THE EVOLUTION OF RESISTANCE TO _Bacillus thuringiensis_ IN GREENHOUSE _Trichoplusia ni_ POPULATIONS

by

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Abstract

The microbial insecticide, *Bacillus thuringiensis (Bt)*, has become the mainstay of nonchemical control of Lepidopteran pests either as sprays or through the incorporation of *Bt* toxins into transgenic crops. Findings in the present study, report the frequent and rapid development of resistance to *Bt* subsp. *kurstaki* in populations of cabbage loopers, *Trichoplusia ni*, in commercial greenhouses in British Columbia, Canada. Studies of the genetic inheritance of resistance to *Bt* (DiPel) in these populations suggest that the *Bt* resistance is inherited as an autosomal, partially recessive trait and is due to more than one gene. However in a second study, dominance of *Bt* resistance varied with the host plant on which *Bt* was provided suggesting that the host plant will impact resistance evolution.

Cucumber, tomato and sweet pepper are the three principal crops grown in commercial greenhouses. In laboratory studies, *T. ni* performance varied considerably among the three crops with the most rapid growth and highest fecundity on cucumber leaves and the least rapid growth and lowest fecundity on pepper leaves. This finding suggests that there is intense selection pressure on *T. ni* populations in pepper environments. Suprisingly, a negative relationship between fecundity and offspring size was observed across the three host plant treatment groups. Offspring of the most fecund cucumber treatment group were significantly smaller than offspring of the least fecund pepper treatment group.

Resistance traits are often assumed to be associated with fitness costs and the presence of such costs may depend on the environment. In herbivorous insects, the host plant is a pivotal component of the herbivore's environment and it is likely that
resistance-associated fitness costs are magnified by poor nutritional resources. Therefore, the performance of four genotypic lines (resistant, susceptible and reciprocal hybrids) and their progeny were compared among the three greenhouse crops. Interestingly, the magnitude of fitness costs associated with \textit{Bt} resistance increased with declining host plant suitability. Moreover, no viable progeny were produced by resistant lines fed the least suitable host plant. Therefore, tritrophic interactions between \textit{T. ni}, \textit{Bt}, and the host plant will play a significant role in the evolution of resistance.
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Chapter I

1.1 Introduction

The war against insect pests has been waged since the beginning of agriculture. People have devised many ingenious methods of insect control from manual searching, mechanical trapping, chemical weapons to even excommunication. No effort has been as successful in the short term as the use of chemical insecticides. However, the supremacy of these chemical weapons has often been thwarted by the insect pests themselves. Many targeted insect species have evolved resistance to chemical insecticides in less than 10 years and often in as little as two (Georghiou & Taylor 1986). New chemicals are continually being developed for use against resistant insects in an escalating chemical battle between man and pest.

A similar battle has been ongoing in nature between insects and disease-causing organisms. A bacterium, *Bacillus thuringiensis kurstaki* (*Bt*), has the ability to produce toxins that are lethal to caterpillar pests. The presence of the insecticidal toxin was discovered in association with dead caterpillars in silkworm colonies in the early 1900s (Milner 1994). Studies then began on the use of the bacterial toxins as insecticides but were waylaid when chemical insecticides were introduced. As the negative impacts of chemical pesticides on the environment became apparent, interest in the natural insecticide *Bt* was renewed. However, much work was needed to improve the efficacy of natural insecticides to allow them to compete with chemical insecticides. The advent of new improved *Bt* formulations with efficacy nearing that of chemical insecticides, as well as the emerging resistance of insect pests to chemical insecticides, greatly increased the use of *Bt* around the world.
Bt comprises the majority of the market of "natural" insecticides worldwide which also includes insect viruses and plant essential oils (Cannon 1993). The specificity of Bt as an insecticide is one reason why it is a very safe alternative to chemical insecticides. The subspecies of Bt determines which toxins are produced and which insect is targeted. Bt kurstaki (Btk) is the most widely used Bt against Lepidopteran pests and is not toxic to other insect orders. Bt israelensis (Bti) is widely used to control mosquito larvae and is only toxic to some Dipteran species. A few other Bt subspecies that target either Coleoptera (Bt subsp. tenebrionis) or Lepidoptera (Bt subsp. aizawai) have also been formulated for insect control but are used less frequently than Btk or Bti. Due to each Bt subspecies' specificity, Bt causes little or no harm to humans, most beneficial insects (ie. pollinators), and other organisms and is an excellent alternative to chemical insecticides (Croft 1990; Flexner, Lighthart, & Croft 1986).

Bt is vital to organic agriculture as the primary weapon used against Lepidopteran pests. Few other alternatives are available to organic growers and research into new organic methods is costly and often lags behind research into new chemical controls. Furthermore, the cost of chemical control is often prohibitive to growers in developing countries. However, local production of Bt in countries such as Brazil, Cuba and Peru has made the use of Bt economically viable to growers. These factors attest to the importance of Bt to agriculture both locally and on a worldwide scale. However, the threat of insect pests developing resistance to Bt is ever present (Tabashnik 1994).

For over 30 years, Bt was used successfully without any evidence of resistance developing in targeted pest populations. During this long "honeymoon period", many thought that resistance to Bt would not develop because of the complexity of the Bt
toxins. Where chemical insecticides are based on one chemical that targets a specific biological process, Bt toxins are often composed of more than five different toxins (Tabashnik 1994).

In 1990, however, resistance to Bt was detected in populations of the diamondback moth, Plutella xylostella, infesting cabbage farms in Hawaii (Tabashnik et al. 1990). Since then, resistance to Bt has been detected in diamondback populations worldwide from Japan, USA, Mexico, the Philippines to Malaysia (Ferre & van Rie 2002). The honeymoon period was over.

Worldwide efforts then began to monitor for development of Bt resistance in other pests in the field. Surprisingly, resistance was not been detected in any other species, despite the selection for Bt-resistance in numerous species in the laboratory (Tabashnik 1994). This lack of resistance is particularly striking in light of the rapid development of resistance of over 500 arthropod species to chemical insecticides and acaricides since their inception (Voss 1988).

**Bt Toxicity and Resistance Mechanisms**

The δ-endotoxins of Bt are primarily responsible for Bt toxicity in commercial Bt products used against Lepidopteran pests. The δ-endotoxins present in the majority of the commercial Bt products for Lepidopteran control are produced by the Bt subspecies kurstaki HD-1 during sporulation (ie. Dipel, Foray, Thuricide). These products are composed of individual δ-endotoxins and Bt spores along with formulation ingredients. The δ-endotoxin is made up of five subunits (Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B) that target the brush border membrane of the midgut epithelium (Rosenheim & Tabashnik 1990). Other Bt subspecies that also target Lepidoptera produce a different assortment of
δ-endotoxins, such as *Bta*, the second most commonly used *Bt* product for Lepidoptera control, which produces Cry1Aa, Cry1Ab, Cry1C, Cry1D (Tabashnik 1994). Following ingestion of a *Bt* spore or toxin molecule, the toxin is activated by proteolytic enzymes in the insect's alkaline midgut. The subunits bind to the midgut brush border causing pores to form and allowing gut contents to leak across the deteriorated membrane. Subsequent changes in the insect's haemolymph alkalinity lead to paralysis and eventual death (Faust & Bulla Jr. 1984). Ingestion of a lethal dose of *Bt* toxins/spores coupled with appropriate gut conditions are required for larval mortality.

The complex mode of action of *Bt* toxins is thought to have deterred the rate of resistance evolution and may eventually give rise to a number of resistance mechanisms. The most common form of *Bt* resistance in Lepidoptera confers high levels of resistance to Cry1A toxins with no cross resistance to Cry1C toxins and is referred to as ‘mode 1’ resistance (Tabashnik et al. 1998). Mode 1 resistance is characterized by recessive inheritance and reduced or absent binding of at least one Cry1A toxin in completely resistant homozygous insects, whereas the presence of a susceptible allele in heterozygous individuals renders them susceptible. This form of resistance has been reported for four independent resistant strains of *P. xylostella*, laboratory-selected *Heliothis virescens*, the tobacco budworm, and laboratory-selected *Plodia interpunctella*, the Indianmeal moth (Tabashnik et al. 1998). Resistance in laboratory-selected *Helicoverpa armigera*, the cotton bollworm, corresponded to the lack of the high affinity binding site for Cry1Ac (Akhurst et al. 2003). However, not all resistant moth species contain resistance traits that correspond to mode 1 resistance or to reduced binding of Cry
toxins suggesting that alternative resistance mechanisms are present (Tabashnik et al. 1998).

Alteration of the midgut proteases, that either reduce the activation of Cry prototoxins or increase the degradation of the activated toxins, are also thought to be involved in Bt resistance. For example, midgut proteases of a P. interpunctella laboratory colony exhibited 11-fold higher resistance to Cry1Ab proto-toxins than to the activated form of the toxins (Herrero, Oppert, & Ferré 2001), and a genetic linkage was found between the reduced susceptibility to Cry1Ab and a major gut protease (Oppert et al. 1997). Midgut extracts of larvae of a resistant H. virescens laboratory colony exhibited both slower activation and enhanced degradation of the Cry1Ab toxin than susceptible insects (Forcada et al. 1996). In addition, reduced activation of the Cry1Ca prototoxin was implicated as a minor mechanism of resistance in another resistant P. xylostella population (Liu et al. 2000). Therefore, altered proteolytic activity is another potential resistance mechanism that may be present alone or in concert with reduced binding.

Despite the prevalence of the mode 1 resistance mechanism and the potential of altered proteolytic processing, similar levels of midgut damage were observed in two laboratory-selected resistant H. virescens populations, relative to susceptible populations, indicating that Bt-toxins were still able to damage the midgut cells of resistant individuals (Forcada et al. 1999; Martinez-Ramirez, Gould, & Ferré 1999). Rapid repair of damaged midgut cells was suggested as a possible resistance mechanism in these populations. Furthermore Bt resistance mechanisms may also include an insect’s immune response. For example, tolerance to low to medium levels of Bt was induced in a laboratory colony
of *Ephestia kuehniella*, the flour moth, and was correlated with an elevated immune response (Rahman et al. 2004). These examples illustrate the underlying complexity of *Bt* resistance and suggest that multiple mechanisms are likely responsible for resistant phenotypes.

**Inheritance of Resistance**

In general, resistance to pesticides in insects, mites, plants and vertebrates is attributed to allelic variants at one or two loci due to the intense selection pressure introduced by a new insecticide (Roush & McKenzie 1987). The number of resistance loci selected upon is thought to depend on whether resistance is selected for within or outside of the existing tolerance distribution of the population. It is predicted that polygenic resistance will be selected from within the normal tolerance distribution due to the combination of existing resistance alleles of minor effect, whereas selection outside of this distribution will select for monogenic responses that are typically rare mutations of major effect (McKenzie & Batterham 1994; McKenzie 1996).

Given the complexity of the mode of action of *Bt* and the potential for multiple resistance mechanisms, it is likely that polygenic traits exist in many populations. However, if a major monogenic trait for resistance is present in a population, it will quickly become the most prevalent resistance trait (Groeters & Tabashnik 2000; Orr 1998). Therefore, it is expected that most resistance traits will be due to one or a few major loci, however this does not preclude the possibility of the role of polygenes in further increases in resistance. The evolution of resistance to pesticides may be a “two-phase” response that first involves mutations of relatively major effect, which confer responses outside of existing phenotypic distributions, followed by a second phase that
incorporates the effects of many minor genes (McKenzie 1996). A similar two-phase process has been suggested as perfecting the evolution of Batesian mimicry in the peppered moth, *Biston betularia* (Charlesworth 1994).

In studies of *Bt* resistance, monogenic models are consistent with results of backcross data, the most common method used to examine inheritance patterns. For example, *Btk* resistance in *P. xylostella* (Tabashnik et al. 1992; Tang et al. 1997; Gould et al. 1995; Sayyed, Ferré, & Wright 2000) and in laboratory-selected *Ostrinia nubilalis* (Huang et al. 1999) corresponded to monogenic models of resistance. However, resistance to Cry1Ab in a laboratory colony of *H. virescens* (Sims & Stone 1991) and in a field-derived strain of *P. xylostella* (Sayyed & Wright 2001) did not correspond to monogenic inheritance. In laboratory-selected *Pectinophora gossypiella*, mutant cadherin alleles at three loci were shown to be involved in resistance to Cry1Ac and two resistant alleles in any combination are required for resistance (Morin et al. 2003). This recent example illustrates the underlying complexity of the resistant genotype.

The rate of *Bt* resistance evolution depends on the dominance, initial allele frequency, and relative fitness of the various genotypes in the population (Roush & McKenzie 1987). Dominance is not an intrinsic property of a *Bt*-resistance allele, and it is dependent not only on the concentration of *Bt* used but on the environmental parameters. The most commonly described measure of dominance is based on the position of the mortality curve of heterozygous genotypes relative to susceptible and resistant genotypes at a specified mortality level, typically 50% (Bourguet, Genissel, & Raymond 2000). Using this measure of dominance, genetic studies demonstrate that dominance of *Bt* resistance can vary from being co-dominant to being completely

The dominance of Bt resistance affects the competitiveness of heterozygous individuals in the presence of Bt and is particularly important to the effectiveness of strategies designed to delay resistance (McGaughey & Whalon 1992; Tabashnik 1994; Roush 1989; Tabashnik 1989). Due to the variety of possible resistance mechanisms, it is likely not possible to make a general statement regarding the dominance of Bt resistance. It is speculated that resistance traits that are due to loss of binding of the toxin to the midgut receptor are likely recessive if minimal toxin binding results in sufficient midgut damage in heterozygotes for susceptibility (Bourguet, Genissel, & Raymond 2000). Consistent with this speculation, resistance that corresponds to mode 1 in Lepidoptera is associated with recessive inheritance (Tabashnik et al. 1998). In contrast, altered proteolytic processing may result in higher dominance due to the activity of altered midgut proteases in heterozygote individuals (Bourguet, Genissel, & Raymond 2000). Furthermore, the dominance of a trait itself is subject to evolution and may increase over evolutionary time through the action of dominance modifiers (Bourguet 1999; Otto & Bourguet 1999). Therefore, the inheritance of Bt resistance will likely differ both spatially and temporally between resistant populations due to differences in resistant genotypes and interacting environmental effects and on the selection of dominance modifiers.

**Fitness Costs**

The current lack of observed Bt resistance in field populations may be due to an inherent instability of resistance in the absence of Bt exposure. It is generally assumed
that insecticide resistance traits are associated with fitness costs in the absence of the insecticide (Roush & McKenzie 1987). Because resistance genes are rarely fixed in natural populations, the frequency of new resistance mutations is thought to be maintained via counterbalanced selection pressures that decrease resistance in insecticide-free environments (Coustau, Chevillon, & ffrench-Constant 2000). As mentioned previously, selection for insecticide resistance is expected to occur outside of the normal phenotypic distribution in the field. In this case, evolution will likely involve the selection of mutations with large effects (Mcnair 1991). Because the phenotypic distribution prior to insecticide use was shaped by natural selection, any new mutation with a large effect is expected to be associated with significant costs in the ancestral (i.e. insecticide-free) environment (Fisher 1958). These negative effects are presumably due to the pleiotropic action of the resistance trait on other phenotypic characters as a consequence of altered biochemical and physiological processes (McKenzie 1996). It is further predicted that resistance mechanisms that disrupt metabolic pathways through effects on receptors, such as mode 1 resistance to Bt, will be associated with severe deleterious effects (Uyenoyama 1990).

Bt resistance has been reported to decline in the absence of selection in a number of laboratory colonies (Hama, Suzuki, & Tanaka 1992; McGaughey & Beeman 1988; Sayyed & Wright 2001; Tabashnik, Finson, & Johnson 1991). This decline in resistant genotype frequencies has been attributed to resistance-related fitness costs, such as decreased growth rate (Liu et al. 1999), survival (Groeters et al. 1994; Carrière et al. 2001a), fecundity, and mating success (Groeters et al. 1993) of resistant versus susceptible individuals in the absence of Bt. For the pink bollworm, Pectinophora
gossypiella, fitness costs over the winter period were particularly severe such that resistant individuals exhibited a 71% reduction in emergence in the spring as compared to susceptible individuals (Carrière et al. 2001b). However, in other studies significant fitness costs were not associated with Bt resistance. For example, estimates of overall intrinsic growth rates of resistant P. xylostella populations were not found to differ from susceptible populations (Sayyed & Wright 2001). Furthermore, no differences in survival or larval weight were found between resistant and susceptible H. virescens in the absence of Bt (Gould & Anderson 1991).

It is predicted that resistance-associated fitness costs will be ameliorated over time due to competition among resistant genotypes (Coustau, Chevillon, & ffrench-Constant 2000). Reduced fitness costs may arise through allelic replacement or the accumulation of compensatory alleles (Cohan, King, & Zawadzki 1994). In the sheep blowfly, Lucilia cuprina, diazinon-resistant individuals exhibited increased overwintering mortality, lowered fitness (as determined from population cage studies), and increased asymmetry in morphological structures as compared to wild-type individuals early in the evolution of diazinon resistance (McKenzie & Clarke 1988). Ten years after the first resistant individuals appeared, field-collected resistant flies were similar in fitness to susceptible flies in non-treated environments due to the actions of a modifier gene (Clarke 1997). In contrast, fitness costs associated with resistance to organophosphates in Culex pipiens were ameliorated through the replacement of the costly allele with a less costly allele at the resistant locus (Raymond et al. 2001). Therefore, fitness costs need not be permanently associated with resistance traits.
The presence of resistance-associated fitness costs does suggest that there is often a trade-off between resistance and other fitness-related functions (Bergelson & Purrington 1996; Carrière et al. 1994). This trade-off is evident in the prolonged lifespan, reduced size and reduced fecundity of many resistant individuals relative to susceptible individuals (Carrière et al. 1994). In studies of parasite resistance, fitness costs are thought to result from the direction of resources toward the maintenance of immune function or to the production of defense mechanisms (Coustau, Chevillon, & ffrench-Constant 2000). Such trade-offs form the basis of life-history theory and can be used to predict the rate at which particular strategies (i.e. resistance vs susceptible) evolve under different environmental conditions and the level that will be maintained in the population (Boots & Begon 1993).

Using life-history theory, it is predicted that the trade-off between resistance and other life-history traits, such as reproduction, will be most evident when resources are limiting (Bergelson & Purrington 1996). Therefore, the rate of resistance evolution and the stability of the resistance trait in the absence of selection pressure will depend on environmental stress. Resistance evolution is predicted to be most rapid in the least stressful environment due to the reduction in life-history trade-offs and the least stable in stressful environments.

**Tritrophic Interactions: Bt, Insect Herbivores, and Host Plants**

The interactions between insect herbivores and natural enemies, including insect pathogens like *Bt*, are known to be significantly influenced by host plants (Price et al. 1980). Much research has provided evidence to support the notion that plants can use predators and parasitoids as bodyguards to protect against insect herbivory (Price et al.
and the bodyguard hypothesis is currently being extended to include entomopathogens (Elliot et al. 2000). Plants may influence the interaction between insects and entomopathogens by increasing pathogen numbers or enhancing pathogen effectiveness (Elliot et al. 2000). For example, high population densities of \( Bt \) on phylloplanes of plants have been proposed to be due to the maintenance of insect pathogens by plants as bodyguards (Smith & Couche 1991). In opposition to the bodyguard hypothesis, antimicrobial compounds produced by plants may reduce pathogen numbers. Many plant compounds are known to exhibit antibacterial properties that can decrease the persistence of \( Bt \) on leaf material (Ludlum, Felton, & Duffey 1991), which is an important component of \( Bt \) efficacy (Brand et al. 1976). For example, survivorship of \( Manduca sexta \) larvae exposed to \( Bt \) increased with increasing levels of nicotine, an allelochemical in tobacco plants, due to bactericidal effects of nicotine on \( Bt \) (Krischik, Barbosa, & Reichelderfer 1988). These influences, either positive or negative, will ultimately affect the selection pressure of the pathogen on the insect population and the evolution of resistance.

