:R1	-5.56666667	0.000000	***	-7.86745680	-3.26587653
CC:AD2:R2 - SC:AD1:R2	2.32222222	0.045521	*	0.02143209	4.62301236
CC:AD2:R2 - SC:AD1:R3	4.46666667	0.000000	***	2.16587653	6.76745680
CC:AD2:R2 - SC:AD2:R1	-9.02222222	0.000000	***	-11.32301236	-6.72143209
CC:AD2:R2 - SC:AD2:R2	-0.91111111	0.979495		-3.21190125	1.38967902
CC:AD2:R2 - SC:AD2:R3	2.18888889	0.079906	.	-0.11190125	4.48967902
CC:AD2:R3 - SC:AD1:R1	-8.84444444	0.000000	***	-11.14523458	-6.54365431
CC:AD2:R3 - SC:AD1:R2	-0.95555556	0.970526		-3.25634569	1.34523458
CC:AD2:R3 - SC:AD1:R3	1.18888889	0.871636		-1.11190125	3.48967902
CC:AD2:R3 - SC:AD2:R1	-12.30000000	0.000000	***	-14.60079013	-9.99920987
CC:AD2:R3 - SC:AD2:R2	-4.18888889	0.000000	***	-6.48967902	-1.88809875
CC:AD2:R3 - SC:AD2:R3	-1.08888889	0.925981		-3.38967902	1.21190125
SC:AD1:R1 - SC:AD1:R2	7.88888889	0.000000	***	5.58809875	10.18967902
SC:AD1:R1 - SC:AD1:R3	10.03333333	0.000000	***	7.73254320	12.33412347
SC:AD1:R1 - SC:AD2:R1	-3.45555556	0.000064	***	-5.75634569	-1.15476542
SC:AD1:R1 - SC:AD2:R2	4.65555556	0.000000	***	2.35476542	6.95634569
SC:AD1:R1 - SC:AD2:R3	7.75555556	0.000000	***	5.45476542	10.05634569
SC:AD1:R2 - SC:AD1:R3	2.14444444	0.095291	.	-0.15634569	4.44523458
SC:AD1:R2 - SC:AD2:R1	-11.34444444	0.000000	***	-13.64523458	-9.04365431
SC:AD1:R2 - SC:AD2:R2	-3.23333333	0.000291	***	-5.53412347	-0.93254320
SC:AD1:R2 - SC:AD2:R3	-0.13333333	1.000000		-2.43412347	2.16745680
SC:AD1:R3 - SC:AD2:R1	-13.48888889	0.000000	***	-15.78967902	-11.18809875
SC:AD1:R3 - SC:AD2:R2	-5.37777778	0.000000	***	-7.67856791	-3.07698764
SC:AD1:R3 - SC:AD2:R3	-2.27777778	0.055222	.	-4.57856791	0.02301236
SC:AD2:R1 - SC:AD2:R2	8.11111111	0.000000	***	5.81032098	10.41190125
SC:AD2:R1 - SC:AD2:R3	11.21111111	0.000000	***	8.91032098	13.51190125
SC:AD2:R2 - SC:AD2:R3	3.10000000	0.000687	***	0.79920987	5.40079013

Harmonic Mean of Cell Sizes 90.08249
Honestly Significant Difference: 2.299736
Means with the same letter are not significantly different.

Groups, Treatments and means

a	CC:AD1:R1	24.51
ab	CC:AD2:R1	24.28
b	SC:AD2:R1	22.09
c	SC:AD1:R1	18.63
d	SC:AD2:R2	13.98

Table A.2 (Continued)

de	CC:AD2:R2	13.07
ef	SC:AD2:R3	10.88
ef	CC:AD1:R2	10.81
f	SC:AD1:R2	10.74
fg	CC:AD2:R3	9.789
fg	SC:AD1:R3	8.6
g	CC:AD1:R3	7.911

A.1.1.2 One-way ANOVAs

Table A.3 Contest competition: varying growth rate, D1 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	3681	1840.5	77.58	<2e-16 ***
Residuals	87	2064	23.7		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-12.100	1.258	-9.622	<1e-04 ***
R3 - R1 == 0	-14.667	1.258	-11.663	<1e-04 ***
R3 - R2 == 0	-2.567	1.258	-2.041	0.109

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.4 Contest competition: varying growth rate, D2 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	5218	2608.8	45.44	2.78e-14 ***
Residuals	88	5052	57.4		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-14.665	1.941	-7.557	<1e-04 ***
R3 - R1 == 0	-17.031	1.941	-8.777	<1e-04 ***
R3 - R2 == 0	-2.367	1.956	-1.210	0.451

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.5 Contest competition: varying growth rate, D3 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	5451	2725.4	96.01	<2e-16 ***
Residuals	87	2470	28.4		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-14.300	1.376	-10.395	<1e-04 ***
R3 - R1 == 0	-18.067	1.376	-13.133	<1e-04 ***
R3 - R2 == 0	-3.767	1.376	-2.738	0.0203 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.6 Scramble competition: varying growth rate, D1 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
RType         2  1581.7    790.8    86.17  <2e-16 ***
Residuals    87   798.4      9.2
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:
                 Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -7.8333     0.7822 -10.015     <1e-04 ***
R3 - R1 == 0  -9.6667     0.7822 -12.358     <1e-04 ***
R3 - R2 == 0  -1.8333     0.7822  -2.344     0.0551 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.7 Scramble competition: varying growth rate, D2 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
RType         2    1782   891.1    69.34  <2e-16 ***
Residuals    87    1118    12.9
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:
                 Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -8.1000     0.9256  -8.751     <0.001 ***
R3 - R1 == 0 -10.3667     0.9256 -11.200     <0.001 ***
R3 - R2 == 0  -2.2667     0.9256  -2.449     0.0428 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"c" "b" "a"
```

Table A.8 Scramble competition: varying growth rate, D3 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	1666	832.9	66.54	<2e-16 ***
Residuals	87	1089	12.5		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-7.7333	0.9135	-8.465	<1e-04 ***
R3 - R1 == 0	-10.0667	0.9135	-11.020	<1e-04 ***
R3 - R2 == 0	-2.3333	0.9135	-2.554	0.0328 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.9 Contest competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	3619	1809.3	72.02	<2e-16 ***
Residuals	87	2186	25.1		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-12.400	1.294	-9.582	<1e-04 ***
R3 - R1 == 0	-14.300	1.294	-11.050	<1e-04 ***
R3 - R2 == 0	-1.900	1.294	-1.468	0.311

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.10 Contest competition: varying growth rate, D2 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
RType         2    3108   1554.2    70.34  <2e-16 ***
Residuals    87   1 922     22.1
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -10.367      1.214  -8.541  <1e-04 ***
R3 - R1 == 0  -13.833      1.214 -11.397  <1e-04 ***
R3 - R2 == 0   -3.467      1.214  -2.856  0.0146 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"c" "b" "a"
```

Table A.11 Contest competition: varying growth rate, D3 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
RType         2    3731   1865.7    113.4  <2e-16 ***
Residuals    87    1431     16.4
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -10.867      1.047 -10.377  < 1e-04 ***
R3 - R1 == 0  -15.333      1.047 -14.643  < 1e-04 ***
R3 - R2 == 0   -4.467      1.047  -4.266  0.000152 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"c" "b" "a"
```

Table A.12 Scramble competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	1734	867.0	45.12	3.61e-14 ***
Residuals	87	1672	19.2		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-7.133	1.132	-6.302	< 1e-04 ***
R3 - R1 == 0	-10.533	1.132	-9.306	< 1e-04 ***
R3 - R2 == 0	-3.400	1.132	-3.004	0.00955 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.13 Scramble competition: varying growth rate, D2 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	1937	968.6	44.27	5.48e-14 ***
Residuals	87	1904	21.9		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-7.267	1.208	-6.017	< 1e-04 ***
R3 - R1 == 0	-11.200	1.208	-9.274	< 1e-04 ***
R3 - R2 == 0	-3.933	1.208	-3.257	0.00456 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.14 Scramble competition: varying growth rate, D3 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
RType	2	2442	1220.7	70.94	<2e-16 ***
Residuals	87	1497	17.2		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ RType, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-9.933	1.071	-9.274	<1e-04 ***
R3 - R1 == 0	-11.900	1.071	-11.111	<1e-04 ***
R3 - R2 == 0	-1.967	1.071	-1.836	0.164

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

A.1.2 RIB analyses

A.1.2.1 Multi-way ANOVAs

Table A.15 Four-way ANOVA for HD RIB simulations, DD x R x D x AD

Response: TotalGen

	Sum Sq	Df	F value	Pr(>F)
ADtype	176.0	1	30.8902	3.466e-08 ***
DD	121.3	1	21.2944	4.428e-06 ***
Dtype	2.0	2	0.1786	0.8364785
Rtype	3482.8	2	305.6097	< 2.2e-16 ***
ADtype:DD	10.0	1	1.7576	0.1852172
ADtype:Dtype	0.7	2	0.0587	0.9430289
DD:Dtype	1.3	2	0.1142	0.8920586
ADtype:Rtype	107.5	2	9.4351	8.689e-05 ***
DD:Rtype	92.6	2	8.1218	0.0003162 ***
Dtype:Rtype	7.6	4	0.3321	0.8564350
ADtype:DD:Dtype	9.0	2	0.7880	0.4550457
ADtype:DD:Rtype	6.0	2	0.5296	0.5890102
ADtype:Dtype:Rtype	50.6	4	2.2197	0.0649656 .
DD:Dtype:Rtype	10.7	4	0.4682	0.7591512
ADtype:DD:Dtype:Rtype	17.6	4	0.7724	0.5432416
Residuals	5948.8	1044		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A.16 Multiple comparisons of means using Tukey contrasts, HD RIB simulations, R x AD

Mean Square Error: 5.698084
Rtype:ADtype, means

	TotalGen	std	r	Min	Max
R1:AD1	12.577778	2.888922	180	7	23
R1:AD2	12.505556	2.880288	180	8	25
R2:AD1	9.150000	2.159751	180	6	17
R2:AD2	10.266667	2.469026	180	6	22
R3:AD1	7.522222	1.611604	180	4	14
R3:AD2	8.900000	2.252497	180	5	17

alpha: 0.05 ; Df Error: 1044
Critical Value of Studentized Range: 4.037602
Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
R1:AD1 - R1:AD2	0.07222222	0.999738		-0.6461535	0.7905980
R1:AD1 - R2:AD1	3.42777778	0.000000	***	2.7094020	4.1461535
R1:AD1 - R2:AD2	2.31111111	0.000000	***	1.5927354	3.0294869
R1:AD1 - R3:AD1	5.05555556	0.000000	***	4.3371798	5.7739313
R1:AD1 - R3:AD2	3.67777778	0.000000	***	2.9594020	4.3961535
R1:AD2 - R2:AD1	3.35555556	0.000000	***	2.6371798	4.0739313
R1:AD2 - R2:AD2	2.23888889	0.000000	***	1.5205131	2.9572646
R1:AD2 - R3:AD1	4.98333333	0.000000	***	4.2649576	5.7017091
R1:AD2 - R3:AD2	3.60555556	0.000000	***	2.8871798	4.3239313
R2:AD1 - R2:AD2	-1.11666667	0.000146	***	-1.8350424	-0.3982909
R2:AD1 - R3:AD1	1.62777778	0.000000	***	0.9094020	2.3461535
R2:AD1 - R3:AD2	0.25000000	0.920058		-0.4683758	0.9683758
R2:AD2 - R3:AD1	2.74444444	0.000000	***	2.0260687	3.4628202
R2:AD2 - R3:AD2	1.36666667	0.000001	***	0.6482909	2.0850424
R3:AD1 - R3:AD2	-1.37777778	0.000001	***	-2.0961535	-0.6594020

Means with the same letter are not significantly different.
Groups, Treatments and means

a	R1:AD1	12.58
a	R1:AD2	12.51
b	R2:AD2	10.27
c	R2:AD1	9.15
c	R3:AD2	8.9
d	R3:AD1	7.522

Table A.17 Multiple comparisons of means using Tukey contrasts, HD RIB simulations, R x DD

Study: AnovaModel.1 ~ c("DD", "Rtype")
HSD Test for TotalGen
Mean Square Error: 5.698084
DD:Rtype, means

	TotalGen	std	r	Min	Max
CC:R1	12.616667	2.862345	180	8	23
CC:R2	9.216667	2.431807	180	6	22
CC:R3	7.622222	1.812913	180	4	15
SC:R1	12.466667	2.905206	180	7	25
SC:R2	10.200000	2.233318	180	6	18
SC:R3	8.800000	2.154377	180	5	17

alpha: 0.05 ; Df Error: 1044
Critical Value of Studentized Range: 4.037602

Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
CC:R1 - CC:R2	3.4000000	0.000000	***	2.6816242	4.1183758
CC:R1 - CC:R3	4.9944444	0.000000	***	4.2760687	5.7128202
CC:R1 - SC:R1	0.1500000	0.991302		-0.5683758	0.8683758
CC:R1 - SC:R2	2.4166667	0.000000	***	1.6982909	3.1350424
CC:R1 - SC:R3	3.8166667	0.000000	***	3.0982909	4.5350424
CC:R2 - CC:R3	1.5944444	0.000000	***	0.8760687	2.3128202
CC:R2 - SC:R1	-3.2500000	0.000000	***	-3.9683758	-2.5316242
CC:R2 - SC:R2	-0.9833333	0.001385	**	-1.7017091	-0.2649576
CC:R2 - SC:R3	0.4166667	0.561435		-0.3017091	1.1350424
CC:R3 - SC:R1	-4.8444444	0.000000	***	-5.5628202	-4.1260687
CC:R3 - SC:R2	-2.5777778	0.000000	***	-3.2961535	-1.8594020
CC:R3 - SC:R3	-1.1777778	0.000047	***	-1.8961535	-0.4594020
SC:R1 - SC:R2	2.2666667	0.000000	***	1.5482909	2.9850424
SC:R1 - SC:R3	3.6666667	0.000000	***	2.9482909	4.3850424
SC:R2 - SC:R3	1.4000000	0.000001	***	0.6816242	2.1183758

Groups, Treatments and means

a	CC:R1	12.62
a	SC:R1	12.47
b	SC:R2	10.2
c	CC:R2	9.217
c	SC:R3	8.8
d	CC:R3	7.622

A.1.2.2 One-way ANOVAs

Table A.18 Contest competition: varying growth rate, D1 AD1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	593.4	296.68	54.79	3.98e-16 ***
Residuals	87	471.1	5.41		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-4.6333	0.6008	-7.712	<1e-04 ***
R3 - R1 == 0	-6.0000	0.6008	-9.986	<1e-04 ***
R3 - R2 == 0	-1.3667	0.6008	-2.275	0.0647 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.19 Contest competition: varying growth rate, D2 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	408.9	204.43	36.36	3.33e-12 ***
Residuals	87	489.1	5.62		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-3.2333	0.6122	-5.281	< 1e-04 ***
R3 - R1 == 0	-5.1667	0.6122	-8.439	< 1e-04 ***
R3 - R2 == 0	-1.9333	0.6122	-3.158	0.00609 **

R1 R2 R3
"c" "b" "a"

Table A.20 Contest competition: varying growth rate, D3 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	553.7	276.84	57.53	<2e-16 ***
Residuals	87	418.6	4.81		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-4.4667	0.5664	-7.886	<1e-04 ***
R3 - R1 == 0	-5.8000	0.5664	-10.240	<1e-04 ***
R3 - R2 == 0	-1.3333	0.5664	-2.354	0.0538 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.21 Scramble competition: varying growth rate, D1 AD1

Multiple Comparisons of Means: Tukey Contrasts

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	353.9	176.94	32.62	2.69e-11 ***
Residuals	87	471.9	5.42		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-2.8333	0.6014	-4.712	< 1e-04 ***
R3 - R1 == 0	-4.8333	0.6014	-8.037	< 1e-04 ***
R3 - R2 == 0	-2.0000	0.6014	-3.326	0.00368 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.22 Scramble competition: varying growth rate, D2 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	170.1	85.03	20.35	5.62e-08 ***
Residuals	87	363.5	4.18		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-1.7333	0.5278	-3.284	0.00417 **
R3 - R1 == 0	-3.3667	0.5278	-6.379	< 1e-04 ***
R3 - R2 == 0	-1.6333	0.5278	-3.095	0.00736 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.23 Scramble competition: varying growth rate, D3 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	423.9	211.94	42.19	1.56e-13 ***
Residuals	87	437.1	5.02		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-3.6667	0.5787	-6.336	<1e-04 ***
R3 - R1 == 0	-5.1667	0.5787	-8.927	<1e-04 ***
R3 - R2 == 0	-1.5000	0.5787	-2.592	0.0298 *

> cld(.Pairs) # compact letter display
R1 R2 R3
"c" "b" "a"

Table A.24 Contest competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	200.5	100.23	16.26	1e-06 ***
Residuals	87	536.4	6.17		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-2.1667	0.6411	-3.379	0.00309 **
R3 - R1 == 0	-3.6333	0.6411	-5.667	< 1e-04 ***
R3 - R2 == 0	-1.4667	0.6411	-2.288	0.06288 .

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Table A.25 Contest competition: varying growth rate, D2 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   347.6    173.8    33.44  1.69e-11 ***
Residuals    87   452.2      5.2
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
               Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -2.9667     0.5887  -5.040   < 1e-04 ***
R3 - R1 == 0  -4.7667     0.5887  -8.098   < 1e-04 ***
R3 - R2 == 0  -1.8000     0.5887  -3.058   0.00822 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
 R1  R2  R3
"c" "b" "a"
```