Plant defenses may also significantly influence an insect herbivore’s susceptibility to disease. Plant defensive structures, (i.e. trichomes) and defensive chemistry may directly impact an insect’s physiology and impair immunity to disease. For example, survivorship of \( M. sexta \) larvae decreased with increasing L-canavanine levels, an allelochemical in leguminous plants, presumably due to negative effects of L-canavanine on insect gut permeability (Felton & Dahlman 1984). Tannic acids, a commonly produced plant defensive compound, can absorb to the peritrophic membrane of insect species and damage the midgut epithelium (Bernays & Chamberlain 1980). Because the
peritrophic membrane is one of the primary physical barriers to bacteria and virus infection, it is no surprise that tannic acid increased the efficacy of $Bt$ towards *Heliothis virescens* and *T. ni* (Gibson et al. 1995). Furthermore, physical defenses, such as trichomes, are also known to cause damage to the peritrophic membrane and may, therefore, influence the toxicity of insect pathogens (Price et al. 1980).

Host plant defenses may also indirectly impact an insect’s immunity through effects on nutritional quality via the production of plant defensive compounds or simply through variation in plant nutrient content (Price et al. 1980). It has commonly been observed that the susceptibility of insect herbivores to entomopathogens generally increases as host plant suitability decreases (Meade & Hare 1994). For example, a decrease in quality of dietary protein enhanced the toxicity of the $Bt$ toxin Cry1Ac to *Manduca sexta* (Neal 1996), and the susceptibility of *Spodoptera exigua* to $Bt$ was found to increase with more resistant celery cultivars (Meade & Hare 1993; Meade & Hare 1994). These differences in $Bt$ virulence have translated into poor efficacy in the field, which could ultimately impact resistance evolution. For example, plant defensive compounds in fruiting cotton genetically modified to produce Cry1Ac were pinpointed as causing an observed reduction in efficacy of $Bt$ toxins in field plantations (Olsen & Daly 2000).

Often what is disregarded in laboratory studies of resistant traits and correlated fitness phenotypes is the possibility of interactions between the environment and the genotype of the resistant individuals. Little is known about the sensitivity of fitness parameters correlated with resistant genotypes under different environmental conditions. As mentioned previously, life-history theory predicts that severity of fitness costs associated with resistant traits will increase under conditions of environmental stress
(Bergelson & Purrington 1996). For example, *Drosophila melanogaster* resistant to attack by the endoparasitoid, *Asobara tabida*, experience reduced survival as compared to susceptible flies when subject to high intraspecific competition (Kraaijeveld & Godfray 1997). However, when intraspecific competition was minimal there were no differences in survivorship between the two lines. Therefore, the severity of the environment can significantly increase the magnitude of resistance-associated fitness costs. The overwintering period is a time of severe environmental stress for many insects and therefore resistance associated fitness costs often increase in severity during this period. For example, fitness costs were observed to increase in magnitude in *Bt* resistant pink bollworm, *P. gosypiella* (Carrière et al. 2001b), Colorado potato beetle resistant to Cry3A (Alyokhin & Ferro 1999), and in *L. cuprina* resistant to dieldrin (McKenzie & Batterham 1994) during the overwintering period.

Host plant quality is another important environmental stressor due to its significant impact on the growth and reproduction of phytophagous insects (Awmack & Leather 2002). Since the availability of resources to insects is directly impacted by the host plant, this in turn will alter the production or maintenance of defensive traits expressed by the insect. Thus, fitness costs associated with pesticide resistance in herbivorous insects are expected to vary with the suitability of the host plant and thereby alter *Bt* resistance evolution.

### 1.2 Study System

The evolution of resistance to *Bt* was studied in commercial vegetable greenhouse populations of *Trichoplusia ni*. This study system is unique in that there are multiple discrete *T. ni* populations, each of which feed almost exclusively on one of three host
plants: cucumber, tomato and pepper. This system, therefore, provided a perfect opportunity to examine the effects of host plant on the evolution of resistance to Bt and to specifically examine how resistance associated fitness costs vary among the host plant environments. Details on the biology of *T. ni*, the greenhouse environment, and the three host plants are provided in the following sections.

**Trichoplusia ni**

*Trichoplusia ni* is a polyphagous Lepidopteran that is an economic pest in a wide variety of crops in the Fraser Valley, British Columbia (BC), Canada. *T. ni* is an important pest in field cole crops (ie. cauliflower, broccoli, and cabbage) and is the only Lepidopteran pest in commercial vegetable greenhouses in BC. *T. ni* populations can grow rapidly due to the high reproductive potential of the species. Fecundity of female moths is reported to range from 300 eggs per female (McEwen & Hervey 1960) to over 1,000 eggs (Mitchell & Chalfant 1984) with hatching success ranging between 50 and 80%. Eggs are laid singly and females are reported to mate with an average of two males and up to six (Shorey, Andres, & Hale Jr. 1962).

*T. ni* is considered to be a subtropical insect and temperatures below 15.6°C drastically reduce adult mating and flight (Mitchell & Chalfant 1984). Therefore, *T. ni* is ideally suited to growth in greenhouse environments. Temperatures lower than 7.2°C prevent development of immature stages (Toba et al. 1973). Catches of adult *T. ni* occur from June to September with a peak in August in Southwestern BC (Mitchell & Chalfant 1984). *T. ni* are not generally thought to overwinter in BC and populations of *T. ni* are thought to move up from overwintering populations in the Southern USA. However, there are reports of overwintering *T. ni* in Ontario and the Northeastern states (Lingren,
It seems possible that *T. ni* overwinter in southwestern British Columbia, particularly close to heated urban areas. Local *T. ni* populations may then be comprised of both overwintering and migrating populations.

*T. ni* moths and larvae can occur in greenhouse environments from the beginning of the growing season (Dec.-Feb.) to the end of season (Nov.-Dec.). Typically, the crop is removed at the end of the year and the greenhouse structure is cleaned and fumigated to prevent the carry over of insect pests between growing seasons. The early presence of a *T. ni* population in greenhouses may be due to inadequate clean-up by the grower or the possible introduction of *T. ni* with the new seedlings brought in from a propagator. Populations of *T. ni* typically increase in greenhouses during the summer months in accordance with elevated trap catches in field crops. *T. ni* populations can then continue to cycle in greenhouses to the end of the growing season due to greenhouse temperature control. The generation time, from egg to adult, of *T. ni* is dependent on temperature and ranges from 73.5 days at 16.6°C to 21.5 days at 27.2°C (Toba et al. 1973). Growers attempt to regulate temperatures within greenhouses structures to within 18-25°C and therefore *T. ni* can cycle almost monthly throughout the growing season.

There is little available literature on the incidence of *Bt* resistance in *T. ni* populations. In one study, selection for *Bt* resistance in the laboratory increased the LC$_{50}$ (*Bt* concentration that results in 50% mortality) 26-fold in a laboratory reared *T. ni* population (Estada & Ferré 1994), indicating that some *T. ni* populations do have the genetic propensity to develop *Bt* resistance.
Greenhouse Environments and Resistance

Greenhouses provide a relatively constant environment that protects insect herbivores from inclement weather and allows for rapid growth of insect populations. High insect densities then result in a positive feedback with amplifying control measures greatly increasing the selection intensity. In addition, immigration of susceptible individuals into greenhouses is limited (although not entirely absent due to the presence of numerous vents in the roofs of greenhouses of Dutch-venlo design) and thus does not mitigate the rate of resistance evolution. Greenhouse environments have been implicated in two studies as contributing to the development of Bt resistance. Populations of *P. xylostella* that originated from crucifer transplants grown in greenhouses in Florida had the highest LC₅₀ (>200-fold higher than field populations) (Shelton et al. 1993). In that study, greenhouse growers were reported to apply Bt 15 times to a single set of crucifer transplants, with up to 50 applications in one growing season. The authors contend that due to the concentration of transplants and sprays in greenhouses, greenhouses may play a major role in the development and distribution of Bt resistance (Shelton et al. 1993). After reported control failures, *P. xylostella* populations collected from intensely treated watercress grown in greenhouses in Osaka displayed 700-fold resistance to Bt (Tanaka & Kimura 1991). Therefore, greenhouse growing conditions do appear to provide the ideal conditions for the evolution of resistance to Bt in Lepidopteran pests.

Contained environments have played a significant role in the evolution of resistance to chemical insecticides. In studies of insecticide-resistant fly populations, animal barns provided the most conducive habitat for resistance selection due to the rapid growth of fly populations coupled with intense control programs (Denholm et al. 1990).
In addition, farms that provided conditions for year-round breeding acted as foci for selection of resistant individuals that subsequently dispersed to other farms in the spring (Denholm, Sawicki, & Farnham 1985). With respect to Bt resistance, it is no coincidence that populations of Indianmeal moth, *Plodia interpunctella*, contained in grain bins and subjected to a high number of Bt sprays, were the first reported species to have developed some Bt resistance (2-fold) in response to Bt applications (McGaughey 1985). High heritability of Bt resistance in this species was attributed to the low environmental variation experienced by populations of *P. interpunctella* in grain bins (Tabashnik 1994). Therefore, greenhouses and similar environments provide ideal conditions for Bt resistance evolution and may act as a source of resistant alleles for surrounding field populations.

**Greenhouse Crops**

The host plant can impact resistance development in a variety of ways, affecting *T. ni* population growth, Bt toxicity, and correlated fitness-costs associated with resistance. *T. ni* is a generalist herbivore that is known to feed on over 160 different host plants, but its growth and reproduction vary considerably among plants (Sutherland & Greene 1984; Sutherland 1966; Soo Hoo, Coudriet, & Vail 1984). Therefore, it is plausible that fitness costs associated with resistance might vary with the host plant. Beefsteak and vine tomatoes, bell peppers and long English cucumbers are the major crops grown in vegetable greenhouses in British Columbia. *T. ni* can be an economic pest in each crop, however preliminary evidence suggests that *T. ni* does not perform equally well on each host plant.
Tomatoes, peppers and cucumbers differ considerably in plant chemistry, which may contribute to the differences observed in *T. ni* growth. With respect to constitutive defensive compounds, tomato leaves are known to contain the glycoalkaloid, a-tomatine, and the catecholic phenols, chlorogenic acid and rutin, each of which are known to negatively affect the growth of insect herbivores, such as *Heliothis zea* (Isman & Duffey 1982a; Isman & Duffey 1982b; Bloem, Hoover, & Duffey 1989; Elliger et al. 1981; Kennedy 2003). These defensive compounds may augment *Bt* toxicity, because *Bt* is more toxic toward *Tuta absoluta* ingested on tomato leaves (Giustolin et al. 2001). Interestingly, chlorogenic acid in combination with peroxidase, an inducible defensive compound in tomato, increased *Bt* toxicity towards *H. zea* (Ludlum, Felton, & Duffey 1991). In contrast, rutin was not found to alter *Bt* toxicity towards *M. sexta* (Krischik, Barbosa, & Reichelderfer 1988). In studies on other entomopathogens, catecholic phenolics are thought to damage midgut cells (Hoover et al. 1998) and thereby influence virulence.

Despite being in the same family as tomato, little is known about pepper allelochemicals and their possible interaction with *Bt*. In one study, the crude ethanol extract of a *Capsicum annum* variety exhibited high contact toxicity towards *Tribolium confusum* (Williams & Mansingh 1993), demonstrating the presence of potent defensive compounds. Capsaicinoids, the phenols responsible for the pungency of hot peppers, are phytochemicals common to *Capsicum* species and may be part of pepper’s defensive chemistry (Estrada et al. 2002). In a study on the host range, *T. ni* growth was considerably reduced on pepper relative to other host plants tested (Sutherland 1966) suggesting that pepper leaves are a poor nutritional resource for *T. ni* growth.
In contrast, *T. ni* is known to have a specific behavioral adaptation (i.e. trenching) to avoid the waxy secretions produced by cucumber in response to herbivory, suggesting that *T. ni* has an evolutionary history of feeding on plants with defensive strategies similar to cucumber (Dussord & Denno 1994). Curcurbitacins, tetracyclic triterpendoids, are the primary defensive chemicals contained in cucumber and are known to deter herbivory by generalist herbivores. These compounds do not, however, deter feeding by *T. ni* (Tallamy et al. 1997). Therefore, the suitability of the three different host plants for *T. ni* growth and their effect on *Bt* are expected to vary considerably.
1.3 Thesis Objectives and Overview

The overall objectives of the present study were 1) to examine the incidence of Bt resistance in commercial greenhouse populations of *Trichoplusia ni*, 2) to determine if the inheritance of Bt resistance corresponded to a monogenic trait, and 3) to examine the influence of host plant on resistance associated fitness costs. The thesis is divided into seven chapters, the first of which is the current introduction, followed by five separate data chapters, each of which is formulated into journal manuscripts. The final chapter provides an overall summary of the research findings.

Chapter II presents the results of a three-year survey of Bt resistance in *T. ni* populations collected from commercial vegetable greenhouses in British Columbia, Canada. Two resistant *T. ni* populations (P5 and T2c) that were collected during the survey were established as laboratory colonies. These laboratory populations provided resistant individuals for the studies described in Chapters III-VI. In Chapter III, progeny of reciprocal F₁ crosses between a susceptible laboratory population and the P5 resistant strain were examined to determine the dominance of Bt resistance in the P5 population. The F₁ larvae and parental populations were then backcrossed to determine if Bt resistance corresponded to a monogenic trait in this population. A similar crossing scheme was followed with the P5 line at Cornell University. The results from the Cornell crosses were used to corroborate the findings in Chapter III. The results of both sets of crosses were then compared to expectations from single- and 2-locus models of inheritance in the present study.

In Chapter IV, the effect of host plant on the magnitude and dominance of resistance to Bt in resistant (Pᵣ), reciprocal hybrids (F₁f, F₁m) and susceptible (Pₛ)
genotypes derived from the T2c population was examined. The growth rates of the four T2c genotypes on untreated host plants were further examined in Chapter V to determine if resistance-associated fitness costs varied with the host plant. In Chapter VI, transgenerational effects associated with the different host plants and four genotypes were examined. In this chapter, the four genotypes (PR, PS, F1f, F1m) were derived from the P5 population. Each genotype was reared on three different host plants in the absence of Bt and the subsequent growth of the progeny generation in a common diet environment was examined. In the final chapter, the significance of the research findings are discussed with respect to the role of tritrophic interactions in the evolution of resistance to Bt in an insect herbivore.
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Chapter II: Bt resistance in greenhouse *T. ni* populations

2.1 Introduction

*Bacillus thuringiensis* (*Bt*) has been used successfully for over 30 years for the control of insect pests, but surprisingly only one species of target pest, diamondback moth, *Plutella xylostella*, has been shown to have evolved resistance to this microbial control in the field (Ferre & van Rie 2002). We report here the second occurrence of *Bt* resistance in an agricultural situation: the resistance of cabbage loopers, *Trichoplusia ni*, in vegetable greenhouses in British Columbia, Canada. In addition, we show that this resistance is rapidly lost when selection ceases, which implies that high costs are associated with this resistance.

The mode of action of *Bt* is based on the production of protein crystals that are toxic to particular insect groups. Due to its specificity and limited environmental impact, *Bt* has become the primary alternative to chemical insecticides for control of moth pests of forests and agriculture. The use of transgenic plants engineered to produce *Bt* endotoxins is on the rise globally. However, the continued use of both *Bt* sprays and *Bt* transgenic crops depends on preventing the evolution of resistance in target pest populations (Ferre & van Rie 2002). Resistance to *Bt* in field situations has been predicted from the results of laboratory experiments involving over 16 pest species for which resistance to *Bt* has been selected (Tabashnik 1994). As an example, the genetic potential for *Bt* resistance evolution in *T. ni* is demonstrated by the successful selection of laboratory populations for resistance (Estada & Ferre 1994). However, the predicted

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evolution of resistance has thus far not been borne out outside of the laboratory (Tabashnik 1994; Ferré & van Rie 2002).

The current lack of documented *Bt* resistance in the field may be due to an inherent instability of resistance in the absence of *Bt* exposure. Newly arisen resistance traits are often assumed to be associated with a fitness cost (Coustau, Chevillon, & ffrench-Constant 2000). This assumption arises from the observation that resistance genes are rarely fixed in populations and are often lost in the absence of the parasite or pathogen and the maintenance of genetic polymorphisms are thought to be due to counter balanced selection pressures (Coustau, Chevillon, & ffrench-Constant 2000). *Bt* resistance has been reported to decline in the absence of selection in a number of laboratory colonies (Hama, Suzuki, & Tanaka 1992; McGaughey & Beeman 1988; Sayyed & Wright 2001; Tabashnik, Finson, & Johnson 1991) and this decline has been attributed to fitness costs, such as decreased growth rate (Liu et al. 1999), survival (Groeters et al. 1994), or fecundity and mating success (Groeters et al. 1993) of resistant versus susceptible individuals in the absence of *Bt*. However, estimates of overall intrinsic growth rates of field derived resistant *P. xylostella* populations were not found to differ from susceptible populations (Sayyed & Wright 2001). Furthermore, no differences in survival or larval weight were found between resistant and susceptible *H. virescens* in the absence of *Bt* (Gould & Anderson 1991). Despite the uncertainty of fitness costs associated with resistance, many proposed resistance management strategies rely on their presence (Ferré & van Rie 2002; Tabashnik et al. 1994). It is, therefore, imperative to identify and measure if there are fitness costs for the development of appropriate resistance management strategies.
Commercial greenhouse vegetable growers in British Columbia, Canada, rely heavily on Bt for the control of cabbage loopers, T. ni, because it is compatible with other control agents. These greenhouse T. ni populations most likely originate from immigrants from field populations which enter through ceiling vents during summer months. T. ni moths can then cycle continuously throughout the growing season with multiple overlapping generations per year. Resistance has been detected in field P. xylostella populations that undergo multiple generations per year in regions such as Hawaii, Malaysia, Philippines, Florida, and Thailand (Tabashnik 1994). The relative containment of T. ni populations in large greenhouses may be highly conducive to resistance development. In another example of contained populations, Plodia interpunctella collected from Bt-treated grain bins were modestly more resistant (1.2-fold) to Bt than populations from untreated bins (McGaughey 1985).

The use of Bt-based sprays against T. ni provides an ideal environment for the evolution of resistance, and selection for resistance can be intense following multiple sprays of high concentrations of Bt used to control severe pest outbreaks. Following reports of poor Bt efficacy in commercial greenhouses, we surveyed Bt resistance in T. ni populations to ascertain whether resistance was indeed evolving. In addition, life history characteristics of T. ni were measured to determine if selection for resistance to Bt was associated with a fitness cost.
2.2 Materials and Methods

T. ni collection

We surveyed the Bt resistance of cabbage loopers in commercial vegetable greenhouses in the lower mainland of British Columbia between Vancouver and Abbotsford, 100 km to the east. We sampled greenhouses ranging in size from 7,000 m$^2$ to 81,000 m$^2$ with an average growing area of 44,800 m$^2$, that were reported to have T. ni infestations. Sample dates and collections are enumerated in Table 1. In 2001, three broccoli fields situated >1km from commercial greenhouses and treated with 1 or fewer Bt applications were also sampled for T. ni and assayed for comparison.

All T. ni larvae seen during the greenhouse visit were collected, placed in 473 ml paper cups with collected leaves and returned to the laboratory for rearing using a method modified from (Ignoffo 1963). T. ni larvae were reared in individual 30 ml cups containing 2 ml of wheat-germ-based artificial diet in a controlled temperature room at 26°C with a 16:8 (L:D) photoperiod. The number of larvae collected per greenhouse depended on the level of the infestation and the final number of parents depended on the proportion of collected larvae that pupated successfully in the laboratory (Table 1). Collected larvae were weighed at pupation (only in 2001-02) and placed into a cage for emergence and mating. Cages were supplied with paper toweling for oviposition and a 10% sucrose solution contained in 30 ml plastic cups with cotton wicks. Once the first eggs were laid, egg sheets were harvested every 2 days until less than 10 adults remained in the cage. Egg sheets were maintained at 4°C until use for a maximum of 12 days in 2000. In 2001 and 2002, eggs were only stored for 7 days due to lower observed fertility of eggs stored longer than 7 days in 2000. In the majority of collections, development
time to pupation of collected larvae differed by less than 7 days. To account for any differences in resistance due to the parental lifestage at collection, multiple egg sheets from throughout the egg laying period were assayed per population.