Table A.26 Contest competition: varying growth rate, D3 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   325.4   162.71    23.66  6.23e-09 ***
Residuals    87   598.2     6.88
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
               Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -2.9333     0.6771  -4.332   0.00011 ***
R3 - R1 == 0  -4.6000     0.6771  -6.794   < 1e-04 ***
R3 - R2 == 0  -1.6667     0.6771  -2.462   0.04140 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
 R1  R2  R3
"c" "b" "a"
```

Table A.27 Scramble competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	161.2	80.58	9.996	0.000124 ***
Residuals	87	701.3	8.06		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-1.8667	0.7331	-2.546	0.0334 *
R3 - R1 == 0	-3.2667	0.7331	-4.456	<1e-04 ***
R3 - R2 == 0	-1.4000	0.7331	-1.910	0.1420

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1 R2 R3
"b" "a" "a"

Table A.28 Scramble competition: varying growth rate, D2 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	159.8	79.88	14.13	4.86e-06 ***
Residuals	87	491.9	5.65		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-2.4333	0.6140	-3.963	0.000417 ***
R3 - R1 == 0	-3.1000	0.6140	-5.049	< 1e-04 ***
R3 - R2 == 0	-0.6667	0.6140	-1.086	0.525361

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
 R1 R2 R3
"b" "a" "a"

Table A.29 Scramble competition: varying growth rate, D3 AD2