*T. ni* larvae from a laboratory colony that had been maintained for > 10 years without exposure to *Bt* were used to initiate a new laboratory colony to serve as an unselected reference for the greenhouse populations. The lab colony was susceptible to *Bt* and was assayed 10 times over the three years with LC₅₀ values ranging from 0.9 to 5.5 kInternational Units/ml diet and a mean ±s.e.m. of 2.2±0.4 kIU/ml diet. In the remainder of this paper, different greenhouses are designated by crop (T = tomato, P = pepper, C = cucumber, and B = broccoli fields), greenhouse number and year.

**Bioassays**

Five day old first generation larvae obtained from parents collected as larvae from greenhouses were used for bioassays. *Bt* concentration mortality assays were performed by incorporating *Bt* (DiPel, Abbott laboratories) into freshly made artificial diet. DiPel was diluted serially in distilled water and mixed in a 1:10 ratio (*Bt* solution:diet) with diet that had cooled to 50°C. Two ml of diet plus *Bt* was dispensed into 30 ml plastic cups and one larva was placed into each cup. Each population was assayed at five different doses ranging from 2.5 to 320 kIU/ml diet and, if possible, assays were repeated a minimum of two times (Table 1).

The number of concentrations tested for each population varied depending on the number of larvae available. A minimum of 3 concentrations was tested in any one replicate of the assay per population, with 10 or more larvae per concentration. The number of larvae tested per population is presented in Table 1. Larval mortality was
observed 3 days following the experimental set-up. Mortality was assessed visually and any suspect larvae were prodded gently with a toothpick to ensure a correct evaluation. Five day old larvae in the control treatment were weighed at the time of the experimental set-up.

Five resistant greenhouse populations were maintained in the laboratory in the absence of *Bt* exposure on artificial diet. Two hundred larvae were maintained per generation. For each generation, eggs were chosen from the peak of the egg laying period when the largest number of adult moths were present. Populations were re-assayed for *Bt* resistance following 3, and either 7 or 8 generations of laboratory rearing. The rate of decrease of resistance (R) was calculated as described in Tabashnik (1994) with respect to the LC$_{50}$ of the first generation larvae and the LC$_{50}$ of subsequent generations where the inverse of R is the number of generations required for a 10-fold change in LC$_{50}$.

*Statistical Analysis*

Probit analysis was performed using Genstat 5 (Rothamstead Experimental Station 1998) to calculate the LC$_{50}$ and 95% fiducial limits for each greenhouse population. The probit analysis procedure in Genstat utilizes methods outlined in Finney (1971). The average mortality in the control treatment groups was less than 5% after 3 days and assays with greater than 20% control mortality at this assessment were not included in the analysis. LC$_{50}$ ratios are reported for 3 days of feeding unless otherwise stated and calculated with respect to the mean LC$_{50}$ of the reference laboratory colony (LC$_{50}$=2.2 kIU/ml diet). The 3 day assessment was reported preferentially since mortality due to other factors became apparent after 6 days of feeding.
Mean parental pupal weights and mean first generation 5 day larval weights were regressed against log transformed population LC$_{50}$ (JMPIN 4.0). Populations with high mortality in the control treatment group (>40%) at day 6 following the experimental set-up were discarded from the pupal and larval weight analyses due to potential sublethal effects of disease such as nuclearpolyhedrovirus infections on growth and pupal size. Larvae that hatched from eggs stored for longer than 10 days (in 2000) were also discarded from the larval and pupal weight analyses as prolonged storage at 4°C decreased larval growth rates (Milks 2002). Pupal weights of populations treated with chemical insecticides in the greenhouse were also not included due to potential sublethal effects. Pupal weights and larval weights were compared between collection dates of the same greenhouse populations using t-tests (JMPIN 4.0). Mean LC$_{50}$s of field and untreated greenhouse populations were compared using multiple comparison procedures (student t’s in JMPIN 4.0). LC$_{50}$ results are reported as mean ±95% fiducial limits unless otherwise indicated.
2.3 Results

Surveys for Bt resistance

In each of the survey years, populations of T. ni were found that were significantly more resistant to Bt than the reference laboratory colony (Figure 1). All sampled greenhouse populations that had been treated with Bt displayed elevated levels of resistance. Among the collections, the resistance ratio (greenhouse LC50/lab colony LC50) varied by more than 100-fold (Table 2.1). Growers of greenhouses that harboured the two most resistant populations in 2000, the three most resistant populations in 2001, and the two most resistant populations in 2002, had reported poor Bt efficacy.

The field populations of T. ni sampled in 2001 had a mean LC50 of 4.7±1.6 kIU/ml diet which did not significantly differ from the reference colony (Figure 2.1). Over the three survey years, 8 greenhouse populations had not been treated with Bt prior to the initial collection of the growing season. Collections of T. ni larvae from the same greenhouse in separate growing seasons were treated as separate populations. This assumption seems reasonable since at the end of each year greenhouse crops are removed and structures are cleaned and fumigated to eradicate any insects. Five of the untreated populations surveyed had similar resistance levels to the sampled field populations with a mean LC50 of 7.4±1.2 kIU/ml diet but had significantly higher resistance than the reference lab colony. The remaining three untreated populations had resistance levels that were higher than the field populations with a mean LC50 of 37±7.5 kIU/ml diet. The most resistant untreated population in 2001 (P5) was physically located between two treated greenhouses (P6 and C5a) with similar resistance levels suggesting that resistant moths immigrated into P5.
Figure 2.1: LC$_{50}$ and fiducial limits of $T. ni$ populations collected from greenhouses 2000-02. Greenhouse populations treated with $Bt$ are shown as $\bullet$, and $\diamond$ when untreated, field populations as $\square$, and the laboratory colony as $\circ$. 
Table 2.1: Summary of the surveys of *T. ni* greenhouse population for *Bt* resistance survey. The number of collected larvae pupating in the laboratory (parental number), the number of first generation larvae assayed, and the number of assays per greenhouse collection are listed. Greenhouse collections are represented by greenhouse crop (C=cucumber, P=pepper, T=tomato, B=broccoli (field)), year and date of collection.

<table>
<thead>
<tr>
<th>Year</th>
<th>Greenhouse</th>
<th>Sampling Date</th>
<th># Parents</th>
<th>Total assayed</th>
<th># assays</th>
<th>Ratio <em>a</em></th>
<th>Res. Probit Slope (±s.e.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>C1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8/30</td>
<td>76</td>
<td>589</td>
<td>7</td>
<td>14</td>
<td>0.86 ±0.01</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>8/15</td>
<td>94</td>
<td>292</td>
<td>3</td>
<td>26</td>
<td>0.55 ±0.11</td>
</tr>
<tr>
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<td>C3</td>
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<td>69</td>
<td>243</td>
<td>4</td>
<td>23</td>
<td>0.74 ±0.15</td>
</tr>
<tr>
<td></td>
<td>C4</td>
<td>8/10</td>
<td>68</td>
<td>181</td>
<td>2</td>
<td>12</td>
<td>0.75 ±0.16</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>6/23</td>
<td>36</td>
<td>413</td>
<td>4</td>
<td>47</td>
<td>0.69 ±0.09</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>6/29</td>
<td>31</td>
<td>364</td>
<td>3</td>
<td>16</td>
<td>1.10 ±0.18</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>6/19</td>
<td>16</td>
<td>218</td>
<td>2</td>
<td>15</td>
<td>0.56 ±0.11</td>
</tr>
<tr>
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<td>8/16</td>
<td>133</td>
<td>403</td>
<td>4</td>
<td>25</td>
<td>0.56 ±0.09</td>
</tr>
<tr>
<td></td>
<td>T1</td>
<td>8/24</td>
<td>97</td>
<td>245</td>
<td>4</td>
<td>55</td>
<td>1.13 ±0.38</td>
</tr>
<tr>
<td>2001</td>
<td>P4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8/1</td>
<td>43</td>
<td>364</td>
<td>3</td>
<td>4</td>
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* a ratios were calculated relative to an LC<sub>50</sub> of 2.2 kIU/ml diet for the reference population
  * b no Bt sprays prior to larval collection of *T. ni* within the year indicated
  * c treated with a chemical insecticide one week prior to collection
Four of the treated greenhouse populations (T2-2001, T2-2002, T3-2002, C5-2001) were sampled repeatedly within each growing season. (As mentioned earlier, larval collections from the same greenhouse in different years were treated as separate populations.) Three of these populations were frequently treated with *Bt* for the entire growing season and this was reflected in significant increases in the LC$_{50}$s from the first to the last collections (Figure 2.2). The fourth population had become uncontrollable with *Bt* and was treated with a chemical insecticide. Larvae were collected a week following the chemical application and the LC$_{50}$ was 5-fold lower than that of the initial collection (Table 2.1). Four of the 8 untreated greenhouse populations were also sampled multiple times within a growing season. Three of these remained untreated and displayed no change in LC$_{50}$ between collection dates (P4-01, T1-01, T4-01) (Figure 2.2). Following the initial collection, the remaining untreated *T. ni* population (C4-2002) increased beyond economic threshold levels which necessitated *Bt* treatment. After several *Bt* applications, the population was re-sampled and the LC$_{50}$ had significantly increased from the initial collection.

Three greenhouse populations were sampled toward the end of 2000 following *Bt* applications and again in 2001 prior to any *Bt* treatments. The change in LC$_{50}$ between years was examined to determine if *Bt* resistance was carried over between years. The LC$_{50}$s changed from 54 to 12, 25 to 7 and from 122 to 7 kIU/ml diet for the three greenhouse populations (P4, C4 and T1 respectively). All populations declined to resistance levels equivalent to that of the field populations sampled in 2001. Growers experienced poor economic conditions at the beginning of 2001 due to high gas prices
Figure 2.2: The change in LC$_{50}$ over time of greenhouse $T. ni$ populations in 2001-02.

Populations with solid lines were treated with $Bt$ and populations with dashed lines were not. LC$_{50}$ values plus 95% fiducial limits were calculated using Probit analysis in Genstat 5.
and many delayed the planting of their crops by up to four weeks which may have affected the survivorship of *T. ni* between years.

To determine if the assayed LC$_{50}$ was related to grower management practices, the total amount of *Bt* applied prior to the first larval collection in each growing season was regressed against the population LC$_{50}$ at the time of the first collection. The quantity of International Units per hectare was calculated for each *Bt* application by converting kg applied to IU for both *Bt* formulations used by growers (16 x $10^9$ IU/Kg Dipel and 10.6 x $10^9$ IU/L Foray 48B, Abbott laboratories) from grower reported application rates in kg/ha or L/ha for Dipel or Foray respectively. The total amount of *Bt* applied prior to the larval collection per greenhouse was significantly related to the *T. ni* resistance level identified in the *Bt* dose-response assays ($R^2=0.78$, df=1, p=0.0086) (Figure 2.3). Only initial collections per greenhouse per growing season were included in the analysis to avoid pseudo-replication. No significant difference was found in the relationship between the amount of *Bt* applied and the assayed LC$_{50}$ among the different years (df=2, p=0.66) and therefore year was not included in the overall analysis.

*Stability of Bt Resistance*

In the absence of *Bt* exposure in the laboratory, resistance declined in all 5 established colonies (Figure 2.4). The two most resistant of these colonies were initiated from separate collections in 2001 from the same greenhouse. Resistance declined rapidly in one colony from 248 to 4.2 kIU/ml diet (T2c-2001) after 7 generations with a rate of decline of −0.25 which indicates that 4 generations were required for a 10-fold decrease in LC$_{50}$. In the other colony established from the 2$^{nd}$ collection from T2 in 2001 (T2b-2001), resistance decreased less than 2-fold from 199 to 147 kIU/ml diet in 2 generations.
Figure 2.3: The relationship between the Bt dose-response assay LC$_{50}$s of the first generation offspring of greenhouse collected $T. ni$ and the total amount of Bt applied in the greenhouse. Only the results of the collections conducted between May and September and the first collection per greenhouse per year are included to avoid pseudoreplication. Populations with high mortality in the control treatment group were not included in the analysis.

\[ R^2 = 0.80 \]
Figure 2.4: Decline in resistance of greenhouse *T. ni* populations reared in the laboratory in the absence of *Bt* exposure. LC$_{50}$s and 95% fiducial limits are shown.
(R= -0.05) and subsequently died out prior to 8 generations. A third line established from the same greenhouse (T2a-2002) in 2002 had an LC$_{50}$ after the first lab generation of 104 kIU/ml diet which declined to 3 kIU/ml diet after 2 generations for a rate of decline of −0.80 or a 10-fold decrease in LC$_{50}$ in less than 2 generations.

The remaining two of the five colonies were established from two separate resistant greenhouse populations. One colony (C4-2001) was resistant at an LC$_{50}$ of 86 kIU/ml diet and declined to 63 kIU/ml diet after 2 generations for a rate of decline of −0.07 (10-fold decrease in 15 generations). The final colony (P5) was moderately resistant at establishment with an LC$_{50}$ of 50 kIU/ml diet and declined to 8 kIU/ml diet after 2 generations (R= -0.40) and to 4.3 kIU/ml diet after 7 generations (R= -0.15).

**Pupal and Larval Weights**

Parental pupal weights were significantly associated to log-transformed population LC$_{50}$ (R$^2$=0.91) (Figure 2.5a). The mean pupal weight (± s.e.m.) of the most resistant population was 199±4 mg, over 20% lower than the mean pupal weight of the untreated populations of 256±2 mg. Pupal weights were observed to decrease by 17% between collections for two greenhouse populations that changed in resistance from 96 to 248 kIU/ml diet (T2-2001) and 104 to 352 kIU/ml diet (T2-2002) (t=12.9, df=380, p<0.0001 and t=7.9, df=275, p<0.0001). For three untreated greenhouse populations that exhibited no change in LC$_{50}$ over time, pupal weights were stable with a 0-5% change between collections.

Weights of first generation larvae at 5 days were also negatively related to population resistance levels (R$^2$=0.61, slope=−0.0027, p=0.001) (Figure 2.5b). The mean larval weight of the most resistant population (LC$_{50}$>100,000 IU/ml diet) was 3.45±0.05
mg. Moderately resistant (30,000<LC₅₀<65,000 IU/ml diet) mean larval weights were 5.0±0.6 mg, as compared to the mean larval weights of non-resistant colonies (LC₅₀<12,000 IU/ml diet) of 7.2±0.8 mg.
Figure 2.5: Relationship between (a) mean pupal weights of collected larvae and (b) mean 5-day larval weights of offspring and the population LC$_{50}$. Only the results of the last collection per greenhouse per year are included to avoid pseudoreplication. Values of $r^2$ were calculated for a second-order polynomial equation ($y=0.394-0.031x-0.024(x-4.602)^2$, $F=46.7$, $P=0.0001$) for pupal weights and first-order polynomial equation ($y=17.8-2.75x$, $F=18.57$, $p=0.001$) for 5 day larval weights using JMPIN 4.0.
2.4 Discussion

Resistance to *Bt* clearly develops in cabbage loopers in commercial vegetable greenhouses in response to grower spray regimes. The rate of resistance development was similar between years and resistance alleles are apparently widespread in surrounding field populations and in the founding populations in greenhouses. Thus far, most cases of *Bt* resistance in Lepidoptera appear to be primarily associated with an autosomal, recessive allele with an average estimated frequency of 0.001 to 0.0015 in field populations (Reviewed by Ferre & van Rie 2002). One might predict relatively high frequencies of resistance alleles among founding populations of *T. ni* in greenhouses, since resistance develops rapidly in these populations and they are likely to have been initiated by a small number of immigrants.

Resistance allele frequencies are often estimated through the use of a diagnostic dose that causes 99% mortality of a susceptible reference population (ffrench-Constant & Roush 1990). As the variation in the susceptibility of different laboratory strains and unexposed field populations is considerable (Gonzalez-Cabrera et al. 2001, Robertson et al. 1995) it is often difficult to decide on the appropriate diagnostic dose (Marcon et al. 1999). In the present study, a dose of 40 kIU/ml diet was sufficient to kill 99% of the reference laboratory colony and no survivors were found at 80 kIU/ml diet. Given that reports of poor *Bt* efficacy corresponded to populations with LC₅₀s at 48 kIU/ml diet or greater, 48 kIU/ml diet or higher may be a suitable diagnostic dose. By combining the assay results of the three sampled field populations, 4 larvae out of 100 (4%) and 8 larvae out of 160 (5%) survived at 80 and 40 kIU/ml diet respectively. Therefore, in the most simplistic case if the resistant trait was due to a single recessive allele then the allele
frequency may be 0.20 in the wild population when using a discriminating dose of 80 kIU/ml diet. Since foliar *Bt* applications contain a variety of *Bt* toxins, it seems likely that resistance may be due to more than one gene and this assumption may not be valid. However, high allele frequencies in the invading *T. ni* populations would further explain the rapid increase in *Bt* resistance in response to selection pressure and therefore more work is needed to address this issue. High allele frequencies of resistance alleles are not unheard of. In field populations of *Pectinophora gossypiella* in Arizona, frequencies were estimated to be as high as 0.18 (Tabashnik et al. 2000).

Various physiological mechanisms associated with the steps in the mode of action of the *Bt* toxin proteins could be associated with resistance (Taylor & Feyereisen 1996, Ferré & van Rie 2002). These include solubilization, proteolytic processing, passage through the peritrophic membrane, receptor binding, membrane insertion, pore formation, and osmotic lysis of midgut cells. Due to this complexity in the mode of action of *Bt*, a variety of associated fitness costs are possible. The major mechanism observed in field derived, resistant *P. xylostella* populations is alteration in the binding of *Bt* toxins to the gut receptor molecules (Ferré & van Rie 2002). Altered target sites could induce deleterious effects due to the disruption of pre-existing pathways (Uyenoyama 1990).

The presence of resistance-associated fitness costs should result in counterselection in the absence of *Bt* and a subsequent decline in resistance. This supposition was borne out as resistance declined rapidly in three field derived resistant colonies, whereas two other highly resistant colonies exhibited limited decreases in resistance. The initial high resistance levels and slow decline of resistance in these colonies suggests that they may have had a high frequency of resistance alleles and
therefore few or no susceptible individuals. Both of these colonies subsequently died after 4 generations of laboratory rearing due to poor fecundity and reduced growth that may have been caused by negative pleiotropic effects.

Two indicators of reduced fitness of resistant *T. ni* are slower larval growth and smaller pupal size. The mean pupal weight of the most resistant strain was 20% lower than the mean pupal weight of untreated populations and larval weights were 49% smaller. Since pupal weights in *T. ni* are proportional to fecundity (Milks, Burnstyn, & Myers 1998), any decrease in pupal weight would confer a negative fitness effect. Furthermore, a decrease in growth rate will increase the period in which larvae are vulnerable to predators, diseases, and weather further affecting fitness (Gould, Kennedy, & Johnson 1991). Therefore, the decrease in pupal weight and larval growth rates observed with increasing resistance demonstrate that *Bt* resistance in *T. ni* populations is associated with severe deleterious pleiotropic effects and this provides an explanation for the lack of resistance stability in the resistant colonies.

It is possible that sublethal effects or maternal effects due to prior exposure to *Bt* are responsible for the observed negative relationship between the growth rates of offspring and LC50 of the parental greenhouse population. It is known that *Bt* is a feeding inhibitor that can reduce growth rates and pupal weights (Salama & Sharaby 1988). However, sublethal effects were not observed to affect pupal weights of spruce budworm, *Choristoneura fumiferana* (Ramachandran et al. 1993), and were absent or in fact opposite in resistant individuals feeding on *Bt* or *Bt*-transgenic crops (Ramachandran et al. 1998; Gould et al. 1995). Few studies have tested potential maternal effects due to *Bt* exposure. However in one study, strains of pink bollworm, *Pectinophora gossypiella*,
with different levels of resistance showed no maternal effects on development time and larval weight (Carrière et al. 2001). In a preliminary study, we compared the growth characteristics between a colony initiated from a resistant population in 2001 that had reverted to susceptibility, and a hybrid of the susceptible colony and its sister colony that had been selected to maintain resistance. Parents of the hybrids were grown for one generation without exposure to \textit{Bt} to reduce any potential sublethal effects. Maternal effects were reduced by examining hybrid offspring of matings between reverted susceptible females and resistant males. Mean hybrid pupal weights (± s.e.m.) (217 ± 6.7 g) were significantly smaller than those from the susceptible colony (250 ± 5.7 g; t = 3.6, df = 43, p < 0.001) which supports the existence of a resistance correlated fitness cost rather than sublethal effects. In contrast, resistant \textit{P. xylostella} derived from Malaysian field collections exhibited increased growth rates and larger pupal weights relative to unselected subpopulations (Sayyed & Wright 2001).

Despite the uncertainty of resistance-associated fitness costs, many proposed resistance management strategies, particularly those for \textit{Bt} transgenic crops, rely on their presence. Using insecticidal rotations (Ferré & van Rie 2002) or temporal refuges (periods with no \textit{Bt} exposure) (Tabashnik et al. 1994) as management strategies require that resistance declines when selection ceases. Given that resistance to \textit{Bt} rapidly declined in several of the resistant populations studied here and that a decrease in resistance was correlated with the use of an insecticidal spray, the use of insecticidal rotations may be an important tool in managing \textit{Bt} resistance in greenhouse \textit{T. ni} populations.
Populations of *T. ni* in greenhouses are likely to be initiated each year from a small number of individuals either having survived overwinter in the greenhouse or having immigrated from field populations. The presence of resistance genes in either wild or overwintered populations will allow the rapid development of resistance to be a continuing occurrence. Deleterious fitness costs associated with *Bt* resistance in *T. ni* populations would be predicted to cause the resistance of moths in both greenhouses and field populations to decline in the absence of *Bt* sprays. However, the estimate of relatively high frequencies of resistance alleles in wild populations doesn’t support this prediction. Either the frequency of resistance alleles is overestimated or fitness costs are not as deleterious in the wild as in the laboratory. The continued selection for *Bt* resistance in greenhouses may lead to selection of resistance alleles with minimal pleiotropic effects or modifier genes that could ameliorate fitness costs and thus could stabilize resistance in the absence of *Bt* applications (Roush & McKenzie 1987). These possibilities put at risk the long term viability of both foliar *Bt* applications and *Bt* transgenic crops.
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Chapter III: Inheritance of Bt resistance in T. ni

3.1 Introduction

Insecticides based on Bacillus thuringiensis are presently the most widely used natural insecticides in the world against Lepidopteran pests. However, the potential for resistance to develop in targeted insect populations is a continual threat to the long term use of B. thuringiensis based products. The first report of resistance to B. thuringiensis subsp. kurstaki (Bt) outside of the laboratory was of diamondback moth populations, Plutella xylostella, in Hawaii (Tabashnik et al. 1990). Within a decade resistance to Bt in P. xylostella has been observed worldwide (Ferre & van Rie 2002). Recently, resistance to Bt was found in commercial greenhouse populations of the cabbage looper, Trichoplusia ni (Janmaat & Myers 2003). This finding provides a unique opportunity to compare the inheritance of a newly evolved resistant trait in T. ni to the inheritance of resistance in P. xylostella.

In order to devise strategies to delay the evolution of resistance to Bt, knowledge of the genetic inheritance of Bt resistance is required. The most widely publicized resistance management strategy is the high-dose/refuge strategy that has been employed in conjunction with the planting of transgenic crops expressing B. thuringiensis toxins (Shelton et al. 2000). The success of this strategy depends on a variety of assumptions, one of which is that the resistance trait in the insect population is recessive at the dose expressed by the transgenic plant (McGaughey & Whalon 1992; Tabashnik 1994). Furthermore, resistance management strategies such as the use of toxin mixtures or

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2 This chapter has been accepted for publication in Applied and Environmental Microbiology and is co-authored with Judith Myers, senior supervisor, and Ping Wang, Wendy Kain, and Jian-Zhou Zhao from Cornell University.
rotations with different toxins are more likely to succeed if the inheritance of resistance to each toxin is recessive (Roush 1989; Tabashnik 1989).

Dominance relationships are measured in a variety of ways, the most common of which is the comparison of dose-mortality curves of susceptible homozygous, resistant homozygous and heterozygous individuals (Bourguet, Genissel, & Raymond 2000). By using this method, the inheritance of resistance to Bt products or toxins in the diamondback moth varied from being almost completely recessive to partially recessive (Ferré & van Rie 2002). However, resistance to Bt in a laboratory population of Ostrinia nubilalis was incompletely dominant (Huang et al. 1999) and resistance to the Cry1Ab toxin of Bt in Heliothis virescens was found to be codominant (Sims & Stone 1991). These exceptions demonstrate that species-specific knowledge of the inheritance of Bt resistance is required to devise appropriate resistance management strategies.

Much debate has centered on the role of monogenic or polygenic traits in the evolution of resistance to insecticides in the field (McKenzie 1996). The majority of the examples of field-evolved resistance to synthetic insecticides involve monogenic traits (Roush & McKenzie 1987; Mallet 1989). It is, therefore, commonly assumed in resistance management strategies that resistance is due to one gene with a susceptible and resistant allele (Tabashnik 1986). Unlike synthetic insecticides, foliar insecticides based on B. thuringiensis are composed of a suite of bacterial toxins (Hofte & Whiteley 1989). Therefore, it is possible that resistance to Bt may arise due to a suite of genes as opposed to a single monogenic response.

In previous studies, monogenic models of Bt resistance correspond fairly well to backcross data. For example, studies of the inheritance of Bt resistance in P. xylostella
(Tabashnik et al. 1992; Tang et al. 1997; Gould et al. 1995; Sayyed, Ferré, & Wright 2000) and in *Ostrinia nubilalis* (Huang et al. 1999) were consistent with monogenic models of resistance. However, exceptions have been noted in resistance to the individual *Bt* toxin Cry1Ab in a laboratory colony of *H. virescens* (Sims & Stone 1991) and in a field-derived strain of *P. xylostella* (Sayyed & Wright 2001). Similar exceptions have been noted in resistance to the *B. thuringiensis aizawai* toxin Cry1C in *P. xylostella* (Shelton, Zhao, & Roush 2002) and Cry1Ca in *Spodoptera littoralis* (Chaufaux et al. 1997). These exceptions further emphasize the need for knowledge of the genetic inheritance of *Bt* resistance in *T. ni* to develop a species’ specific or even population specific resistance management strategy.

In the present study, the inheritance of *Bt* resistance in a *T. ni* colony derived from a commercial vegetable greenhouse population was examined. Reciprocal F₁ crosses between a susceptible laboratory population and a resistant strain were performed to examine the dominance of *Bt* resistance. F₁ larvae and parental populations were backcrossed to determine if *Bt* resistance corresponded to a monogenic trait.
3.2 Materials and Methods

Insects

To study the genetic inheritance of Bt resistance in T. ni, a susceptible colony obtained from laboratory culture was crossed with a field-derived resistant colony. These crosses were conducted simultaneously at two separate locations (University of British Columbia (UBC), Canada and Cornell University (Cor), New York) to compare inheritance results between laboratories. Two different susceptible laboratory colonies were used at the two different sites (P$_S$-UBC, P$_S$-Cor), and both were obtained from laboratory colonies that had been reared in the absence of Bt for over 10 years. The susceptible colonies were obtained from the Department of Plant Sciences, UBC and the Department of Entomology, Geneva, Cornell University.

The resistant T. ni colony (P$_R$) was initiated from 74 individuals collected from a commercial greenhouse in British Columbia (labeled P5 in our previous paper (Janmaat & Myers 2003), Canada in 2001 and showed 24-fold higher resistance to Bt than a reference susceptible laboratory colony. In the 4$^{th}$ generation of laboratory culture in the absence of Bt, two lines were initiated at UBC. One line was subjected to selection with Bt each generation (P$_R$-UBC) and the other was reared without any Bt exposure (unsel$_1$-UBC). Selected lines were exposed to the Bt formulation DiPel WP (Abbott Laboratories), a product used in commercial vegetable greenhouses, which contained 16,000 International Units (IU) per mg. International units are a standardized method of indicating Bt activity. In the US, the standard Bt serotype (HD-1-S-1971) is assigned the value 18,000 IU as it is 18 times more effective against T. ni than the French standard (E-61) which was assigned 1,000 IU (Burgerjon & Dulmage 1976). The unsel$_1$-UBC line
died out after 7 generations in the laboratory due to disease. At this point, another unselected line (unsel2-UBC) was initiated from the P_R-UBC colony.

After six generations of selection at UBC, eggs of the P_R-UBC colony were shipped to Cornell and an additional selected (P_R-Cor) and unselected line (unsel-Cor) were initiated from the original resistant line. At Cornell, the lines were selected with a DiPel 0.86% WP formulation (Bonide) that contained 4,320 IU per mg. At UBC, unselected T. ni larvae were reared in groups of 15 larvae in 175 ml styrofoam cups on a wheat-germ based diet at 26°C with a 16:8 (L:D) photoperiod using methods described in (Janmaat & Myers 2003). A minimum of 200 larvae were reared each generation. Selected larvae were reared under similar conditions for 5 days prior to selection. Similarly at Cornell, T. ni larvae were reared in 480 ml Styrofoam cups with 80 ml high wheat-germ based diet with 35 larvae per cup (Bell et al. 1981). A minimum of 150 unselected larvae were reared for each generation. Cups were kept in an environmental chamber at 27-29°C, 50% RH and a photoperiod of 16:8 h (L:D).

Selection and Survival Bioassays

P_R-UBC was selected for resistance by placing groups of 20-25 five-day-old larvae (2nd and 3rd instars) onto 10 ml of diet mixed with a Bt (DiPel WP) dose contained in a 175 ml styrofoam cup. All live larvae were transferred to new diet without Bt after 2 days. Survivorship was recorded at pupation, and pupae were collected and pooled in a mating cage to produce progeny for the next generation (Table 1). At each generation, 500-1000 larvae were selected. At Cornell University, selection was performed on neonates with a diet overlay assay. In each cup, 2 ml of a DiPel solution were distributed
over the diet surface. The concentration of DiPel was 10 to 80 kIU/ml diet in the second to ninth generation of rearing at Cornell.

At UBC the susceptibility of 5 day old larvae to Bt was assessed by incorporating Bt into the artificial diet using methods described by Janmaat and Myers (2003). All assays were performed with a minimum of 5-7 doses ranging from 1.25-160 kIU/ml diet depending on the expected resistance level and a control. Twenty to forty larvae were assayed per dose for each bioassay. Larval mortality was observed 3 days following the experimental set-up. At Cornell University, a modified diet overlay assay method (Zhao et al. 2002) was used to test the susceptibility of neonates to Bt. Five to six concentrations plus a control and four cups for each concentration were included in each bioassay. Ten neonates were transferred into each cup. Cups were covered with lids and held at 27± 1°C, 50 ± 2% RH, and a photoperiod of 16:8 h (L:D) for 4 days to determine mortality or growth inhibition. Preliminary results at Cornell indicated that growth inhibition (neonates reaching second instar after 4 days) was a better indicator of neonate susceptibility to Bt than mortality. Therefore, IC50 values are reported for Cornell assays and LC50 values are reported for UBC assays.

Inbred Lines

After 3 rounds of selection at UBC, twenty-two pairs were chosen at random from the PR-UBC colony and were then sib-mated for 3 generations to create inbred lines. Inbred lines were established to increase homogeneity for genetic analysis as the assumption of homozygous parental lines is critical to the determination of inheritance from F1 and backcross generations. Pupae were sexed and single pairs were placed into 16 oz paper cups supplied with 10% sugar solution and lined with paper towelling for
oviposition. Of the twenty-two crosses, 17 produced sufficient viable offspring for bioassays. The LC$_{50}$ per pair was assayed over 3 subsequent generations. Several inbred lines exhibited poor fecundity and were not able to be maintained.

To further ensure that few susceptible alleles remained in the inbred lines, any line that exhibited a decrease in LC$_{50}$ between generations or had an evident plateau in the concentration-mortality line was assumed to contain susceptible genes and was terminated. The presence of a plateau at concentrations lower than the family LC$_{50}$ indicated that the inbred line was not genetically homogenous. Five inbred lines [6, 12, 13, 16, 17] exhibited stable resistance over 3 generations and adequate fecundity and four of the five lines were used for the genetic analysis [6, 12, 13, 17]. The inbred lines [6, 12, 13, 16] were maintained without Bt exposure for 14 generations and repeatedly assayed after 6, 10 and 14 generations to examine resistance stability.

**Analysis of Inheritance**

To examine maternal effects, sex linkage and dominance, F$_1$ larvae from reciprocal crosses between susceptible and resistant lines were tested. At Cornell, F$_1$ larvae from reciprocal mass crosses (50 pupae per sex) between the P$_S$-Cor and selected P$_R$-Cor strains were assessed. Whereas at UBC, the inheritance of resistance was examined in reciprocal single-pair crosses between the four resistant inbred lines and the P$_S$-UBC strain. Pupae were obtained following three generations of sib-mating from each inbred line and were paired with P$_S$ -UBC pupae to produce F$_1$ hybrids. To examine the number of factors involved in resistance, hybrid larvae were backcrossed to parental resistant lines (F$_1$ x P$_R$) at both locations and to P$_S$ at UBC. Progeny of mass crosses
between 75 F1 females and 50 resistant males were tested for susceptibility to *Bt* at Cornell and progeny of single-pair crosses were assayed at UBC.

At UBC, two single pairs per inbred line were crossed for each of two reciprocal backcrosses. All pairs producing sufficient numbers of viable offspring were assayed. For the F1 x P_R backcross, 3 pairs each for line 6 and 12 were assayed, 2 pairs for line 13, and 1 pair for line 17 were assayed. For the F1 x P_S-UBC backcross, 2 pairs were assayed for line 6, 12 and 17, and 3 pairs were assayed for line 13. In addition, five single-pair crosses within two of the inbred lines (resistant x resistant) were performed to examine any remaining variation in resistance in the inbred lines.

**Data Analysis**

LC\(_{50}\) values and slopes of concentration-mortality lines were estimated using the probit analysis procedure in Genstat 5 Release 4.1 (Rothamsted Experimental Station 1998) at UBC. The POLO program (LeOra software 1997) was used for probit analysis of dose-response data (Russell, Robertson, & Savin 1977) at Cornell University. Mortality was corrected using Abbott's formula (Abbott 1925) for each probit analysis. In the UBC assays, no mortality was recorded in the majority of the control treatment groups and if mortality occurred it was less than 5%. The LC\(_{50}\) values of different crosses or genetic lines were considered significantly different if their 95% fiducial limits did not overlap. Resistance ratios were calculated by dividing the LC\(_{50}\) of the strain by the LC\(_{50}\) of the respective P_S population. LC\(_{50}\) values were rounded to the nearest hundredth. All LC\(_{50}\) or IC\(_{50}\) values in the text and tables are represented as kIU/ml diet or water.
Deviance statistics were used to test for differences in mortality over the dose range between groups using the accumulated display setting in Genstat 5. Deviance ratios (devratio) and approximate Chi-square probabilities are shown in the text. To test for dominance, responses of F₁ offspring were compared to the parental resistant family and the susceptible parent. Dominance was estimated as described in (Liu & Tabashnik 1997) where the estimation of dominance (D) based on the LC₅₀ was used (Stone 1968). D ranges from -1 (completely recessive) to 1 (completely dominant).

Indirect methods based on estimated mortalities from normal distributions with the mean and standard deviation corresponding to the LC₅₀ and reciprocal of the probit slope respectively of different genotypes were used to compare responses of backcross progeny to responses predicted from models with 1, or 2 loci (Tabashnik et al. 1992; Tabashnik et al. 2002). The assumptions of the models were 1) each locus had one resistant and one susceptible allele and 2) the parental susceptible and resistant strains (Pₛ and Pᵣ) were homozygous for susceptible or resistant alleles respectively.

Additional monogenic models with non-homozygous parental lines were examined where the frequency of the resistant allele p varied from 0.5 to 1.0 in the Pᵣ population or from 0 to 0.3 in the Pₛ population in increments of 0.05. The expected proportion of susceptible, hybrid and resistant genotypes were estimated for the F₁ and subsequent backcross generations and utilized to adjust the expected backcross LC₅₀ in the following 3 scenarios.

**Case 1:** The expected genotypic frequencies from a monogenic model in the F₁ x Pᵣ backcross generation with a non-homozygous Pᵣ and a homozygous susceptible Pₛ line where R = resistant allele and S=susceptible allele were:
Frequency RR genotype = P = \( \frac{1}{2} p^2 \)

where p = Freq(R) and q = Freq(S) in PR

Frequency RS genotype = H = \( \frac{1}{2} (3 - 2p) p \)

Frequency SS genotype = Q = \( \frac{1}{2} q (1 + q) \)

**Case 2:** The expected genotypic frequencies from a monogenic model in the F1 x Ps backcross generation with a non-homozygous PR and a homozygous susceptible Ps line were:

\[ P = 0 \]
\[ H = \frac{1}{2} p \]
\[ Q = \frac{1}{2} (1 + p q) \]

**Case 3:** The expected genotypic frequencies from a monogenic model in the F1 x Ps backcross generation with a non-homozygous Ps and a homozygous resistant PR line were:

\[ P = \frac{1}{2} p (1 + p) \]

where p = Freq(R) and q = Freq(S) in Ps

\[ H = \frac{1}{2} (3 - 2q) q \]
\[ Q = \frac{1}{2} q^2 \]

For the two-locus model, four models with epistasis (nonadditive interactions between loci) were also tested and were analogous to models A, B, C and D described by Tabashnik et al 1992. In Model A, individuals heterozygous at one locus and homozygous resistant at the other were fully resistant (R1S1R2R2 and R1R1R2S2), whereas in model B the same genotypes responded like F1 progeny (R1S1R2S2). In model C, R1S1R2R2 responded like F1 progeny, and the LC50 for R1R1R2S2 was the geometric mean.
of the LC50s for the F1 progeny and the resistant parent (assumed to be R1R1R2R2). In model D, R1R1R2S2 was fully resistant and the LC50 of R1S1R2R2 was the geometric mean of the F1 progeny and the resistant parent. For all model comparisons, expected and observed mortalities at each concentration were compared using a 2 x 2 test for independence at each of the concentrations used in the bioassay (Tabashnik et al. 1992). Overall model $\chi^2$ values were calculated by summing the $\chi^2$ values over all doses for each model. The model with the lowest $\chi^2$ value was determined to have the best fit to the observed data. Results of the four inbred lines from the UBC backcrosses were pooled in the analyses, since the 95% confidence interval of the LC50 of the four lines overlapped.
3.3 Results

Response to Selection and Genetic Variation within Resistant Colonies

An increase in resistance of the P_{R}-UBC T. ni colony to Bt was observed in response to selection with increasing Bt doses (Table 3.1). Prior to selection, there was 0% survival to pupation at 80 kIU/ml diet. Following 5 generations of selection, survivorship to pupation increased to 17% at 80 kIU/ml diet. Prior to selection, the P_{R}-UBC colony’s LC_{50} after 3 days of larval feeding was 8 kIU/ml diet (6.0-10.3 kIU/ml diet) which increased to 44.8 kIU/ml diet (36.5-54.7 kIU/ml diet) for a resistance ratio of 44.8 as compared to P_{S}-UBC (ie. LC_{50} P_{R}/ LC_{50} P_{S}). After 8 - 9 generations of selection at Cornell University, the IC_{50} of the P_{R}-Cor neonates was 5.8 kIU/ml for a resistance ratio of 37.7 relative to P_{S}-Cor (Table 3.2 and 3.3).

Bioassays of offspring of 17 single pair crosses between P_{R}-UBC resistant individuals demonstrated that genetic variation for Bt resistance remained in the population after selection at 80 kIU/ml diet (Pair: devratio=2.34, p=0.002). The LC_{50} values of the pairs ranged from 16.7 ±3.0 to 42 ±6.0 kIU/ml diet.