```
            Df Sum Sq Mean Sq F value  Pr(>F)
Rtype        2   77.2   38.58   6.488 0.00236 **
Residuals   87  517.3    5.95
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Linear Hypotheses:
             Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -1.0667     0.6296  -1.694  0.21316
R3 - R1 == 0  -2.2667     0.6296  -3.600  0.00152 **
R3 - R2 == 0  -1.2000     0.6296  -1.906  0.14311
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
  R1   R2   R3
 "b" "ab"  "a"
```

A.2 Low dose results

A.2.1 Block analyses

A.2.1.1 Multi-way ANOVAs

Table A.30 Four-way ANOVA for LD block simulations, D x R x DD x AD

Response: TotalGen

	Sum Sq	Df	F value	Pr(>F)
ADtype	16.9	1	1.9055	0.16776
DD	334.4	1	37.7658	1.133e-09 ***
kernel	1.0	2	0.0540	0.94748
Rtype	2288.2	2	129.1947	< 2.2e-16 ***
ADtype:DD	3.7	1	0.4150	0.51959
ADtype:kernel	7.0	2	0.3927	0.67532
DD:kernel	4.1	2	0.2313	0.79356
ADtype:Rtype	5.7	2	0.3240	0.72331
DD:Rtype	1128.6	2	63.7206	< 2.2e-16 ***
kernel:Rtype	31.8	4	0.8979	0.46451
ADtype:DD:kernel	22.0	2	1.2434	0.28883
ADtype:DD:Rtype	24.2	2	1.3648	0.25589
ADtype:kernel:Rtype	17.2	4	0.4867	0.74557
DD:kernel:Rtype	86.7	4	2.4488	0.04469 *
ADtype:DD:kernel:Rtype	28.0	4	0.7906	0.53131
Residuals	9245.4	1044		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table A.31 Multiple comparisons of means using Tukey contrasts, LD block simulations, DDx D x R

Study: AnovaModel.3 ~ c("DD", "kernel", "Rtype")
HSD Test for TotalGen
Mean Square Error: 8.855779
DD:kernel:Rtype, means

	TotalGen	std	r	Min	Max
CC:D1:R1	18.26667	4.660933	60	10	30
CC:D1:R2	14.28333	2.255627	60	10	19
CC:D1:R3	14.48333	2.325188	60	10	20
CC:D2:R1	19.50000	5.000000	60	12	31
CC:D2:R2	13.43333	2.257692	60	8	21
CC:D2:R3	13.91667	2.165145	60	9	19

Table A.31 (Continued)

CC:D3:R1	19.70000	5.567155	60	10	36
CC:D3:R2	13.53333	1.741485	60	11	19
CC:D3:R3	13.91667	2.172959	60	10	21
SC:D1:R1	15.33333	2.703837	60	11	22
SC:D1:R2	14.25000	2.055377	60	10	20
SC:D1:R3	13.83333	2.132609	60	10	20
SC:D2:R1	15.11667	2.840934	60	10	27
SC:D2:R2	14.71667	2.693946	60	10	22
SC:D2:R3	14.20000	2.523113	60	9	21
SC:D3:R1	15.11667	2.477640	60	11	22
SC:D3:R2	14.43333	2.235057	60	10	20
SC:D3:R3	14.01667	2.037709	60	10	20

alpha: 0.05 ; Df Error: 1044
Critical Value of Studentized Range: 4.946299
Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
CC:D1:R1 - CC:D1:R2	3.98333333	0.000000	***	2.0830510	5.8836156687
CC:D1:R1 - CC:D1:R3	3.78333333	0.000000	***	1.8830510	5.6836156687
CC:D1:R1 - CC:D2:R1	-1.23333333	0.705004		-3.1336157	0.6669490020
CC:D1:R1 - CC:D2:R2	4.83333333	0.000000	***	2.9330510	6.7336156687
CC:D1:R1 - CC:D2:R3	4.35000000	0.000000	***	2.4497177	6.2502823353
CC:D1:R1 - CC:D3:R1	-1.43333333	0.426140		-3.3336157	0.4669490020
CC:D1:R1 - CC:D3:R2	4.73333333	0.000000	***	2.8330510	6.6336156687
CC:D1:R1 - CC:D3:R3	4.35000000	0.000000	***	2.4497177	6.2502823353
CC:D1:R1 - SC:D1:R1	2.93333333	0.000012	***	1.0330510	4.8336156687
CC:D1:R1 - SC:D1:R2	4.01666667	0.000000	***	2.1163843	5.9169490020
CC:D1:R1 - SC:D1:R3	4.43333333	0.000000	***	2.5330510	6.3336156687
CC:D1:R1 - SC:D2:R1	3.15000000	0.000001	***	1.2497177	5.0502823353
CC:D1:R1 - SC:D2:R2	3.55000000	0.000000	***	1.6497177	5.4502823353
CC:D1:R1 - SC:D2:R3	4.06666667	0.000000	***	2.1663843	5.9669490020
CC:D1:R1 - SC:D3:R1	3.15000000	0.000001	***	1.2497177	5.0502823353
CC:D1:R1 - SC:D3:R2	3.83333333	0.000000	***	1.9330510	5.7336156687
CC:D1:R1 - SC:D3:R3	4.25000000	0.000000	***	2.3497177	6.1502823353
CC:D1:R2 - CC:D1:R3	-0.20000000	1.000000		-2.1002823	1.7002823353
CC:D1:R2 - CC:D2:R1	-5.21666667	0.000000	***	-7.1169490	-3.3163843313
CC:D1:R2 - CC:D2:R2	0.85000000	0.985309		-1.0502823	2.7502823353
CC:D1:R2 - CC:D2:R3	0.36666667	1.000000		-1.5336157	2.2669490020
CC:D1:R2 - CC:D3:R1	-5.41666667	0.000000	***	-7.3169490	-3.5163843313
CC:D1:R2 - CC:D3:R2	0.75000000	0.996309		-1.1502823	2.6502823353
CC:D1:R2 - CC:D3:R3	0.36666667	1.000000		-1.5336157	2.2669490020
CC:D1:R2 - SC:D1:R1	-1.05000000	0.898901		-2.9502823	0.8502823353
CC:D1:R2 - SC:D1:R2	0.03333333	1.000000		-1.8669490	1.9336156687

Table A.31 (Continued)

CC:D1:R2 - SC:D1:R3	0.45000000	0.999996		-1.4502823	2.3502823353
CC:D1:R2 - SC:D2:R1	-0.83333333	0.988065		-2.7336157	1.0669490020
CC:D1:R2 - SC:D2:R2	-0.43333333	0.999998		-2.3336157	1.4669490020
CC:D1:R2 - SC:D2:R3	0.08333333	1.000000		-1.8169490	1.9836156687
CC:D1:R2 - SC:D3:R1	-0.83333333	0.988065		-2.7336157	1.0669490020
CC:D1:R2 - SC:D3:R2	-0.15000000	1.000000		-2.0502823	1.7502823353
CC:D1:R2 - SC:D3:R3	0.26666667	1.000000		-1.6336157	2.1669490020
CC:D1:R3 - CC:D2:R1	-5.01666667	0.000000	***	-6.9169490	-3.1163843313
CC:D1:R3 - CC:D2:R2	1.05000000	0.898901		-0.8502823	2.9502823353
CC:D1:R3 - CC:D2:R3	0.56666667	0.999898		-1.3336157	2.4669490020
CC:D1:R3 - CC:D3:R1	-5.21666667	0.000000	***	-7.1169490	-3.3163843313
CC:D1:R3 - CC:D3:R2	0.95000000	0.956561		-0.9502823	2.8502823353
CC:D1:R3 - CC:D3:R3	0.56666667	0.999898		-1.3336157	2.4669490020
CC:D1:R3 - SC:D1:R1	-0.85000000	0.985309		-2.7502823	1.0502823353
CC:D1:R3 - SC:D1:R2	0.23333333	1.000000		-1.6669490	2.1336156687
CC:D1:R3 - SC:D1:R3	0.65000000	0.999362		-1.2502823	2.5502823353
CC:D1:R3 - SC:D2:R1	-0.63333333	0.999544		-2.5336157	1.2669490020
CC:D1:R3 - SC:D2:R2	-0.23333333	1.000000		-2.1336157	1.6669490020