Inbreeding Results

Five lines, derived from the single-pair crosses, were sib-mated successfully for three generations and reared in the absence of selection with Bt. In the first generation, six lines were discarded due to prominent plateaus corresponding to mid-range Bt doses in the assays which suggests that the lines were not genetically homogeneous for resistance (McKenzie 1996). Two lines were discarded in generation 2 and four lines in generation 3, due to significant decreases in LC_{50} between generations. Two thirds of the lines displayed poor fecundity and adult survival after the
Table 3.1: History of selection of the PR-UBC strain.

<table>
<thead>
<tr>
<th>Lab Generation</th>
<th>No. selected</th>
<th>Selective Dose [kIU/ml diet]</th>
<th>% survivorship to Day 3</th>
<th>% survivorship to pupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>200</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>661</td>
<td>5.0</td>
<td>35.4</td>
<td>22.7</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>550</td>
<td>10</td>
<td>84</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>560</td>
<td>80</td>
<td>20</td>
<td>7.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>1028</td>
<td>80</td>
<td>9.3</td>
<td>2.9</td>
</tr>
<tr>
<td>9</td>
<td>543</td>
<td>80</td>
<td>29</td>
<td>16.9</td>
</tr>
<tr>
<td>10</td>
<td>800</td>
<td>80</td>
<td>20.6</td>
<td>6.6</td>
</tr>
</tbody>
</table>

a. 1 mg DiPel WP = 16,000 IU
b. Surviving pupae were used to initiate inbred lines
c. Progeny of the 10<sup>th</sup> lab generation were sent to Cornell

Table 3.2: History of selection of the PR-Cornell strain.

<table>
<thead>
<tr>
<th>Lab Generation</th>
<th>No. Selected</th>
<th>Selective Dose&lt;sup&gt;b&lt;/sup&gt; [kIU DiPel/ml]</th>
<th>% Survivorship to Pupation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>900</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>3</td>
<td>1500</td>
<td>10</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>510</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>560</td>
<td>20</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>675</td>
<td>40</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>500</td>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>625</td>
<td>40</td>
<td>17</td>
</tr>
<tr>
<td>9</td>
<td>1500</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>10</td>
<td>150</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>11</td>
<td>1500</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>12</td>
<td>1000</td>
<td>40</td>
<td>9</td>
</tr>
<tr>
<td>13</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>40</td>
<td>N/A&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>14</td>
<td>775</td>
<td>40</td>
<td>8</td>
</tr>
<tr>
<td>15</td>
<td>720</td>
<td>40</td>
<td>7</td>
</tr>
</tbody>
</table>

a. 1<sup>st</sup> lab generation began with the original eggs received from UBC
b. 2 ml of DiPel suspension was added to the surface (~50 cm<sup>2</sup>) of the diet
c. No. selected was not recorded for this generation
Table 3.3: Response of susceptible (Ps-UBC), resistant (PR-UBC, PR-Cor), F₁ and backcross *Trichoplusia ni* larvae to *Bacillus thuringiensis kurstaki* (DiPel). The response of resistant inbred lines after 1 (G1) and 14 (G14) generations of rearing in the absence of selection are shown. UBC results are the means of four inbred lines and their respective crosses.

<table>
<thead>
<tr>
<th>Inbred Line</th>
<th>Cross</th>
<th>n</th>
<th>Slope ± SE</th>
<th>LC₅₀ (95% fiducial limits) [kIU/ml]</th>
<th>RRᵃ</th>
<th>Dᵇ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>UBC</strong></td>
<td>Ps-UBC</td>
<td>180</td>
<td>0.761 ±0.13</td>
<td>1.0 (0.6-1.4)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PR-G1</td>
<td>396</td>
<td>1.32 ±0.11</td>
<td>23.9 (21.2-26.4)</td>
<td>23.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PR-G14</td>
<td>800</td>
<td>0.77 ±0.06</td>
<td>18.3 (15.7-21.7)</td>
<td>18.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>480</td>
<td>1.53 ±0.23</td>
<td>2.4 (2.2-2.6)</td>
<td>2.4</td>
<td>-0.44</td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>480</td>
<td>1.32 ±0.20</td>
<td>2.3 (2.0-2.8)</td>
<td>2.3</td>
<td>-0.48</td>
</tr>
<tr>
<td></td>
<td>F₁ x P₁</td>
<td>1204</td>
<td>0.80 ±0.04</td>
<td>5.1 (4.6-5.7)</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₁ x Pₛ</td>
<td>1325</td>
<td>1.03 ±0.11</td>
<td>2.5 (2.2-2.7)</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td><strong>Cornell</strong></td>
<td>Ps-Cor</td>
<td>160</td>
<td>2.26 ±0.42</td>
<td>0.15e (0.11-0.20)</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PR-Cor</td>
<td>240</td>
<td>2.96 ±0.49</td>
<td>5.8e (4.6-7.0)</td>
<td>37.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>240</td>
<td>1.87 ±0.20</td>
<td>0.35e (0.21-0.62)</td>
<td>2.3</td>
<td>-0.55</td>
</tr>
<tr>
<td></td>
<td>F₁</td>
<td>240</td>
<td>1.40 ±0.18</td>
<td>0.35e (0.26-0.48)</td>
<td>2.3</td>
<td>-0.55</td>
</tr>
<tr>
<td></td>
<td>F₁ x P₁</td>
<td>400</td>
<td>1.57 ±0.18</td>
<td>0.90e (0.65-1.16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. LC₅₀ of PR divided by the LC₅₀ of the respective Ps
b. D=Dominance using Stone (1968) where D ranges from -1 (completely recessive) to 1 (completely dominant) at the LC₅₀
c. F₁ cross between Ps(f) x PR or F₁ where f=female
d. F₁ cross between Ps x PR(f)
e. IC₅₀ dose at which 50% were growth inhibited with units of kIU/ml of DiPel inoculum
third generation of inbreeding presumably due to inbreeding effects. Four inbred lines were chosen and sib-mated for an additional eleven generations.

In the absence of selection pressure, the LC50 values of the four inbred lines remained relatively stable over 14 generations (Figure 3.1). A regression of the natural logarithm of the LC50 values over time showed a negative change in LC50 (F=4.15, df=19, p=0.057, JMPIN 4.03). An examination of the change over time in the individual lines revealed that line 6 displayed a significant decrease in LC50. If line 6 was excluded from the analysis, there was no change in LC50 over time in the remaining lines (F=1.09, df=14, p=0.32).

In contrast, the resistance of PR-UBC following field collection declined from an LC50 of 52 kIU/ml diet to 8.0 kIU/ml diet in 3 generations (Janmaat & Myers 2003). In the absence of selection, the LC50 of unsel1-UBC continued to decrease to 4.3 kIU/ml diet after 7 generations. The LC50 of the unsel2-UBC which was initiated from the 8th generation of the selected PR-UBC colony (LC50: 44.8 kIU/ml diet) also rapidly declined to 2.9 kIU/ml diet after 9 unselected generations (Figure 3.2). Similarly, the non-selected strain at Cornell exhibited a decrease in resistance to an IC50 of 0.9 kIU/ml (95% fiducial limits: 0.7-1.2 kIU/ml) after 11 generations of rearing in the absence of Bt. The decrease in line 6 was much slower than the non-selected colonies as it displayed a decrease in LC50 from 27 to 13 kIU/ml diet over 14 generations. Therefore, susceptible alleles or costly resistant alleles were excluded from the inbred lines which stabilized the LC50 values over time. However, bioassays of offspring of single-pair crosses within two of the inbred lines (i.e. line 13 x line 13; line 17 x line 17) after 3 generations of inbreeding revealed that significant variation in resistance remained in the two inbred lines (Line 13
Figure 3.1: Change in LC$_{50}$ over 14 unselected generations of four inbred P$_R$ lines [line 6, 12, 13, 16].
Figure 3.2: Change in LC$_{50}$ over time of a selected field derived strain (P$_R$-UBC) of *Trichoplusia ni* and two unselected lines (unsel$_1$-UBC & unsel$_2$-UBC).

Selection with *Bt* began after 3 generations of laboratory rearing.
devratio=4.5, p=0.004; Line 17 devratio=17.4, p<0.001). LC$_{50}$ values varied from 27.4 to 48.5 kIU/ml diet and 18.9 to 61.1 kIU/ml diet within lines 13 and 17 respectively.

**Evaluation of Dominance and Maternal Effects**

No difference was found between the LC$_{50}$ values and slopes of the concentration-mortality lines of the hybrid progeny for the two reciprocal crosses (Cross) between the inbred lines and the P$_5$-UBC strain (Cross devratio=0.005, p=0.946; Cross*Dose devratio=0.62, p=0.432) (Table 3.3). Little variation in LC$_{50}$ was observed between the 8 F$_1$ hybrid lines (LC$_{50}$ range: 1.5 ±0.7 to 3.0±0.4 kIU/ml diet) (p=0.95). Similarly, the IC$_{50}$ values of the Cornell reciprocal F$_1$ crosses were identical at 0.35 kIU/ml of DiPel suspension (Table 3.3). Therefore, no maternal effects or sex linkage were evident and inheritance of *Bt* resistance is assumed to be autosomal.

The mean resistance ratio of the UBC F$_1$ hybrids was 2.4±0.2 as compared to a mean resistance ratio of the resistant parents of 23.9±1.4. The resistance ratio of the Cornell F$_1$ hybrids was 2.3 relative to the ratio of 37.7 of the resistant strain. The mean dominance value calculated for the UBC F$_1$ hybrids pooled over the inbred lines was -0.46 over the two reciprocal F$_1$ crosses as compared to -0.55 for the Cornell crosses (Table 3.3). Therefore, resistance to *Bt* at the LC$_{50}$ or IC$_{50}$ was partially recessive assuming that the parental lines were homozygous.

**Backcross Results**

For the F$_1$ x P$_R$ UBC backcross, the slope of the concentration-mortality line of the backcross progeny was lower than that of the F$_1$ hybrid, indicating that variation in resistance levels increased, as is expected when the inheritance is due to one or a few loci (slope: F$_1$-UBC 1.54±0.12, F$_1$-UBC x resistant line 0.80±0.04). However, a decrease in
slope was not observed between the concentration-mortality lines of the Cornell F₁ hybrids and the backcross progeny (slope: F₁-Cor 1.62±0.13; F₁-Cor x Pᵣ-Cor 1.57±0.18).

For all F₁ x Pᵣ backcrosses, progeny exhibited higher mortality than expected under a model of monogenic inheritance (Figure 3.3). There were significant deviations between observed and expected mortalities near the expected LC₅₀ (Table 3.4). Under the assumption of homozygous parental lines, this result suggests that a monogenic model did not adequately fit the observed data (Tabashnik 1991). However, relaxing the assumption of homozygosity in the parental lines increased the correspondence between expected and observed results. This was shown by the reduction in model χ² values from a monogenic model with a resistant allele frequency of 1.0 in Pᵣ to a frequency of 0.8 for both F₁ x Pᵣ backcrosses (Table 3.4). Therefore, the discrepancy between predicted and observed results can be modeled by either the presence of more than one resistance locus or the presence of susceptible alleles in the resistant parental lines.

To distinguish between these two plausible hypotheses, the results of the UBC F₁ x Pₛ backcross were examined. Unlike the F₁ x Pᵣ progeny, progeny of the F₁ x Pₛ backcross exhibited lower mortality than expected at all concentrations (Figure 3.3). However if Pᵣ lines contained susceptible alleles, it would be predicted that F₁ x Pₛ backcross progeny would have a higher mortality than expected. Some of the F₁ individuals would be homozygous susceptible and therefore a higher proportion of backcross progeny would also be homozygous susceptible. As expected, including a non-homozygous Pᵣ line in the F₁ x Pₛ backcross model did not improve the fit to the
Table 3.4: Indirect tests of a monogenic model of Bt resistance inheritance by comparing observed and expected mortalities of backcrosses between F1 and PR, the resistant parent. Monogenic models were adjusted for the presence of a non-homozygous resistant parental population (PR) that had a resistant allele frequency of 0.9 or 0.85.

<table>
<thead>
<tr>
<th>Line</th>
<th>Dose</th>
<th>n</th>
<th>Observed</th>
<th>Expected (p=1.0)</th>
<th>Expected (p=0.9)</th>
<th>Expected (p=0.8)</th>
<th>$\chi^2$</th>
<th>$\chi^2$</th>
<th>$\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Monogenic Model</td>
<td>Monogenic Model</td>
<td>Monogenic Model</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UBC</td>
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<td>172</td>
<td>9.3</td>
<td>9.9</td>
<td>0.04</td>
<td>1.17</td>
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<td>14.0</td>
<td>1.86</td>
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<td>172</td>
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<td>37.4</td>
<td>5.58</td>
</tr>
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<td>5.00</td>
<td>174</td>
<td>56.3</td>
<td>42.8</td>
<td>6.37*</td>
<td>51.4</td>
<td>0.83</td>
<td>58.4</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>174</td>
<td>71.3</td>
<td>52.6</td>
<td>12.82**</td>
<td>61.3</td>
<td>3.87*</td>
<td>68.8</td>
<td>0.25</td>
</tr>
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<td></td>
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<td>172</td>
<td>88.4</td>
<td>64.9</td>
<td>26.39**</td>
<td>88.4</td>
<td>14.58***</td>
<td>77.8</td>
<td>6.85**</td>
</tr>
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<td>93.5</td>
<td>83.4</td>
<td>8.49**</td>
<td>86.4</td>
<td>4.79*</td>
<td>89.4</td>
<td>1.89</td>
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<tr>
<td>Cornell</td>
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<td>5.1</td>
<td>2.11</td>
<td>7.7</td>
<td>1.05</td>
<td>12.8</td>
<td>0.46</td>
</tr>
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<td>40</td>
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<td>38.5</td>
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<td>52.5</td>
<td>48.7</td>
<td>0.11</td>
<td>56.4</td>
<td>1.22</td>
<td>66.7</td>
<td>0.30</td>
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<td></td>
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<td>52.5</td>
<td>48.7</td>
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<td>0.59</td>
</tr>
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<td>51.2</td>
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<td>69.2</td>
<td>0.14</td>
</tr>
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<td>51.3</td>
<td>5.93*</td>
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</tr>
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<td>2.88</td>
<td>40</td>
<td>85</td>
<td>53.8</td>
<td>9.06**</td>
<td>61.5</td>
<td>5.57*</td>
<td>71.8</td>
<td>4.91**</td>
</tr>
<tr>
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<td>82.5</td>
<td>64.1</td>
<td>3.42</td>
<td>69.2</td>
<td>1.90</td>
<td>76.9</td>
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<td>40</td>
<td>87.5</td>
<td>79.5</td>
<td>0.92</td>
<td>84.6</td>
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<td>87.2</td>
<td>0.11</td>
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<td>97.5</td>
<td>94.9</td>
<td>0.37</td>
<td>94.9</td>
<td>0.37</td>
<td>97.4</td>
<td>0.35</td>
</tr>
</tbody>
</table>

a. Values represent kIU/ml diet for the UBC crosses and kIU/ml of DiPel inoculum for the Cornell crosses
b. Where * indicates that the $\chi^2$ value is significant and suggests that the observed values deviate from the expected values at a level <0.05 and ** and *** indicate a significance level of < 0.01 and <0.001 respectively.
Figure 3.3: Comparison of the mortality observed from assays of backcross progeny ($F_1 \times P_R; F_1 \times P_S$) relative to the mortality expected from a monogenic model with homozygous parental lines. The mean ratio (± stderr) of observed to expected mortality is shown for the four $P_R$ inbred lines.
observed data (Table 3.5). In contrast, including resistant alleles in the P<sub>S</sub> line (in a F<sub>1</sub> x P<sub>S</sub> model with homozygous P<sub>R</sub>), decreased the overall χ<sup>2</sup> value from 22.3 to 10.6 with a change in the resistant allele frequency from 0 to 0.05 in the P<sub>S</sub> population (Table 3.5). However, the possibility of a non-homozygous P<sub>S</sub> population would not account for the higher than expected mortality observed in the F<sub>1</sub> x P<sub>R</sub> backcross. Therefore, the lack of correspondence between the backcross results and those predicted from a monogenic model suggest that more loci are involved in resistance.

To further elucidate the inheritance of resistance, the P<sub>R</sub> x F<sub>1</sub> backcross results were compared to mortalities predicted from a 2-locus model with additive or epistatic effects. The 2-locus additive model yielded a similar χ<sup>2</sup> value to the monogenic model with the non-homozygous P<sub>R</sub> line (where p=0.8) for the Cornell crosses but a higher χ<sup>2</sup> value was obtained for the UBC crosses. For both the Cornell and UBC backcrosses, the 2-locus model with epistatic effects yielded the lowest overall model χ<sup>2</sup> value (Table 3.6). In this model, the R<sub>1</sub>S<sub>1</sub>R<sub>2</sub>R<sub>2</sub> genotype responded like F<sub>1</sub> progeny, and the LC<sub>50</sub> for R<sub>1</sub>R<sub>1</sub>R<sub>2</sub>S<sub>2</sub> was midway between the F<sub>1</sub> hybrid and the resistant parent. None of the 2-locus models improved the correspondence between the observed and expected mortalities of the F<sub>1</sub> x P<sub>S</sub> backcross. However, the limited difference in LC<sub>50</sub> between the F<sub>1</sub> and P<sub>S</sub> populations and the high dose range chosen may not have been adequate to effectively compare the different multi-locus models.
Table 3.5: Indirect test of a monogenic model of Bt resistance inheritance by comparing observed and expected mortalities of backcrosses between F\(_1\) and the susceptible parent (P\(_S\)). The model was adjusted for the presence of a non-homozygous resistant (P\(_R\)) parental population with a resistant allele frequency of 0.85 or a non-homozygous susceptible (P\(_S\)) parental population with a resistant allele frequency of 0.05.

<table>
<thead>
<tr>
<th>Dose [\mu\text{g DiPel/ml diet}]</th>
<th>n</th>
<th>Observed</th>
<th>Monogenic Model (p=1.0 in P(_R))</th>
<th>Monogenic Model (p=0.8 in P(_R))</th>
<th>Monogenic Model (p=0.05 in P(_S))</th>
</tr>
</thead>
<tbody>
<tr>
<td>[\text{Expected}]</td>
<td>[\chi^2^a]</td>
<td>[\text{Expected}]</td>
<td>[\chi^2^a]</td>
<td>[\text{Expected}]</td>
<td>[\chi^2^a]</td>
</tr>
<tr>
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<td>29.4</td>
<td>22.0</td>
<td>3.6</td>
<td>22.7</td>
</tr>
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<td>2.5</td>
<td>255</td>
<td>44.7</td>
<td>57.1</td>
<td>7.8**</td>
<td>57.5</td>
</tr>
<tr>
<td>5.0</td>
<td>255</td>
<td>83.1</td>
<td>87.0</td>
<td>1.5</td>
<td>87.1</td>
</tr>
<tr>
<td>10.0</td>
<td>255</td>
<td>94.5</td>
<td>98.0</td>
<td>4.4*</td>
<td>98.1</td>
</tr>
<tr>
<td>20.0</td>
<td>255</td>
<td>98.0</td>
<td>100.0</td>
<td>5.0*</td>
<td>99.9</td>
</tr>
<tr>
<td>[\Sigma \chi^2]</td>
<td></td>
<td></td>
<td>22.3</td>
<td>24.0</td>
<td></td>
</tr>
</tbody>
</table>

a. Where * indicates that the \(\chi^2\) value is significant and suggests that the observed values deviate from the expected values at a level \(\leq 0.05\) and ** and *** indicate a significance level of \(\leq 0.01\) and \(\leq 0.001\) respectively.
Table 3.6: Indirect tests of a 2-locus model of *Bt* resistance inheritance by comparing observed mortalities of backcrosses between *F*₁ and the resistant parental population (*P*ᵣ) with mortalities predicted from a 2-locus model with additive or non-additive effects (Model C in Tabashnik et al. 1992).

<table>
<thead>
<tr>
<th>Line</th>
<th>Dose*</th>
<th>n</th>
<th>Observed</th>
<th>2-locus Model (additive)</th>
<th>2-locus Model C (with epistasis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UBC</td>
<td>1.25</td>
<td>172</td>
<td>9.3</td>
<td>5.3</td>
<td>9.9</td>
</tr>
<tr>
<td></td>
<td>2.50</td>
<td>172</td>
<td>25.6</td>
<td>16.4</td>
<td>4.38*</td>
</tr>
<tr>
<td></td>
<td>5.00</td>
<td>174</td>
<td>56.3</td>
<td>35.8</td>
<td>14.65***</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>174</td>
<td>71.3</td>
<td>58.9</td>
<td>5.78*</td>
</tr>
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<td>20.0</td>
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<td>88.4</td>
<td>77.8</td>
<td>6.85**</td>
</tr>
<tr>
<td></td>
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<td>93.5</td>
<td>91.1</td>
<td>0.69</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
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</tbody>
</table>

<table>
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<th>Cornell</th>
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<th>7.51</th>
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<td></td>
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<td>25.6</td>
</tr>
<tr>
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<td></td>
<td>0.40</td>
<td>40</td>
<td>37.5</td>
<td>25.6</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>40</td>
<td>52.5</td>
<td>33.3</td>
</tr>
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<td></td>
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<td>40</td>
<td>52.5</td>
<td>46.2</td>
</tr>
<tr>
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<td>40</td>
<td>65</td>
<td>61.5</td>
</tr>
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<td>40</td>
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<td>69.2</td>
</tr>
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<td>74.4</td>
</tr>
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<tr>
<td></td>
<td></td>
<td>9.72</td>
<td>40</td>
<td>97.5</td>
<td>97.4</td>
</tr>
</tbody>
</table>

| Σχ² | 7.93 | 4.61 |

a. Values represent klU/ml diet for the UBC crosses and klU/ml of DiPel inoculum for the Cornell crosses.

b. Where * indicates that the χ² value is significant and suggests that the observed values deviate from the expected values at a level <0.05 and ** and *** indicate a significance level of < 0.01 and <0.001 respectively.
3.4 Discussion

Resistance to Bt in a moderately resistant T. ni population appears to be due to an autosomal partially recessive trait as determined by comparisons between dose-mortality curves of resistant, susceptible, hybrid and backcross progeny. The similarity of the results of the two separate laboratories was striking considering the differences in methodology. For example, the dominance value varied from -0.46 to -0.55 between the two locations and, therefore Bt resistance in this T. ni population conformed to a partially recessive trait. In other studies, resistance has varied from partial to complete recessivity in laboratory selected strains of Plodia interpunctella (McGaughey 1985; McGaughey & Beeman 1988), and field derived strains of P. xylostella (Tabashnik et al. 1992; Tang et al. 1997; Sayyed, Ferré, & Wright 2000), whereas, in an Ostrinia nubilalis laboratory population resistance to Bt was found to be co-dominant (Huang et al. 1999).

One general assumption of studies of the inheritance of resistance is that the parent populations are homozygous. In previous studies, variation in resistance of progeny of F1 hybrid crosses has shown that resistant alleles were present in susceptible laboratory populations (Gonzalez-Cabrera, Herrero, & Ferré 2001; Tabashnik et al. 1997) and that susceptible alleles were present in selected resistant populations (Liu & Tabashnik 1997). In the present study, to increase homogeneity, four inbred lines derived from single-pair crosses were maintained by sib-mating for 3 generations and were used to produce F1 and backcross progeny. Inbred lines which exhibited a decrease in resistance over time were discarded. A possible effect of this procedure was the exclusion of major resistance alleles that were heterozygous in either parent of the initial
cross or the exclusion of minor alleles. However, the results of the single-pair crosses in this study agreed with the mass cross results strengthening the overall conclusions.

The relative stability of resistance over 14 generations in the inbred lines suggests that they were homozygous for resistance, however significant variation in LC\textsubscript{50} did remain in at least two of the inbred lines. Therefore, it is unclear if susceptible alleles or multiple resistant loci were present in the inbred lines. However, the decrease in slope of the concentration-mortality lines from the F\textsubscript{1} to the backcross progeny for the UBC crosses suggested that resistance was due to a few major loci rather than a quantitative trait (Tabashnik 1991; Tabashnik et al. 1992; McKenzie 1996).

In the majority of studies on the inheritance of Bt resistance, resistance has corresponded to one or a few major loci (Caprio & Tabashnik 1992; Tang et al. 1997; Huang et al. 1999; Sayyed, Ferré, & Wright 2000). The primary method to determine the number of loci involved in resistance would be to compare the backcross results to mortalities predicted from a monogenic model. However, non-homozygous parental lines would obscure the results of hybrid and backcross mortality assays and could lead to spurious rejection of monogenic models of resistance. Therefore, both the effect of non-homozygous parental lines and multiple resistant loci were examined in this study.

No correspondence was found between the predictions of a monogenic model and backcross results in this study. Both the inclusion of a non-homozygous P\textsubscript{R} population in a monogenic model or an additional resistance locus, increased the correspondence between the observed and predicted results. A monogenic model with a resistance allele frequency of 0.80 resulted in one dose where there was a significant deviation between observed and predicted mortalities as opposed to significant deviations at 2 to 4 doses.
when p was equal to 1.0. Whereas, a 2-locus model with epistatic effects (Model C) produced a significant deviation at one dose in the UBC F₁ x Pᵣ backcross and no deviations with the Cornell F₁ x Pᵣ backcross. To distinguish between a model with a non-homozygous resistant line and one with multiple resistant loci, the results of the F₁ x Pₛ backcross were utilized. Given that the observed mortality of the F₁ x Pₛ progeny was lower than expected, a model with a proportion of susceptible alleles in the resistant population would not adequately describe the F₁ x Pₛ results. Therefore, the discrepancy between the monogenic model and the F₁ x Pᵣ backcross was most likely due to the presence of more than one locus or more than two alleles in the resistant T. ni population.

Similar discrepancies between backcross results and models of monogenic inheritance have been found in other studies. For example, resistance to Cry3A toxin of B. thuringiensis subspecies tenebrionis of Leptinotarsa decemlineata (Rahardja & Whalon 1995), and resistance to Cry1C of Spodoptera littoralis (Chaufaux et al. 1997) did not correspond to monogenic inheritance. Resistance to Cry1Ac in Pectinophora gossypiella corresponded to a single resistance gene with three alleles or to more than one resistance locus (Tabashnik et al. 2002). In field derived populations of the diamondback moth, resistance to Cry1Ac did not correspond to monogenic inheritance in a population from Malaysia (Sayyed & Wright 2001) and two different genes that confer resistance to Cry1Ab were present in a population originating from the Philippines (Gonzalez-Cabrera, Herrero, & Ferré 2001). Direct tests of monogenic inheritance of Cry1C resistance in a P. xylostella population originating from New York suggested that significant deviations between observed and expected mortalities were the result of non-additive polygenic
inheritance or experimental error (Zhao et al. 2000) and further tests indicated that it might be polygenic inheritance (Zhao et al. 2002).

The presence of multiple resistance loci in *T. ni* is not surprising because the *Bt* toxin is comprised of 5 different toxic Cry proteins (Cry1Aa, Cry1Ab, Cry1Ac, Cry2A, Cry2B) (Hofte & Whiteley 1989). *T. ni* larvae have been shown to be most susceptible to Cry1Ac, followed by Cry1Ab and Cry2Aa, whereas Cry1Aa toxicity has varied from moderate to little toxicity (Moar 1990; Iracheta et al. 1994). In a previous study, a laboratory population of *T. ni* was selected for resistance to Cry1Ab, and no cross resistance to Cry1Ac was found (Estada & Ferre 1994). Similarly, a strain of *P. xylostella* from the Philippines was shown to harbor multiple resistance genes that either confer resistance to Cry1Ab only (Ballester et al. 1999) or combined resistance to Cry1Ab and Cry1Ac (Gonzalez-Cabrera, Herrero, & Ferré 2001; Tabashnik et al. 1997). Therefore, it is possible that two separate loci that confer resistance to either Cry1Ac and/or Cry1Ab were present in the *T. ni* population due to selection with the multi-toxin *Bt* formulation. In a selection of P_R-Cor with Cry1Ac only, monogenic resistance to Cry1Ac was found supporting the prediction that multiple resistance loci for the different toxins are present in this population (P. Wang personal communication).

A two-locus model with non-additive effects (Model C) provided the best fit to the observed backcross mortalities, however a two-locus model with additive effects was adequate for the Cornell results. In Model C, the $R_1S_1R_2R_2$ genotype responded similarly to the F1 hybrid progeny, thereby elevating the expected mortalities of the F1 x P_R backcross from that of an additive two-locus model. In Tabashnik’s (1991) analysis of the determination of inheritance from backcross experiments, two-locus models with
additive effects yielded equal and opposite expected differences on either side of the backcross LC₅₀. The differences observed with non-additive two-locus models were consistently positive or negative over the dose range. In the present study, observed mortalities were consistently higher for the F₁ x Pᵣ progeny than expected from a two-locus model suggesting the presence of epistatic effects.

A two-gene model, with non-additive effects, of the inheritance of resistance in T. ni is undoubtedly more simplistic than the true nature of inheritance; however, it raises the possibility of epistatic interactions between loci. What is remarkable is that two genes for resistance to Bt with complex non-additive interactions may have evolved in a T. ni population outside of the laboratory. The probability of the evolution of resistance to two toxins is considered to be an extremely rare event and this assumption provides the rationale for utilizing pesticide mixtures and rotations, and the pyramiding of toxin genes in transgenic plants as resistance management strategies. It will, therefore, be pertinent to determine if epistatic interactions between loci that facilitate the evolution of resistance to multiple Bt toxins in the field are a common occurrence. If epistatic interactions are common, then the prevalent models of resistance evolution may need to be re-examined.
3.5 Literature Cited


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Chapter IV: Effect of host plant on dominance of Bt resistance

4.1 Introduction

The level of dominance of alleles for resistance to insecticides determines the rate of resistance evolution (Georghiou 1972). Resistance management strategies often depend on the assumption that these alleles are recessive (Roush 1989; Tabashnik 1989). However, this assumption may not always be valid. It is commonly assumed that dominance is an intrinsic property of an allele. However dominance is the relationship among the phenotypes of three different genotypes (Mayo & Burger 1997; Bourguet 1999; Bourguet, Genissel, & Raymond 2000). Since the phenotype is determined by both the genotype and the environment, dominance may therefore vary among environments.

In studies of insecticide resistance, dominance (D) is generally described by the relative position of the concentration-mortality line of F1 hybrids relative to resistant (PR) and susceptible (Ps) parental populations (Stone 1968; Bourguet, Genissel, & Raymond 2000). In the majority of studies on the inheritance of resistance to Bt formulations or toxins, the Bt resistance trait has displayed recessive inheritance (Ferré & van Rie 2002). In contrast, resistance to Bt in a laboratory population of Ostrinia nubilalis was incompletely dominant (Huang et al. 1999) and resistance to the Cry1Ab toxin of Bt in Heliothis virescens was found to be co-dominant (Sims & Stone 1991). The variation in observed dominance levels may be due to a variety of factors, from differences in the genetic trait, genetic background among strains, and environmental factors (Stone 1968). Few studies have examined the potential for the environment to influence dominance of insecticide resistance, although such interactions may be common (Bourguet, Prout, & Raymond 1996).
The host plant is a pivotal component of a phytophagous insect’s environment and can significantly influence the toxicity of Bt. For example, toxicity of Bt towards *Lymantria dispar* is positively correlated with levels of phenolic glycosides in aspen (Hwang et al. 1995). In contrast, nicotine, a defensive compound of tobacco, decreased *Manduca sexta* mortality following ingestion of Bt (Krischik, Barbosa, & Reichelderfer 1988). Variability in Bt toxicity towards a target insect herbivore has been observed even among varieties of a plant species, such as celery cultivars (Meade & Hare 1993) and cabbage varieties (Schuler & van Emden 2000). These effects may influence the expression of the resistant trait in different genotypes and thereby alter the expression of dominance among individuals feeding on different host plants. Therefore, in order to develop appropriate resistance management strategies for different insect-crop systems, it must be determined if the dominance of Bt resistance varies among host plants.

Recently, resistance to Bt was detected in populations of cabbage loopers, *Trichoplusia ni*, occurring in commercial greenhouses (Janmaat & Myers 2003). *T. ni*, a generalist herbivore, is a pervasive pest of three primary greenhouse crops (in British Columbia, Canada): tomatoes, bell peppers and cucumbers. The growth rates of *T. ni* larvae vary considerably among the three different host plants and are the slowest with the smallest resulting pupae on pepper leaves, intermediate on tomato leaves, and the fastest on cucumber leaves (see Chapter V and VI). Given the numerous examples of interactions between host plant allelochemicals and Bt activity (Krischik, Barbosa, & Reichelderfer 1988; Ludlum, Felton, & Duffey 1991; Meade & Hare 1994; Olsen & Daly 2000), it was hypothesized that the dominance of Bt resistance in *T. ni* might also vary with the host plant.
4.2 Methods

A resistant *T. ni* colony was initiated from 90 individuals collected from a commercial greenhouse population in British Columbia, Canada in 2001 (Janmaat & Myers 2003). In the 1st generation of laboratory culture, two lines were initiated and reared on a wheat-germ based artificial diet (Ignoffo 1963). One line was subjected to selection for seven generations up to a maximum dose of 160 kIU of *Bt* (DiPel WP Valent Biosciences) mixed per ml of artificial diet. The other line was reared without any *Bt* exposure for 16 generations. A rapid reduction in resistance was observed in the unselected line and it is identified as the reverted susceptible population (Janmaat & Myers 2003). In addition, a long-term laboratory colony that had no previous exposure to *Bt* was maintained for genetic analysis and this line is identified as the long-term susceptible population.

One generation prior to crossing, individuals from the resistant colony were reared in the absence of *Bt* exposure to reduce potential sublethal or maternal effects. At pupation, pupae were sexed and mass crosses of 40 males and 40 females were set-up for four different phenotypic lines: resistant by resistant (P*R*), susceptible by susceptible (P*S*) and two reciprocal F1 hybrid crosses.

In the first set of crosses, the resistant population was crossed with the long-term susceptible population and exposed to *Bt*-artificial diet mixtures. In the second set of crosses, the resistant population was crossed with the reverted susceptible population and exposed to *Bt* on leaf discs. Due to the poor survivorship of the long-term susceptible laboratory colony on pepper and tomato leaf discs noted in previous experiments, the reverted susceptible population was used in the leaf disc experiment. The higher survival
of the reverted susceptible population on leaf discs was presumably due to its origin from a tomato greenhouse.

Progeny of each of the crosses were reared on artificial diet prior to transfer to the different Bt treatments. In the first set of crosses, progeny were reared on diet without Bt for five days, whereas progeny were reared for three days in the second set of crosses. The progeny of the first set of crosses were then transferred to different doses of Bt mixed in wheat-germ diet (methods in Janmaat & Myers 2003). Mortality was 0% in the control treatment group (i.e. no Bt) for all genetic crosses. The progeny of the second set of crosses were transferred to Bt treated leaf discs. Three different host plants were grown: pepper (444, Enza Zaden), tomato (Rapsodie, Novartis) and cucumber (Ventura, RZ). Leaf discs (35 mm in diameter) cut from new fully expanded leaves from each host plant were dipped in one of 6 different doses of Bt serial diluted in distilled water (0, 5, 10, 20, 40, 80 kIU/ml water). Excess droplets were removed through gentle agitation and leaf discs were air dried on wire mesh racks. Dry leaf discs were placed into individual 59.2 ml plastic soufflé cups (Solo Cup Company). Five larvae were transferred to each leaf disc for a total of 50 larvae per treatment. Larvae were maintained at 26°C with a 16:8 (L:D) photoperiod for three days at which time larval mortality was assessed. Host plant, Bt dose and population were randomly assigned to 7 different experimental dates to account for daily variation in mortality. No differences in percent mortality between days were observed and therefore date was not included in the analysis (date[dose, phenotype, plant] F=0.76, df=5, p=0.84, GLM analysis in JMPIN 4.2).

LC_{50} values were calculated for each host-plant phenotype combination using the Probit analysis procedure in Genstat 5 Release 4.1 (Lawes Agricultural Trust,
Rothamsted, UK, 1997). Two LC$_{50}$ values were considered significantly different if their 95% fiducial limits did not overlap. No differences in concentration-mortality lines were observed between the reciprocal hybrid crosses (devratio=0.92; p=0.34 see methods Chapter III). Therefore, the results of the hybrid crosses were pooled in the analyses. A nominal logistic model was used to examine the effects of host plant, Bt dose and phenotype as well as their interaction on the number of alive and dead larvae per treatment group (JMPIN 4.2). A three-way interaction was not included due to insufficient degrees of freedom.

Dominance was estimated as described in Stone (1968) where the estimation of dominance (D) based on the LC$_{50}$ was used and D typically ranges from -1 (completely recessive) to 1 (completely dominant). Mean D values and standard errors were calculated according to Preisler, Hoy & Robertson 1990. Dominance values were compared using bootstrapping techniques in Genstat 5 Release 4.1. A random binomial distribution with the probability of success equal to the observed mortality and the number of trials equal to the sample size per Bt dose-phenotype-plant combination was used to re-estimate 1000 LC$_{50}$ and D values. The significance of the difference between D values for each host-plant group (i.e. D$_{tomato}$-D$_{pepper}$, etc.) was estimated from the distributions of the differences between dominance values derived from the randomization tests. The number of differences equal to or below 0 were divided by the total number of randomizations to obtain a one-tailed p-value. The p-value was further multiplied by 2 to obtain a 2-tailed p-value and by 3 to adjust the p-value for the 3 pairwise comparisons.
4.3 Results and Discussion

In the first experiment, hybrid larvae derived from a cross between a resistant *T. ni* population (T2c) and a long-term susceptible laboratory colony and larvae from both parental populations were exposed to a range of *Bt* doses mixed in a wheat-germ based diet. Responses of the hybrid larvae on artificial diet were similar to that of the susceptible parent population indicating that the inheritance of resistance to *Bt* was at least partially recessive (Table 4.1). The resistant population was 32-fold more resistant than the susceptible population, whereas the hybrid population was only 3-fold more resistant. However, the inheritance pattern observed in *T. ni* larvae feeding on wheat-germ based diet did not extend to larvae feeding on different host plants.

In the second experiment, *Bt* was presented to *T. ni* larvae on leaf discs of the three different greenhouse crops. Larvae from the resistant population, a reverted susceptible population and their hybrid offspring were fed leaf discs treated with a range of *Bt* doses. Mortality in the control treatment group (where *Bt* = 0 IU/ml) was significantly affected by the host plant but did not differ between the genetic crosses (Plant: $\chi^2=6.22$, $p=0.04$; Phenotype: $\chi^2=2.53$, $p=0.28$). The observed mortality in the control treatment group was 3.3%, 10.0% and 5.7% in the cucumber, tomato and pepper groups, respectively. No differences in percent mortality between the set-up days were observed and, therefore, the set-up date was not included in the analysis (date[dose, phenotype, plant] $F=0.76$, df=5, $p=0.84$, GLM analysis in JMPIN 4.2).

In contrast to the first experiment, the susceptible population was from the same initial collection as the resistant population but had reverted to become susceptible in the absence of selection (Janmaat & Myers 2003). A smaller difference was observed
Table 4.1: LC\textsubscript{50} and dominance values for each phenotype, host-plant combination.