CC:D1:R3 - SC:D2:R3	0.28333333	1.000000		-1.6169490	2.1836156687
CC:D1:R3 - SC:D3:R1	-0.63333333	0.999544		-2.5336157	1.2669490020
CC:D1:R3 - SC:D3:R2	0.05000000	1.000000		-1.8502823	1.9502823353
CC:D1:R3 - SC:D3:R3	0.46666667	0.999994		-1.4336157	2.3669490020
CC:D2:R1 - CC:D2:R2	6.06666667	0.000000	***	4.1663843	7.9669490020
CC:D2:R1 - CC:D2:R3	5.58333333	0.000000	***	3.6830510	7.4836156687
CC:D2:R1 - CC:D3:R1	-0.20000000	1.000000		-2.1002823	1.7002823353
CC:D2:R1 - CC:D3:R2	5.96666667	0.000000	***	4.0663843	7.8669490020
CC:D2:R1 - CC:D3:R3	5.58333333	0.000000	***	3.6830510	7.4836156687
CC:D2:R1 - SC:D1:R1	4.16666667	0.000000	***	2.2663843	6.0669490020
CC:D2:R1 - SC:D1:R2	5.25000000	0.000000	***	3.3497177	7.1502823353
CC:D2:R1 - SC:D1:R3	5.66666667	0.000000	***	3.7663843	7.5669490020
CC:D2:R1 - SC:D2:R1	4.38333333	0.000000	***	2.4830510	6.2836156687
CC:D2:R1 - SC:D2:R2	4.78333333	0.000000	***	2.8830510	6.6836156687
CC:D2:R1 - SC:D2:R3	5.30000000	0.000000	***	3.3997177	7.2002823353
CC:D2:R1 - SC:D3:R1	4.38333333	0.000000	***	2.4830510	6.2836156687
CC:D2:R1 - SC:D3:R2	5.06666667	0.000000	***	3.1663843	6.9669490020
CC:D2:R1 - SC:D3:R3	5.48333333	0.000000	***	3.5830510	7.3836156687
CC:D2:R2 - CC:D2:R3	-0.48333333	0.999989		-2.3836157	1.4169490020
CC:D2:R2 - CC:D3:R1	-6.26666667	0.000000	***	-8.1669490	-4.3663843313
CC:D2:R2 - CC:D3:R2	-0.10000000	1.000000		-2.0002823	1.8002823353
CC:D2:R2 - CC:D3:R3	-0.48333333	0.999989		-2.3836157	1.4169490020
CC:D2:R2 - SC:D1:R1	-1.90000000	0.050083	.	-3.8002823	0.0002823353
CC:D2:R2 - SC:D1:R2	-0.81666667	0.990386		-2.7169490	1.0836156687
CC:D2:R2 - SC:D1:R3	-0.40000000	0.999999		-2.3002823	1.5002823353
CC:D2:R2 - SC:D2:R1	-1.68333333	0.158286		-3.5836157	0.2169490020

Table A.31 (Continued)

CC:D2:R2 - SC:D2:R2	-1.28333333	0.637051		-3.1836157	0.6169490020
CC:D2:R2 - SC:D2:R3	-0.76666667	0.995242		-2.6669490	1.1336156687
CC:D2:R2 - SC:D3:R1	-1.68333333	0.158286		-3.5836157	0.2169490020
CC:D2:R2 - SC:D3:R2	-1.00000000	0.931926		-2.9002823	0.9002823353
CC:D2:R2 - SC:D3:R3	-0.58333333	0.999848		-2.4836157	1.3169490020
CC:D2:R3 - CC:D3:R1	-5.78333333	0.000000	***	-7.6836157	-3.8830509980
CC:D2:R3 - CC:D3:R2	0.38333333	1.000000		-1.5169490	2.2836156687
CC:D2:R3 - CC:D3:R3	0.00000000	1.000000		-1.9002823	1.9002823353
CC:D2:R3 - SC:D1:R1	-1.41666667	0.448876		-3.3169490	0.4836156687
CC:D2:R3 - SC:D1:R2	-0.33333333	1.000000		-2.2336157	1.5669490020
CC:D2:R3 - SC:D1:R3	0.08333333	1.000000		-1.8169490	1.9836156687
CC:D2:R3 - SC:D2:R1	-1.20000000	0.747671		-3.1002823	0.7002823353
CC:D2:R3 - SC:D2:R2	-0.80000000	0.992324		-2.7002823	1.1002823353
CC:D2:R3 - SC:D2:R3	-0.28333333	1.000000		-2.1836157	1.6169490020
CC:D2:R3 - SC:D3:R1	-1.20000000	0.747671		-3.1002823	0.7002823353
CC:D2:R3 - SC:D3:R2	-0.51666667	0.999972		-2.4169490	1.3836156687
CC:D2:R3 - SC:D3:R3	-0.10000000	1.000000		-2.0002823	1.8002823353
CC:D3:R1 - CC:D3:R2	6.16666667	0.000000	***	4.2663843	8.0669490020
CC:D3:R1 - CC:D3:R3	5.78333333	0.000000	***	3.8830510	7.6836156687
CC:D3:R1 - SC:D1:R1	4.36666667	0.000000	***	2.4663843	6.2669490020
CC:D3:R1 - SC:D1:R2	5.45000000	0.000000	***	3.5497177	7.3502823353
CC:D3:R1 - SC:D1:R3	5.86666667	0.000000	***	3.9663843	7.7669490020
CC:D3:R1 - SC:D2:R1	4.58333333	0.000000	***	2.6830510	6.4836156687
CC:D3:R1 - SC:D2:R2	4.98333333	0.000000	***	3.0830510	6.8836156687
CC:D3:R1 - SC:D2:R3	5.50000000	0.000000	***	3.5997177	7.4002823353
CC:D3:R1 - SC:D3:R1	4.58333333	0.000000	***	2.6830510	6.4836156687
CC:D3:R1 - SC:D3:R2	5.26666667	0.000000	***	3.3663843	7.1669490020
CC:D3:R1 - SC:D3:R3	5.68333333	0.000000	***	3.7830510	7.5836156687
CC:D3:R2 - CC:D3:R3	-0.38333333	1.000000		-2.2836157	1.5169490020
CC:D3:R2 - SC:D1:R1	-1.80000000	0.087879	.	-3.7002823	0.1002823353
CC:D3:R2 - SC:D1:R2	-0.71666667	0.997847		-2.6169490	1.1836156687
CC:D3:R2 - SC:D1:R3	-0.30000000	1.000000		-2.2002823	1.6002823353
CC:D3:R2 - SC:D2:R1	-1.58333333	0.246343		-3.4836157	0.3169490020
CC:D3:R2 - SC:D2:R2	-1.18333333	0.767984		-3.0836157	0.7169490020
CC:D3:R2 - SC:D2:R3	-0.66666667	0.999119		-2.5669490	1.2336156687
CC:D3:R2 - SC:D3:R1	-1.58333333	0.246343		-3.4836157	0.3169490020
CC:D3:R2 - SC:D3:R2	-0.90000000	0.973886		-2.8002823	1.0002823353
CC:D3:R2 - SC:D3:R3	-0.48333333	0.999989		-2.3836157	1.4169490020
CC:D3:R3 - SC:D1:R1	-1.41666667	0.448876		-3.3169490	0.4836156687
CC:D3:R3 - SC:D1:R2	-0.33333333	1.000000		-2.2336157	1.5669490020
CC:D3:R3 - SC:D1:R3	0.08333333	1.000000		-1.8169490	1.9836156687
CC:D3:R3 - SC:D2:R1	-1.20000000	0.747671		-3.1002823	0.7002823353
CC:D3:R3 - SC:D2:R2	-0.80000000	0.992324		-2.7002823	1.1002823353
CC:D3:R3 - SC:D2:R3	-0.28333333	1.000000		-2.1836157	1.6169490020

Table A.31 (Continued)

CC:D3:R3 - SC:D3:R1	-1.20000000	0.747671	-3.1002823	0.7002823353
CC:D3:R3 - SC:D3:R2	-0.51666667	0.999972	-2.4169490	1.3836156687
CC:D3:R3 - SC:D3:R3	-0.10000000	1.000000	-2.0002823	1.8002823353
SC:D1:R1 - SC:D1:R2	1.08333333	0.871932	-0.8169490	2.9836156687
SC:D1:R1 - SC:D1:R3	1.50000000	0.339962	-0.4002823	3.4002823353
SC:D1:R1 - SC:D2:R1	0.21666667	1.000000	-1.6836157	2.1169490020
SC:D1:R1 - SC:D2:R2	0.61666667	0.999679	-1.2836157	2.5169490020
SC:D1:R1 - SC:D2:R3	1.13333333	0.824079	-0.7669490	3.0336156687
SC:D1:R1 - SC:D3:R1	0.21666667	1.000000	-1.6836157	2.1169490020
SC:D1:R1 - SC:D3:R2	0.90000000	0.973886	-1.0002823	2.8002823353
SC:D1:R1 - SC:D3:R3	1.31666667	0.590093	-0.5836157	3.2169490020
SC:D1:R2 - SC:D1:R3	0.41666667	0.999999	-1.4836157	2.3169490020
SC:D1:R2 - SC:D2:R1	-0.86666667	0.982064	-2.7669490	1.0336156687
SC:D1:R2 - SC:D2:R2	-0.46666667	0.999994	-2.3669490	1.4336156687
SC:D1:R2 - SC:D2:R3	0.05000000	1.000000	-1.8502823	1.9502823353
SC:D1:R2 - SC:D3:R1	-0.86666667	0.982064	-2.7669490	1.0336156687
SC:D1:R2 - SC:D3:R2	-0.18333333	1.000000	-2.0836157	1.7169490020
SC:D1:R2 - SC:D3:R3	0.23333333	1.000000	-1.6669490	2.1336156687
SC:D1:R3 - SC:D2:R1	-1.28333333	0.637051	-3.1836157	0.6169490020
SC:D1:R3 - SC:D2:R2	-0.88333333	0.978275	-2.7836157	1.0169490020
SC:D1:R3 - SC:D2:R3	-0.36666667	1.000000	-2.2669490	1.5336156687
SC:D1:R3 - SC:D3:R1	-1.28333333	0.637051	-3.1836157	0.6169490020
SC:D1:R3 - SC:D3:R2	-0.60000000	0.999777	-2.5002823	1.3002823353
SC:D1:R3 - SC:D3:R3	-0.18333333	1.000000	-2.0836157	1.7169490020
SC:D2:R1 - SC:D2:R2	0.40000000	0.999999	-1.5002823	2.3002823353
SC:D2:R1 - SC:D2:R3	0.91666667	0.968840	-0.9836157	2.8169490020
SC:D2:R1 - SC:D3:R1	0.00000000	1.000000	-1.9002823	1.9002823353
SC:D2:R1 - SC:D3:R2	0.68333333	0.998799	-1.2169490	2.5836156687
SC:D2:R1 - SC:D3:R3	1.10000000	0.856952	-0.8002823	3.0002823353
SC:D2:R2 - SC:D2:R3	0.51666667	0.999972	-1.3836157	2.4169490020
SC:D2:R2 - SC:D3:R1	-0.40000000	0.999999	-2.3002823	1.5002823353
SC:D2:R2 - SC:D3:R2	0.28333333	1.000000	-1.6169490	2.1836156687
SC:D2:R2 - SC:D3:R3	0.70000000	0.998383	-1.2002823	2.6002823353
SC:D2:R3 - SC:D3:R1	-0.91666667	0.968840	-2.8169490	0.9836156687
SC:D2:R3 - SC:D3:R2	-0.23333333	1.000000	-2.1336157	1.6669490020
SC:D2:R3 - SC:D3:R3	0.18333333	1.000000	-1.7169490	2.0836156687
SC:D3:R1 - SC:D3:R2	0.68333333	0.998799	-1.2169490	2.5836156687
SC:D3:R1 - SC:D3:R3	1.10000000	0.856952	-0.8002823	3.0002823353
SC:D3:R2 - SC:D3:R3	0.41666667	0.999999	-1.4836157	2.3169490020

Groups, Treatments and means

a	CC:D3:R1	19.7
a	CC:D2:R1	19.5
a	CC:D1:R1	18.27

Table A.31 (Continued)

b	SC:D1:R1	15.33
b	SC:D2:R1	15.12
b	SC:D3:R1	15.12
b	SC:D2:R2	14.72
b	CC:D1:R3	14.48
b	SC:D3:R2	14.43
b	CC:D1:R2	14.28
b	SC:D1:R2	14.25
b	SC:D2:R3	14.2
b	SC:D3:R3	14.02
b	CC:D2:R3	13.92
b	CC:D3:R3	13.92
b	SC:D1:R3	13.83
b	CC:D3:R2	13.53
b	CC:D2:R2	13.43

Table A.32 Multiple comparisons of means using Tukey contrasts, LD RIB simulations, DD x R

DD:Rtype, means

	TotalGen	std	r	Min	Max
CC:R1	13.40556	2.160326	180	9	19
CC:R2	11.48333	1.635756	180	8	17
CC:R3	11.38889	1.763658	180	8	18
SC:R1	12.38333	2.125333	180	8	19
SC:R2	11.51111	1.754448	180	8	18
SC:R3	11.33333	.796956	180	8	17

alpha: 0.05 ; Df Error: 1044
Critical Value of Studentized Range: 4.037602
Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
CC:R1 - CC:R2	1.92222222	0.000000	***	1.3563443	2.4881001
CC:R1 - CC:R3	2.01666667	0.000000	***	1.4507888	2.5825446
CC:R1 - SC:R1	1.02222222	0.000004	***	0.4563443	1.5881001
CC:R1 - SC:R2	1.89444444	0.000000	***	1.3285665	2.4603223
CC:R1 - SC:R3	2.07222222	0.000000	***	1.5063443	2.6381001
CC:R2 - CC:R3	0.09444444	0.996956		-0.4714335	0.6603223
CC:R2 - SC:R1	-0.90000000	0.000091	***	-1.4658779	-0.3341221
CC:R2 - SC:R2	-0.02777778	0.999992		-0.5936557	0.5381001
CC:R2 - SC:R3	0.15000000	0.974496		-0.4158779	0.7158779
CC:R3 - SC:R1	-0.99444444	0.000009	***	-1.5603223	-0.4285665
CC:R3 - SC:R2	-0.12222222	0.989838		-0.6881001	0.4436557
CC:R3 - SC:R3	0.05555556	0.999767		-0.5103223	0.6214335
SC:R1 - SC:R2	0.87222222	0.000173	***	0.3063443	1.4381001
SC:R1 - SC:R3	1.05000000	0.000002	***	0.4841221	1.6158779
SC:R2 - SC:R3	0.17777778	0.947266		-0.3881001	0.7436557

HSD Test for TotalGen

Groups, Treatments and means

a	CC:R1	13.41
b	SC:R1	12.38
c	SC:R2	11.51
c	CC:R2	11.48
c	CC:R3	11.39
c	SC:R3	11.33

Table A.33 Multiple comparisons of means using Tukey contrasts, LD RIB simulations, DD x D

DD:kernel, means

	TotalGen	std	r	Min	Max
CC:D1	12.23333	2.197256	180	8	19
CC:D2	12.03889	1.981377	180	9	19
CC:D3	12.00556	2.067296	180	8	18
SC:D1	11.76667	2.030769	180	8	18
SC:D2	11.40556	1.832961	180	8	17
SC:D3	12.05556	1.939654	180	8	19

alpha: 0.05 ; Df Error: 1044
Critical Value of Studentized Range: 4.037602
Comparison between treatments means

	Difference	pvalue	sig.	LCL	UCL
CC:D1 - CC:D2	0.19444444	0.924005		-0.37143345	0.7603223
CC:D1 - CC:D3	0.22777778	0.860523		-0.33810012	0.7936557
CC:D1 - SC:D1	0.46666667	0.173568		-0.09921123	1.0325446
CC:D1 - SC:D2	0.82777778	0.000459	***	0.26189988	1.3936557
CC:D1 - SC:D3	0.17777778	0.947266		-0.38810012	0.7436557
CC:D2 - CC:D3	0.03333333	0.999981		-0.53254456	0.5992112
CC:D2 - SC:D1	0.27222222	0.743039		-0.29365567	0.8381001
CC:D2 - SC:D2	0.63333333	0.017985	*	0.06745544	1.1992112
CC:D2 - SC:D3	-0.01666667	0.999999		-0.58254456	0.5492112
CC:D3 - SC:D1	0.23888889	0.834404		-0.32698901	0.8047668
CC:D3 - SC:D2	0.60000000	0.030323	*	0.03412210	1.1658779
CC:D3 - SC:D3	-0.05000000	0.999861		-0.61587790	0.5158779
SC:D1 - SC:D2	0.36111111	0.451972		-0.20476679	0.9269890
SC:D1 - SC:D3	-0.28888889	0.691477		-0.85476679	0.2769890
SC:D2 - SC:D3	-0.65000000	0.013682	*	-1.21587790	-0.0841221

Groups, Treatments and means

a	CC:D1	12.23
a	SC:D3	12.06
a	CC:D2	12.04
a	CC:D3	12.01
ab	SC:D1	11.77
b	SC:D2	11.41

A.2.1.2 One-way ANOVAs

Table A.34 Density dependence comparison at R1 D1 AD1