<table>
<thead>
<tr>
<th>Diet</th>
<th>Population</th>
<th>n</th>
<th>slope</th>
<th>LC\textsubscript{50} [kIU/ml water\textsuperscript{c}]</th>
<th>95% CI</th>
<th>D\textsubscript{50}\textsuperscript{a}</th>
<th>RR\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumber</td>
<td>P\textsubscript{R}</td>
<td>385</td>
<td>0.47 ±0.07</td>
<td>29.1</td>
<td>22.0-42.6</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F\textsubscript{1}</td>
<td>1085</td>
<td>0.38 ±0.05</td>
<td>21.8</td>
<td>17.3-27.4</td>
<td>0.34 ±0.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{S}</td>
<td>450</td>
<td>0.60 ±0.11</td>
<td>12.1</td>
<td>7.0-16.5</td>
<td>0.34 ±0.49</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>P\textsubscript{R}</td>
<td>506</td>
<td>0.34 ±0.06</td>
<td>3.6</td>
<td>1.3-6.1</td>
<td>4.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F\textsubscript{1}</td>
<td>969</td>
<td>0.40 ±0.05</td>
<td>2.8</td>
<td>1.5-4.3</td>
<td>0.69 ±1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{S}</td>
<td>435</td>
<td>0.29 ±0.08</td>
<td>0.7</td>
<td>0.02-2.3</td>
<td>0.34 ±0.49</td>
<td></td>
</tr>
<tr>
<td>Pepper</td>
<td>P\textsubscript{R}</td>
<td>416</td>
<td>0.53 ±0.14</td>
<td>32.2</td>
<td>24.6-43.5</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F\textsubscript{1}</td>
<td>1026</td>
<td>0.41 ±0.04</td>
<td>12.0</td>
<td>9.0-15.1</td>
<td>-1.39 ±0.33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{S}</td>
<td>405</td>
<td>0.40 ±0.10</td>
<td>14.1</td>
<td>9.9-22.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Artificial Diet</td>
<td>P\textsubscript{R}</td>
<td>2126\textsuperscript{d}</td>
<td>0.63 ±0.03</td>
<td>31.9</td>
<td>28.6-35.2</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>F\textsubscript{1}</td>
<td>3196\textsuperscript{d}</td>
<td>0.92 ±0.05</td>
<td>2.9</td>
<td>2.6-3.2</td>
<td>-0.32 ±0.09</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P\textsubscript{S}</td>
<td>180\textsuperscript{d}</td>
<td>0.76 ±0.13</td>
<td>1.0</td>
<td>0.65-1.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. Where D is calculated for a specific mortality level according to Stone (1968) and varies from -1 (completely recessive) to 1 (dominant). Mean D values and standard errors were estimated according to ( Preisler, Hoy, & Robertson 1990).

b. RR represents the resistance ratio of resistant relative to the susceptible phenotype. The susceptible phenotype in the host plant experiment was a reverted susceptible population, whereas a long-term susceptible laboratory population was used in the diet experiment.

c. Leaf discs were dipped in doses of Bt mixed in water whereas Bt diluted in water was mixed into artificial diet in 1:10 ratio (Bt solution:diet).

d. Mean results of single pair crosses are displayed for F\textsubscript{1} (14 crosses) and the resistant population (9 crosses) and the results of a mass cross of 200 individuals are shown for the long-term susceptible laboratory population.
between the susceptible and resistant phenotypes in the leaf disc experiment as compared to the first experiment (Table 4.1). Despite the reduced difference between populations, Bt dose, host plant and phenotype all had significant effects on larval mortality. Mortality increased as expected with Bt dose ($\chi^2=931.4$, df=5, $p<0.0001$). The mortality was lowest over all the doses for the resistant phenotype and highest overall for the susceptible phenotype ($\chi^2=60.5$, df=2, $p<0.0001$) demonstrating that resistance to Bt differed significantly between these phenotypes. No differences, and therefore no maternal effects, were observed between the concentration-mortality lines of the reciprocal hybrid crosses, consequently, the hybrid results were pooled in all analyses.

Bt toxicity differed strikingly between host plants with phenotype-dependent effects (plant effect: $\chi^2=245.0$, df=2, $p<0.0001$; phenotype x plant: $\chi^2=16.2$, df=4, $p<0.0001$) (Figure 4.1). The resistant population exhibited a similar LC$_{50}$ in the cucumber and pepper treatment groups to that obtained in the first experiment. The highest mortality over all phenotypes was observed when Bt was fed to T. ni larvae on tomato leaf discs relative to Bt-treated cucumber or pepper leaf discs (Table 4.1). The LC$_{50}$ of larvae from the resistant population was more than 7-fold lower on tomato than on cucumber or pepper treated leaf discs. This indicates that the resistant individuals were less resistant on tomato discs. The LC$_{50}$ of the susceptible larvae was reduced by more than 12 times on tomato relative to the other host-plant treatments suggesting that the Bt-tomato combination was even more toxic to individuals of the susceptible than the resistant phenotype. This increase in toxicity may be due to a higher deposition of Bt on tomato
Figure 4.1: The LC$_{50}$ for each phenotype host-plant combination where P$_R$ is the resistant phenotype, P$_S$ the susceptible phenotype and F$_1$ represents the pooled results of the reciprocal hybrid crosses. Error bars represent the 95% confidence intervals and LC$_{50}$ values are considered significantly different if the error bars do not overlap.
leaves relative to the other leaf types or due to interactions between tomato leaf chemistry and Bt toxicity.

The resistance of the F1 hybrid relative to the parental phenotypes also differed substantially among host-plant environments. Resistance was co-dominant when Bt was presented to larvae on cucumber leaf discs with D (±se) equal to 0.34 (±0.49). The highest dominance value was estimated for the tomato group (0.69 ±1.0), however there was considerable variation in this estimate which does not preclude the possibility that the dominance in this environment is in fact recessive. However, the dominance of resistance to Bt in the cucumber environment was not completely recessive. This result is in contrast with the partially recessive inheritance observed in the first experiment. This difference may be due to the difference in origin of the susceptible population in the two experiments. In the experiment on leaf pieces, the susceptible population was derived from the same originating population as the resistant line. It is, therefore, possible that differences in the genetic background between the two experiments affected dominance. Similarly, the genetic background has been shown to play a crucial role in the manifestation of fitness costs associated with resistance traits (McKenzie, Whitten, & Adena 1982; Raymond et al. 2001). It is also possible that the difference in diet between the two experiments contributed to the observed increase in dominance when Bt was ingested on cucumber as compared to artificial diet.

Interestingly, there was a significant reduction in dominance when Bt was ingested on pepper leaves as opposed to cucumber (Dcucumber vs Dpepper p=0.018; Bootstrapped p-value (see methods)). Bt resistance was completely recessive or possibly underdominant (D = -1.39 ±0.33) when ingested on pepper leaves. The observed change in dominance
of the resistance trait between the host plant treatment groups demonstrates that dominance may be plastic and dependent on environmental conditions such as host plant.

Only one other study has examined dominance of an insecticide resistance gene in different environments, and this case involved the depth of water and resistance of mosquito larvae to chemical insecticides (Bourguet, Prout, & Raymond 1996). The dominance of resistance to two insecticides (propoxur and chlorpyrifos) varied from almost completely dominant to completely recessive (Bourguet, Prout, & Raymond 1996). Greater water depth and reduced air surface both increased mortality of heterozygous mosquito larvae (Culex pipiens) and thereby reduced dominance. These factors presumably increased larval swimming time, creating a more demanding environment. This finding is similar to our observed low level of dominance of Bt resistance in T. ni on the poorest host plant, pepper, relative to the co-dominance observed in the best environment, cucumber (see results in Chapters V & VI). It is thought that a reduction in dominance with more demanding environments may be due to a pleiotropic cost of resistance (Bourguet, Prout, & Raymond 1996) and, therefore, a more rapid response to selection may be expected in favourable environments.

The response of an insect population to selection with Bt is ultimately dependent on the differentiation between resistant and susceptible phenotypes and hybrid dominance. A rapid response would be expected when there is a large difference between parental phenotypes and the F1 hybrids resemble the selected phenotype. Therefore, it would be expected that resistance to Bt would increase most rapidly in T. ni populations selected on tomato relative to the other host plants if the resistance trait is co-dominant in this environment as estimated. In addition, the increased toxicity of Bt in the tomato
environment would increase the selection pressure on the insect population further contributing to a rapid response. Consistent with this prediction, tomato populations displayed the highest resistance levels in surveys of resistant *T. ni* populations (Janmaat & Myers 2003).

The origin of the resistant population in the second experiment was a tomato greenhouse and it is possible that the genetic background of the population influenced the resistance trait in the native environment. Numerous selection experiments have shown that populations often respond most rapidly to selection in an environment similar to that in which they were previously selected (Kearsey & Pooni 1996). Therefore, it remains to be seen if both dominance and the magnitude of resistance is higher in a common tomato environment for all *T. ni* populations derived from different host plants, or if these factors are higher for all populations on their host plant of origin. In fact, theory predicts that dominance levels should increase over evolutionary time in a given environment, which is consistent with the observed higher dominance on tomatoes (Otto & Bourguet 1999).

Inheritance of resistance to *Bt* in *P. xylostella* derived from resistant field populations has been shown to be partially to completely recessive (Tabashnik et al. 1992; Ferré et al. 1995) and similar patterns of inheritance have been observed across other species (Tabashnik et al. 1998). Surprisingly, *Bt* resistance was not recessive when ingested on cucumber leaves in the present study, and it appeared to vary from complete recessivity to co-dominance with host plant. Because the recessiveness of *Bt* resistance is a pivotal assumption of many resistance management strategies, such strategies will have to be modified to deter resistance evolution in *T. ni* populations feeding on cucumber and possibly on tomato. Furthermore, if dominance of *Bt* resistance is host-plant dependent
in other environments than those studied here, resistance management strategies will not be equally suitable for all insect-crop systems. In particular, the variation in dominance observed here may extend to other generalist insect herbivores, such as the European corn borer that feeds on over 300 different host plants. The effect of host-plant on the dominance of Bt resistance in T. ni emphasizes a need for developing resistance management strategies that are tailored to the specific plant-insect combinations. Moreover, these results likely extend to other insect, pathogen and host plant systems and may provide an additional insight into co-evolutionary processes.
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Chapter V: Host plant dependent fitness costs associated with Bt resistance in T. ni

5.1 Introduction

Traits conferring resistance to challenging environments (i.e. disease, chemical toxins, climatic changes) are generally assumed to be associated with strong deleterious costs (Orr & Coyne 1992; Menair 1991; Carrière et al. 1994). These new genotypes are often at a disadvantage relative to ancestral genotypes under conditions of the ancestral environment due to negative fitness effects associated with the resistance trait. These negative effects often manifest themselves in life-history tradeoffs in which resistant individuals allocate a greater proportion of resources towards the resistance trait as opposed to growth and/or reproduction (Bergelson & Purrington 1996; Carrière et al. 1994). The use of pesticides in agricultural systems strongly selects for resistant mutations that are predicted to be major genes associated with deleterious costs (McKenzie & Batterham 1994; Lande 1983).

Costs associated with insecticide resistance may be condition-dependent such that the trade-off between defense and reproduction becomes more evident under resource poor conditions (Bergelson & Purrington 1996). For example, costs of insecticide resistance are often shown to be more severe during overwintering, a period of extreme environmental stress (McKenzie 1996; Carrière et al. 2001a). Condition-dependent costs were observed for the production of melanin by the butterfly, Pararge aegeria. Melanin is costly to synthesize and has a variety of functions from camouflage to disease resistance (Talloon, van Dyck, & Lens 2004). Differences in environmental quality induced phenotypic differences in wing melanization such that P. aegeria larvae reared
on drought-stressed host grasses developed paler wings than larvae reared on unstressed plants. Therefore, environmental stressors, such as poor nutrition, might alter the manifestation of fitness costs associated with costly phenotypes, such as wing melanization or pesticide resistance.

Host plant quality has a significant effect on the growth and reproduction of phytophagous insects. These effects may be indirect through nutritional quality or through the direct effects of plant defensive compounds on herbivore performance (Awmack & Leather 2002). In either case, the availability of resources to insects will be impacted by the host plant and this in turn may alter the production or maintenance of defensive traits expressed by the insect. Thus, fitness costs associated with pesticide resistance in herbivorous insects are expected to vary with the suitability of the host plant.

Due to concerns regarding the long-term sustainable use of the microbial insecticide Bacillus thuringiensis (Bt), resistance to Bt has been a subject of intense research. However, despite the successful selection of resistance to Bt in multiple laboratory populations, significant resistance has only been detected in populations of Plutella xylostella (Ferré & van Rie 2002) and Trichoplusia ni (Janmaat & Myers 2003) outside of the laboratory. Resistance to Bt is often reported to decline in the absence of selection and therefore it seems likely that Bt resistance is associated with a significant fitness cost (Ferré & van Rie 2002). Previous studies have documented Bt-resistance-associated fitness costs (Liu et al. 1999; Groeters et al. 1994; Groeters et al. 1993). In contrast, the absence of costs and stable resistance to Bt has been reported (Perez & Shelton 1997). The reported absence of fitness costs may have been due to the selection
of resistant alleles that have no negative pleiotropic effects (Guillemaud et al. 1998) or fitness modifiers at other loci (Liu, Tabashnik, & Pusztai-Carey 1996). However, if resistance-associated fitness costs are alleviated in optimal environments, then condition-dependent fitness costs provides an additional explanation.

Cabbage loopers, *Trichoplusia ni*, are pervasive pests of three primary greenhouse crops in British Columbia, Canada: tomatoes, bell peppers and cucumbers. We speculated that significant fitness costs would be associated with *Bt* resistance in *T. ni* populations due to the observed rapid decline of *Bt* resistance in laboratory populations and a negative relationship between resistance and pupal weights of field populations following the cessation of pesticide application (Janmaat & Myers 2003). Furthermore, *T. ni* growth rates differed considerably among the three different host plants in preliminary experiments. We, therefore, hypothesized that fitness costs associated with *Bt* resistance in *T. ni* would differ among host plants.
5.2 Methods

History of T. ni colonies

A Bt-resistant T. ni colony was initiated from 90 individuals collected from a commercial tomato greenhouse in British Columbia, Canada in 2001 (labeled T2c in (Janmaat & Myers 2003). The population was 113-fold more resistant than the laboratory colony at collection. In the 1st generation of laboratory culture, two lines were established on a wheat germ based diet (Ignoffo 1963) according to methods described in (Janmaat & Myers 2003). One line was reared without any Bt exposure and exhibited a significant decrease in resistance after 3 unselected generations (change in LC50 from 248 to 4.1 kIU/ml diet) (Janmaat & Myers 2003). The other line was exposed to Bt kurstaki (DiPel WP, Valent Biosciences) during each generation of lab culture. Groups of 20-25 five-day-old larvae (2nd and 3rd instars) were placed onto 10 ml of artificial diet mixed with Bt. All live larvae were transferred to new diet without Bt after 2 days. Surviving pupae were collected and pooled in a mating cage to produce progeny for the next generation. After four generations of selection, there were considerable decreases in fecundity and fertility of the resistant line. The resistant line was then crossed to the unselected line and the resulting hybrid line was selected with Bt for seven additional generations up to a maximum dose of 160 kIU/ml to produce a new resistant line (Table 5.1).

T. ni Lines

To examine the fitness costs associated with Bt resistance in the three host plant environments, two sets of mass crosses were set up to obtain four genotypic combinations: resistant and susceptible parental generations (PR and PS respectively) and
two reciprocal hybrid generations F_{1f} and F_{1m} (F_{1f}: the resistant parent is the female and F_{1m}: the resistant parent is the male). Individuals from the resistant colony were either reared in the presence (P_{Rsel}) or absence (P_{R}) of Bt exposure for one generation. A comparison of the P_{R} and P_{Rsel} lines will highlight any transgenerational effects of Bt application. Only the P_{R} line was used in the F_{1} crosses. At pupation, pupae were sexed and mass crosses of 40 males and 40 females were set-up for the four different genotypes. Eggs were harvested from caged adults every two days, stored at 4°C, and hatched within 2-4 days of collection.

*Host Plant Effects on Life-history Traits*

Life-history traits of larvae from each genetic cross grown on the three different host plants were measured. The three different crops (pepper: variety 444; tomato: variety Rapsodie; and cucumber: variety Ventura) were grown in a greenhouse at the University of British Columbia, Canada from May to August, 2003. Plants were used ~2 months after planting. Neonates hatched from eggs of each of the genetic generations were placed individually onto leaf pieces contained in 30 ml plastic cups that were placed inside plastic covered seedling flats lined with moistened paper toweling to maintain the turgidity of the leaf pieces. After 3 days of feeding, surviving larvae were transferred to 175 ml Styrofoam cups. In the Styrofoam cups, leaf pieces were hung from wire hooks attached to lids of cups and the size of the leaf piece varied with larval size. The bottom of each cup was removed and the cup was inserted into a 30 ml plastic cup that could be replaced to allow for easy removal of frass. The Styrofoam cups were placed inside covered seedling flats lined with moistened paper toweling. New leaf pieces were provided every two days to early instars and daily to fifth instars in the Styrofoam cups.
Frass was removed when new leaf pieces were provided to avoid buildup of bacteria in the cups. Larvae were maintained at 26°C with a 16:8 (L:D) photoperiod. Eighty to 177 neonates per genotype were put onto leaf pieces over seven different dates. The experimental treatments of host plant and genotype were randomly assigned to the seven different dates to account for daily variation in growth rates and egg viability.

Larval weights were measured at 10 days of age. The number of days to pupation was recorded and pupal weights were recorded two days following pupation. Means and standard errors are reported in the results. Pupae were placed into 30 ml plastic cups until emergence. Mortality was measured as the number of larvae surviving to pupation and was analyzed using the nominal logistic procedure in JMPIN 4.0. Treatment effects were analyzed using a general linear model (glm) in JMPIN 4.0 with host plant and genotype defined as main effects, the day effect nested in host plant and genotype, and the interaction between host plant and genotype. Sex was included in each analysis and reported where significant. Interactions between sex and host plant and sex and genotyype were included in the analysis of each life-history trait but were not found to be significant and were removed from the analyses. Genotype and host plant effects were compared using Student’s t multiple comparisons on least square means produced from the full general linear model. Genotypic effects were further compared within each host plant treatment group using multiple comparisons. Unemerged pupae were not included in the analysis of pupal weight. Response variables were square root or log transformed where appropriate to improve normality and homogeneity of variance (Zar 1996).
5.3 Results

Bt selections

Resistance to Bt increased in 5 of 7 generations of selection of the PR population (Table 5.1). The LC₅₀ of the PR population was 8.0 kIU/ml diet in the first generation which increased to 334 kIU/ml diet after 5 generations of selection. A slight decline in resistance was observed in the following two generations which were selected at the highest Bt dose (160 kIU/ml diet). The PR population was unselected for one generation prior to the establishment of the T. ni lines at which point LC₅₀ values of the parental PR and PS lines were 113 and 2.5 kIU/ml diet respectively.

Host Plant Effects

The host plant had a significant effect on all life-history traits measured (Table 5.2 and 5.3). Over all genotypes, pepper was the poorest host plant for T. ni larvae. The mortality rate was 5-fold higher on pepper leaf pieces as compared to cucumber and tomato leaves, and larval growth rate was 30% slower on pepper as compared to the other host plants. The longer development time on pepper, however, did not result in larger pupae, since pupal weight was 30% smaller in the pepper treatment group as compared to the other host plant groups. Therefore, relative to cucumber and tomato leaves, pepper leaves provide a poor resource for T. ni growth.

Mortality rates of T. ni larvae were similar on tomato and cucumber leaves. There were, however, significant differences in the growth rate and final pupal size between the two host plants. Larvae weighed 110±4 mg at day 10 when feeding on tomato leaves as compared to 161±4 mg when feeding on cucumber. This difference in growth rate corresponded to a decrease in time to pupation from 14.7±0.1 to 13.5±0.1 days in the
Table 5.1: History of selection of the P_R strain after crossing to the unselected sister line.

<table>
<thead>
<tr>
<th>lab generation</th>
<th>no. selected</th>
<th>selective dose (kIU/ml diet)$^a$</th>
<th>% survivorship to pupation</th>
<th>LC$_{50}$ (kIU/ml diet)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>396</td>
<td>0</td>
<td>100</td>
<td>10.9</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>0</td>
<td>100</td>
<td>10.9</td>
</tr>
<tr>
<td>3</td>
<td>684</td>
<td>20</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>679</td>
<td>40</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>785</td>
<td>40</td>
<td>19.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>513</td>
<td>80</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>802</td>
<td>160</td>
<td>36.5</td>
<td>334</td>
</tr>
<tr>
<td>8</td>
<td>402</td>
<td>160</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>480</td>
<td>160</td>
<td>24.8</td>
<td></td>
</tr>
</tbody>
</table>

a. 1 mg DiPel WP = 16 kIU

Table 5.2: Wald Chi-square tests of the survival of larvae of the five genotypic classes on the three different host plants including the effect of day.