```
> summary(AnovaModel.3)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  123.3  123.27       8 0.00641 **
Residuals    58  893.7   15.41
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.35 Density dependence comparison at R1 D2 AD1

```
> summary(AnovaModel.4)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  498.8   498.8   28.94 1.4e-06 ***
Residuals    58  999.8    17.2
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.36 Density dependence comparison at R1 D3 AD1

```
> summary(AnovaModel.5)
             Df Sum Sq Mean Sq F value Pr(>F)
DD            1    322   322.0   12.    83      7e-04 ***
Residuals    58   1456    25.1
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.37 Density dependence comparison at R2 D2 AD1

```
> summary(AnovaModel.1)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  41.7    41.67   7.322 0.00893 **
Residuals    58  330.1    5.69
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.38 Density dependence comparison at R2 D3 AD1

```
> summary(AnovaModel.2)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1   29.4   29.40   7.697 0.00743 **
Residuals    58  221.5    3.82
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.39 Density dependence comparison at R1 D1 AD2

```
> summary(AnovaModel.7)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  135.0   135.0   9.715 0.00284 **
Residuals    58  805.9    13.9
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.40 Density dependence comparison at R1 D2 AD2

```
> summary(AnovaModel.8)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  135.0  135.00   8.818 0.00433 **
Residuals    58  887.9   15.31
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.41 Density dependence comparison at R1 D3 AD2

```
> summary(AnovaModel.9)
             Df Sum Sq Mean Sq F value   Pr(>F)
DD            1  308.3  308.27   24.4  6.98e-06 ***
Residuals    58  732.7   12.63
```

Table A.42 Contest competition: varying growth rate, D1 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	255.1	127.54	11.33	4.23e-05 ***
Residuals	87	979.2	11.26		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-3.8333	0.8662	-4.425	< 1e-04 ***
R3 - R1 == 0	-3.2333	0.8662	-3.733	0.000974 ***
R3 - R2 == 0	0.6000	0.8662	0.693	0.768416

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Table A.43 Contest competition: varying growth rate, D2 AD1

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	846	423.0	31.07	6.57e-11 ***
Residuals	87	1184	13.6		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "a" "a"

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-6.8667	0.9527	-7.208	<1e-05 ***
R3 - R1 == 0	-6.0667	0.9527	-6.368	<1e-05 ***
R3 - R2 == 0	0.8000	0.9527	0.840	0.679

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

Table A.44 Contest competition: varying growth rate, D3 AD1

```
> summary(AnovaModel.3)
            Df Sum Sq Mean Sq F value   Pr(>F)
Rtype        2    718   359.0   21.34 2.87e-08 ***
Residuals   87   1463    16.8
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
                  Estimate Std. Error  t value     Pr(>|t|)
R2 - R1 == 0  -6.1667     1.0589      -5.823      <1e-05 ***
R3 - R1 == 0  -5.8000     1.0589      -5.477      <1e-05 ***
R3 - R2 == 0   0.3667     1.0589       0.346       0.936
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.45 Contest competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	359.0	179.51	17.18	5.13e-07 ***
Residuals	87	908.8	10.45		

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
                  Estimate Std. Error  t value     Pr(>|t|)
R2 - R1 == 0  -4.1333     0.8345      -4.953      <1e-05 ***
R3 - R1 == 0  -4.3333     0.8345      -5.193      <1e-05 ***
R3 - R2 == 0  -0.2000     0.8345      -0.240       0.969
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.46 Contest competition: varying growth rate, D2 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   537.8   268.88    27.58  5.29e-10 ***
Residuals    87   848.2    9.75
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -5.2667     0.8062  -6.533  <1e-05 ***
R3 - R1 == 0  -5.1000     0.8062  -6.326  <1e-05 ***
R3 - R2 == 0   0.1667     0.8062   0.207  0.977
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

 > cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.47 Contest competition: varying growth rate, D3 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   714.4   357.2    37.87  1.48e-12 ***
Residuals    87   820.7   9.4
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error  t value  Pr(>|t|)
R2 - R1 == 0   -6.167      0.793   -7.776  <1e-04 ***
R3 - R1 == 0   -5.767      0.793   -7.272  <1e-04 ***
R3 - R2 == 0    0.400      0.793    0.504  0.869
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.48 Scramble competition: varying growth rate, D1 AD2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Rtype	2	56.4	28.211	4.882	0.00978 **
Residuals	87	502.7	5.779		

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:

	Estimate	Std. Error	t value	Pr(>|t|)
R2 - R1 == 0	-1.1000	0.6207	-1.772	0.18498
R3 - R1 == 0	-1.9333	0.6207	-3.115	0.00696 **
R3 - R2 == 0	-0.8333	0.6207	-1.343	0.37566

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
R1 R2 R3
"b" "ab" "a"

A.2.2 RIB analyses

A.2.2.1 Multi-way ANOVAs

Table A.49 Four-way ANOVA for DD x R x D x AD

Response: TotalGen

	Sum Sq	Df	F value	Pr(>F)
ADtype	14.0	1	3.9620	0.0467985 *
DD	33.1	1	9.3547	0.0022809 **
kernel	20.8	2	2.9386	0.0533792 .
Rtype	518.6	2	73.3418	< 2.2e-16 ***
ADtype:DD	1.6	1	0.4402	0.5071612
ADtype:kernel	3.3	2	0.4627	0.6296822
DD:kernel	22.8	2	3.2314	0.0398992 *
ADtype:Rtype	2.0	2	0.2836	0.7531123
DD:Rtype	61.3	2	8.6712	0.0001841 ***
kernel:Rtype	5.4	4	0.3797	0.8232238
ADtype:DD:kernel	1.3	2	0.1888	0.8279665
ADtype:DD:Rtype	7.3	2	1.0279	0.3581243
ADtype:kernel:Rtype	12.0	4	0.8454	0.4964847
DD:kernel:Rtype	24.1	4	1.7017	0.1473408
ADtype:DD:kernel:Rtype	2.9	4	0.2085	0.9337919
Residuals	3691.2	1044		

A.2.2.2 One-way ANOVAs

Table A.50 Density dependence comparison at R1 D1 AD1

```
> summary(AnovaModel.13)
             Df Sum Sq Mean Sq F value Pr(>F)
DD            1   40.0   40.02       6.882  0.0111 *
Residuals    58  337.2    5.81
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.51 Density dependence comparison at R1 D2 AD1

```
> summary(AnovaModel.14)
             Df Sum Sq Mean Sq F value  Pr(>F)
DD            1  35.27   35.27          7.367   0.00874 **
Residuals    58 277.67    4.79
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.52 Density dependence comparison at R2 D3 AD1

```
> summary(AnovaModel.16)
             Df Sum Sq Mean Sq F value Pr(>F)
DD            1    9.6   9.600           4.609  0.036 *
Residuals    58  120.8   2.083
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.53 Density dependence comparison at R1 D1 AD2

```
> summary(AnovaModel.17)
             Df Sum Sq Mean Sq F value Pr(>F)
DD            1   29.4  29.400          6.839   0.0113 *
Residuals    58  249.3   4.299
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Table A.54 Contest competition: varying growth rate, D1 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2    96.8    48.40    12.56 1.62e-05 ***
Residuals    87   335.3     3.85
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
                  Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0 -2.200e+00  5.069e-01   -4.34  0.000104 ***
R3 - R1 == 0 -2.200e+00  5.069e-01   -4.34  0.000114 ***
R3 - R2 == 0  4.441e-16  5.069e-01    0.00  1.000000
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.55 Contest competition: varying growth rate, D2 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2    82.4    41.21    11.22 4.62e-05 ***
Residuals    87   319.5     3.67
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
          Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -1.7333     0.4948  -3.503  0.00207 **
R3 - R1 == 0  -2.2333     0.4948  -4.513  < 1e-04 ***
R3 - R2 == 0  -0.5000     0.4948  -1.010  0.57230
---
> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.56 Contest competition: varying growth rate, D3 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   79.09    39.54    12.03 2.44e-05 ***
Residuals    87  286.03     3.29
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -2.0667     0.4682  -4.414  < 1e-04 ***
R3 - R1 == 0  -1.9000     0.4682  -4.058 0.000288 ***
R3 - R2 == 0   0.1667     0.4682   0.356 0.932575
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.57 Scramble competition: varying growth rate, D3 AD1

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2    30.2    15.10    4.603  0.0126 *
Residuals    87   285.4     3.28
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
           Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -0.9000     0.4677  -1.925   0.1378
R3 - R1 == 0  -1.4000     0.4677  -2.994   0.0101 *
R3 - R2 == 0  -0.5000     0.4677  -1.069   0.5357
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
  R1   R2   R3
 "b" "ab"  "a"
```

Table A.58 Contest competition: varying growth rate, D1 AD2

```
            Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype        2   140.5    70.23    21.18  3.2e-08 ***
Residuals   87   288.4     3.32
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
                  Estimate Std. Error t value    Pr(>|t|)
R2 - R1 == 0 -2.66667    0.47013  -5.672      < 1e-06 ***
R3 - R1 == 0 -2.63333    0.47013  -5.601     1.06e-06 ***
R3 - R2 == 0  0.03333    0.47013   0.071      0.997
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.59 Contest competition: varying growth rate, D2 AD2

```
            Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype        2    39.2   19.600    6.536  0.00227 **
Residuals   87   260.9    2.999
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
                  Estimate Std. Error t value    Pr(>|t|)
R2 - R1 == 0  -1.0000     0.4471  -2.236      0.0707 .
R3 - R1 == 0  -1.6000     0.4471  -3.578      0.0016 **
R3 - R2 == 0  -0.6000     0.4471  -1.342      0.3761
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
 > cld(.Pairs) # compact letter display
  R1   R2   R3
 "b" "ab"  "a"
```

Table A.60 Contest competition: varying growth rate, D3 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2    59.5   29.733    7.883 0.000714 ***
Residuals    87   328.1    3.772
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -1.8667     0.5014  -3.723  0.000998 ***
R3 - R1 == 0  -1.5333     0.5014  -3.058   0.008306 **
R3 - R2 == 0   0.3333     0.5014   0.665  0.784497
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.61 Scramble competition: varying growth rate, D1 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   40.29   20.144     .113  0.00328 **
Residuals    87  286.70    3.295
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
             Estimate Std. Error t value  Pr(>|t|)
R2 - R1 == 0  -1.3667     0.4687  -2.916  0.01234 *
R3 - R1 == 0  -1.4667     0.4687  -3.129  0.00676 **
R3 - R2 == 0  -0.1000     0.4687  -0.213  0.97523
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.62 Scramble competition: varying growth rate, D2 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2   29.76   14.878    5.081 0.00818 **
Residuals    87  254.73    2.928
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
               Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -1.2667     0.4418  -2.867  0.0142 *
R3 - R1 == 0  -1.1667     0.4418  -2.641  0.0263 *
R3 - R2 == 0   0.1000     0.4418   0.226  0.9722
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> cld(.Pairs) # compact letter display
 R1  R2  R3
"b" "a" "a"
```

Table A.63 Scramble competition: varying growth rate, D3 AD2

```
             Df  Sum Sq  Mean Sq  F value  Pr(>F)
Rtype         2    23.8   11.878    3.118 0.0492 *
Residuals     7   331.4    3.809
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Multiple Comparisons of Means: Tukey Contrasts
Fit: aov(formula = TotalGen ~ Rtype, data = Dataset)
Linear Hypotheses:
               Estimate Std. Error t value Pr(>|t|)
R2 - R1 == 0  -0.8333     0.5039  -1.654  0.2290
R3 - R1 == 0  -1.2333     0.5039  -2.447  0.0429 *
R3 - R2 == 0  -0.4000     0.5039  -0.794  0.7078
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> cld(.Pairs) # compact letter display
  R1   R2   R3
 "b" "ab"  "a"
```