<table>
<thead>
<tr>
<th>Survival</th>
<th>df</th>
<th>$\chi^2$</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>genotype</td>
<td>4</td>
<td>2.6</td>
<td>0.63</td>
</tr>
<tr>
<td>host plant</td>
<td>2</td>
<td>19.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>plant x genotype</td>
<td>8</td>
<td>40.8</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>day</td>
<td>6</td>
<td>73.4</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Table 5.3: Analysis of variance tables for larval weight, growth rate and pupal weights analyses. (Growth rate and pupal weight results are restricted to tomato and cucumber treatments as no \( P_R \) or \( P_R \)sel genotypes pupated on pepper.)

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>SS</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>larval weight</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotype</td>
<td>4</td>
<td>76.3</td>
<td>18.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>host plant</td>
<td>2</td>
<td>1705.4</td>
<td>817.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>plant x genotype</td>
<td>8</td>
<td>74.2</td>
<td>8.9</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>block[plant, genotype]</td>
<td>23</td>
<td>274.9</td>
<td>11.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>time to pupation</strong> [cucumber and tomato only]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotype</td>
<td>4</td>
<td>2.39</td>
<td>12.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>host plant</td>
<td>1</td>
<td>4.01</td>
<td>84.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>plant x genotype</td>
<td>4</td>
<td>1.67</td>
<td>6.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>block[plant, genotype]</td>
<td>16</td>
<td>4.24</td>
<td>5.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>0.49</td>
<td>10.4</td>
<td>0.0013</td>
</tr>
<tr>
<td><strong>pupal weight</strong>  [cucumber and tomato only]</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>genotype</td>
<td>4</td>
<td>11.4</td>
<td>2.7</td>
<td>0.043</td>
</tr>
<tr>
<td>host plant</td>
<td>1</td>
<td>4.1</td>
<td>3.9</td>
<td>0.162</td>
</tr>
<tr>
<td>plant x genotype</td>
<td>4</td>
<td>21.9</td>
<td>5.3</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>block[plant, genotype]</td>
<td>16</td>
<td>134.9</td>
<td>8.1</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>sex</td>
<td>1</td>
<td>105.4</td>
<td>101.3</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
tomato and cucumber environments respectively. In contrast, the final pupal size was smaller in the cucumber treatment group at 213±2 mg as compared to 222±2 mg for the tomato treatment group.

**Genotype-Plant Interactions**

A significant effect of genotype occurred over all the host plant treatment groups on each life-history trait measured except survivorship (Tables 5.2 and 5.3). Furthermore, a significant interaction between the genotype and the host plant demonstrated that the presence and magnitude of resistance-associated fitness costs varied with the host plant environment (Figure 5.1). Male pupae were significantly larger than female pupae and the day effect was highly significant (Table 5.3).

No differences in larval survivorship occurred among the genotypes when fed cucumber leaves, however the resistant genotype (P<sub>R</sub>) experienced significantly lower survival than the other genotypes when fed tomato and pepper leaves (Table 5.4). No larvae of the P<sub>R</sub> genotype survived to pupation on pepper. Similarly, larvae with the P<sub>R</sub>sel genotype exhibited limited survival on pepper leaves and differed significantly from the other genotypes. In contrast, in the tomato environment, survival of P<sub>R</sub>sel was significantly higher than P<sub>R</sub> and equivalent to the other genotypes. This difference suggests the presence of positive transgenerational effects in the tomato environment.

Larval weights of the two P<sub>R</sub> and P<sub>R</sub>sel resistant genotypes were significantly smaller than those of the susceptible P<sub>S</sub> or hybrid genotypes when feeding on pepper. On tomato, larval weights of the P<sub>R</sub> genotype were smaller than the susceptible P<sub>S</sub> genotype, whereas P<sub>R</sub>sel larval weights were equivalent to the susceptible P<sub>S</sub> genotype in this environment. In the cucumber treatment group, no differences in larval weights were
Figure 5.1: Least square mean larval weights (±se) of resistant (P_R), hybrid (F_{1f}, F_{1m}), and susceptible (P_S) genotypes feeding on cucumber, tomato or pepper.

(Significant differences within host-plant groups denoted as different letters).
Table 5.4: Percent mortality, natural log transformed larval weight at day 10, square-root transformed time to pupation and square-root transformed pupal weight are shown for the unselected resistant population (PR), selected resistant population (PRsel), susceptible population (PS) and the reciprocal F1f (resistant female) and F1m (resistant male) hybrids. (Least square means and standard errors from the general linear model analysis are presented. Significant differences between genotypes are denoted as different letters: a,b or c.)

<table>
<thead>
<tr>
<th></th>
<th>PR</th>
<th>PRsel</th>
<th>PS</th>
<th>F1f</th>
<th>F1m</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cucumber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>survivorship [%]</strong></td>
<td>66.3 a</td>
<td>80.9 a</td>
<td>72.0 a</td>
<td>77.1 a</td>
<td>75 a</td>
</tr>
<tr>
<td><strong>larval weight</strong></td>
<td>4.77 ±0.16 ab</td>
<td>4.57 ±0.37 ab</td>
<td>4.36 ±0.12 a</td>
<td>4.98 ±0.10 b</td>
<td>4.99 ±0.11 b</td>
</tr>
<tr>
<td><strong>time to pupation</strong></td>
<td>3.73 ±0.05 a</td>
<td>3.74 ±0.09 a</td>
<td>3.59 ±0.04 b</td>
<td>3.58 ±0.03 b</td>
<td>3.64 ±0.03 ab</td>
</tr>
<tr>
<td><strong>pupal weight</strong></td>
<td>0.463 ±0.007 a</td>
<td>0.461 ±0.004 a</td>
<td>0.452 ±0.005 a</td>
<td>0.462 ±0.004 a</td>
<td>0.462 ±0.004 a</td>
</tr>
<tr>
<td><strong>tomato</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>survivorship [%]</strong></td>
<td>53.6 a</td>
<td>68.3 b</td>
<td>76.4 b</td>
<td>72.9 b</td>
<td>62.7 b</td>
</tr>
<tr>
<td><strong>larval weight</strong></td>
<td>3.58 ±0.10 a</td>
<td>4.18 ±0.10 b</td>
<td>4.17 ±0.11 b</td>
<td>4.89 ±0.10 c</td>
<td>4.14 ±0.12 b</td>
</tr>
<tr>
<td><strong>time to pupation</strong></td>
<td>4.03 ±0.04 a</td>
<td>3.83 ±0.03 b</td>
<td>3.83 ±0.03 b</td>
<td>3.70 ±0.03 c</td>
<td>3.80 ±0.03 b</td>
</tr>
<tr>
<td><strong>pupal weight</strong></td>
<td>0.442 ±0.005 a</td>
<td>0.476 ±0.003 c</td>
<td>0.469 ±0.004 bc</td>
<td>0.477 ±0.003 c</td>
<td>0.465 ±0.004 b</td>
</tr>
<tr>
<td><strong>pepper</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>survivorship [%]</strong></td>
<td>0 a</td>
<td>1.9 a</td>
<td>13.3 b</td>
<td>7.5 b</td>
<td>23.7 6 c</td>
</tr>
<tr>
<td><strong>larval weight</strong></td>
<td>-0.16 ±0.27 a</td>
<td>0.29 ±0.24 a</td>
<td>1.34 ±0.14 bc</td>
<td>0.96 ±0.15 b</td>
<td>1.42 ±0.11 c</td>
</tr>
<tr>
<td><strong>time to pupation</strong></td>
<td>-</td>
<td>-</td>
<td>4.79 ±0.09 a</td>
<td>5.13 ±0.08 b</td>
<td>4.96 ±0.09 a</td>
</tr>
<tr>
<td><strong>pupal weight</strong></td>
<td>-</td>
<td>-</td>
<td>0.388 ±0.014 a</td>
<td>0.392 ±0.013 a</td>
<td>0.392 ±0.013 a</td>
</tr>
</tbody>
</table>

1. Only the susceptible populations and F1 hybrids were included in the analysis of pupal weight and time to pupation.
observed between P_R, P_Rsel and P_S genotypes. Therefore, negative effects of the resistance genotype on larval weight were not observed on the best host plant, cucumber, but were present on the less favorable tomato and pepper host plants. However with respect to time to pupation, the P_R genotype did take significantly longer to reach pupation than the P_S genotype in both the tomato and cucumber environments. Whereas, the P_Rsel resistant genotype only experienced an extended time to pupation in the cucumber environment. Therefore, the presence of resistance-associated fitness costs did not extend to all measured life-history traits in every environment. Furthermore, the presence of resistance-associated fitness costs observed for the P_R genotype did not always extend to the P_Rsel genotype.

The larval weights of the hybrid genotypes were significantly larger than the P_S larvae in the cucumber environment suggesting the presence of heterosis. However, the observed hybrid advantage disappeared in the other environments and the life-history traits appeared to be affected by the genotype of the maternal parent. F_{1f} larvae, derived from a cross between P_R females and P_S males, were significantly larger than the other genotypes in the tomato environment, whereas F_{1f} larvae were significantly smaller than susceptible P_S and the reciprocal hybrid F_{1m} larvae in the pepper environment. Therefore, positive maternal effects were observed in the tomato environment and negative effects were observed in the pepper environment. These parental effects extended through development, as shown by the reduced development time of the F_{1f} genotype on tomato relative to the other genotypes and the extended development time on pepper. Furthermore, the reduced survival of F_{1m} larvae on pepper leaves relative to other genotypes also suggests the presence of negative maternal effects on this host plant.
Interestingly, pupal weights, the primary correlate of fecundity, did not differ between the genotypes in the cucumber treatment group suggesting the absence of sizeable fitness costs in this environment. In contrast, there was a significant reduction in pupal weight observed for the Pr genotype relative to the other genotypes in the tomato environment. This genotypic effect did not extend to the PrSEL genotype which produced the largest pupal size, along with the F1f genotype, in the tomato environment. Therefore, transgenerational effects due to the Bt exposure of the parental PrSEL population appeared to compensate for the small pupal weight that was observed for the Pr line not exposed to Bt in the parental generation. In the pepper treatment group, comparisons could only be made between the Ps and hybrid genotypes and no significant differences were found. A summary table of the host plant and genotypic effects on the magnitude and expression of fitness costs associated with Bt resistance is presented in Table 5.5.
Table 5.5: Summary of the genotype x host plant effects for each life history trait. (Fitness costs were estimated as percent decreases in survival, larval weight, time to pupation, or pupal weight. No PR pupae were obtained in the pepper environment (NA=not available).)

<table>
<thead>
<tr>
<th></th>
<th>survival</th>
<th>larval weight</th>
<th>time to pupation</th>
<th>pupal weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>cucumber</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost</td>
<td>none</td>
<td>none</td>
<td>3.9; 4.1%</td>
<td>none</td>
</tr>
<tr>
<td>maternal effect</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>transgenerational effect</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td><strong>tomato</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost</td>
<td>29; 10%</td>
<td>14; 0%</td>
<td>5.2; 0%</td>
<td>5.7; 0%</td>
</tr>
<tr>
<td>maternal effect</td>
<td>none</td>
<td>+</td>
<td>+</td>
<td>none</td>
</tr>
<tr>
<td>transgenerational effect</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td><strong>pepper</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cost</td>
<td>100; 72%</td>
<td>98; 70%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>maternal effect</td>
<td>none</td>
<td>none</td>
<td>NA</td>
<td>none</td>
</tr>
<tr>
<td>transgenerational effect</td>
<td>none</td>
<td>none</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

1. Maternal effect is positive when the F₁f hybrid measured value is higher than the F₁m hybrid and negative when the F₁f hybrid measured value is lower than the F₁m.
2. The transgenerational effect is positive when the measured fitness cost of the PR sel population is less extreme than that of the PR population that was untreated with Bt in the previous generation.
5.4 Discussion

Host Plant Effects

Despite being a generalist herbivore, the performance of *T. ni* larvae varied considerably among the three different host plants. Larvae exhibited the most rapid growth on cucumber, reduced growth rates on tomato and the slowest growth rate on pepper leaves. Pupal size and larval growth rates are directly correlated with potential fecundity in many phytophagous insects, whereas increases in development time are also associated with an increased risk of predation or parasitism (Awmack & Leather 2002). Since pupal size and development time are often positively correlated, this results in a trade-off between the advantages of large size at maturity and disadvantages of a long development time (Nylin & Gotthard 1998). Therefore, it was not surprising that an increase in development time from the cucumber to the tomato environment was accompanied with an increase in pupal weight. An increase in development time was not associated with an increase in pupal weight in the pepper treatment group, however, and this indicates that pepper was a poor nutritional host for *T. ni*. These results agree with historical studies of the host range of *T. ni* in which *T. ni* took seven days longer to develop on pepper than on tomato (Sutherland 1966). The poor performance of *T. ni* on pepper is likely due to the negative effects of defensive compounds, such as phenolics (Estiarte et al. 1994), or poor nutritional quality. The underlying cause of the extended development time of *T. ni* larvae feeding on tomato is unknown, although tritrophic interactions that reduce the risks associated with development time might provide an answer (Kennedy 2003).
Fitness Costs

The resistant genotype exhibited the slowest growth rate in all host plant environments relative to the other genotypes and the reduced growth rate did not translate into a larger final size. Therefore, Bt resistance in this T. ni population was associated with a fitness cost, and this suggests the presence of a trade-off in the distribution of resources between resistance and growth. In the current study, we are unable to discern if the fitness cost was due to direct effects of the resistance trait on life-history (i.e. pleiotropy) or due to detrimental linkage with other genes. A more rigorous genetic protocol than was conducted in this study, with repeated introductions of the resistant gene into the same genetic background, would be required to distinguish between pleiotropy or detrimental linkage (Bergelson & Purrington 1996). Detrimental linkage rather than pleiotropy was determined to be the cause of fitness costs associated with herbicide resistance in half of the studies reviewed by Bergelson & Purrington 1996.

The dynamics of resistance evolution when resistance frequencies are low is dependent on the relative fitness of the hybrid compared to the susceptible genotype (Roush & McKenzie 1987). When at low frequencies resistance alleles would primarily be present in hybrids, and if fitness costs were dominant this would reduce the rate at which resistance would increase in the population. However for all life-history traits and in all host plant treatment groups examined in this study, fitness costs were not present in the reciprocal hybrids. Similarly, fitness costs associated with resistance to Bt-expressing transgenic cotton in Pectinophora gossypiella were recessive (Carrière et al. 2001a; Carrière et al. 2001b). In contrast, fitness costs associated with resistance to organophosphates in Culex pipiens (Chevillon et al. 1997) or cadmium in Drosophila
(Shirley & Sibly 1999) were dominant, and costs associated with dieldrin resistance varied from additivity to co-dominance in *Lucilia cuprina* (McKenzie 1990). Therefore, the level of dominance of fitness costs varies considerably among different resistance traits and species. In addition, with longterm selection and high resistance allele frequencies, the deleterious effects associated with resistance can be ameliorated through the actions of modifier genes rendering the previously dominant fitness cost recessive (Clarke 1997; McKenzie & O'Farrell 1993; Chevillon et al. 1997).

**Genotype by Environment Interactions**

Because the availability of resources for either defense or growth/reproduction is dependent on the host plant, the differentiation between genotypes is expected to increase in stressful host plant environments (Parsons 1991). As expected, the magnitude of resistance-associated fitness costs was negatively correlated with the suitability of the host plant environment. No resistant individuals survived on pepper leaves, the least suitable host plant, whereas minimal decreases in larval growth rate and no significant difference in pupal size of resistant versus susceptible genotypes were observed in the best host plant environment, cucumber. Intermediate fitness costs were observed in the tomato treatment group. Therefore, the differentiation among genotypes increased with decreasing host plant suitability increasing the selection against resistant genotypes. Similarly, resistance-associated fitness costs have been shown to increase during the overwintering period, a period of extreme environmental stress. Fitness costs were observed to increase in severity in pink bollworm, *Pectinophora gossypiella*, resistant to *Bt* (Carrière et al. 2001a), Colorado potato beetle resistant to Cry3A (Alyokhin & Ferro 1999), some genotypes of the overwintering mosquito species resistant to
organophosphates, (Chevillon et al. 1997) and in L. cuprina resistant to dieldrin (McKenzie & Batterham 1994).

Transgenerational and Maternal Effects

Transgenerational effects resulting from the ingestion of Bt in the parental generation were evident for all life-history traits examined, but only in the tomato environment. Fitness costs were apparent in the P_R population feeding on tomato leaves, but the growth rate and pupal weights of the P_RSsel population did not differ significantly from the P_S population. Therefore, exposure of the parental generation to Bt appeared to increase fitness of the resistant genotype in the tomato environment. Similarly, larvae from the P_RSsel population were larger than the P_R population when grown on diet in a separate preliminary experiment (seven day larval weight: 2.44±0.09; 2.05±0.14 square root transformed least square means for P_RSsel and P_R respectively). In the pepper environment, parental exposure to Bt did not alleviate fitness costs in the resistant genotype. It is possible, that a non-genetic maternal effect such as increased egg provisioning, might have ameliorated the negative genotypic effects in the tomato environment, but these effects might not have been large enough to alter development in the extreme pepper environment. Trade-offs in egg-provisioning and fecundity are a common characteristic of many Lepidopteran species (Awmack & Leather 2002).

Exposure of the parental generation to poor conditions can cause the female to increase provisioning to individual eggs with a subsequent reduction in fecundity. In addition to the transgenerational effect, a significant maternal effect was observed between the genotypes feeding on tomato leaves. The growth rate and pupal size of the F_1f genotype was higher than all other genotypes suggesting that the resistant genotype of the mother
had a positive effect on the development of larvae in the tomato environment. This effect was not observed in the cucumber environment and was negative in the pepper environment with a reduced growth rate of the F₁ genotype relative to the Pₛ genotype.

The presence of both transgenerational and maternal effects in the tomato environment might have been due to the genetic background of the T. ni population, because the original population, which gave rise to the Pₐ and Pₛ lines, was selected for resistance to Bt in a tomato greenhouse. Greenhouses provide an ideal environment for long-term selection on a host plant. T. ni populations undergo multiple generations per year within crop-specific greenhouses (ie. 6+ generations per year) and can survive in greenhouses during the winter clean-up period (V. Cervantes personal communication). These conditions select for the genotype best adapted to growth on a specific host plant, and this selection is coupled with intense selection for Bt resistance. Bt remains active in greenhouses for an extended period (ie. >9 days; preliminary experiment) and is often applied multiple times during each growing season. Therefore, adaptation to both Bt and tomato in the studied T. ni population might explain the presence of complex genetic interactions (ie. maternal and transgenerational effects) observed in the tomato treatment group. The formation of host races in different phytophagous insect species in response to selection has been shown to occur in as little as 10 years (Bernays & Graham 1988). Complex genetic interactions are gaining recognition in both the study of insecticide resistance (McKenzie, Whitten, & Adena 1982) and in the local adaptation of phytophagous insects to alternative host plants (de Jong & Nielsen 2002).

Many proposed resistance management strategies rely on the presence of resistance-associated fitness costs. Using insecticide rotations (Ferré & van Rie 2002) or
temporal refuges (periods with no Bt exposure) (Tabashnik et al. 1994) as management strategies requires that resistance declines when selection ceases. The rate of decline in resistance is associated with the strength of associated fitness costs and the immigration rate of susceptible individuals (Tabashnik & Croft 1982). Because the magnitude of fitness costs varies with host plant, the appropriateness of these resistance management strategies will vary with the crop. Furthermore, these resistance management strategies will be least effective in the host plant environment in which T. ni has the most rapid growth rate (i.e. cucumber). This finding suggests that insecticidal controls will be less effective in the long run and indicates the need for preventative control methods (i.e. biological control agents) that will maintain T. ni population below economic threshold levels.

Due to the presence of associated fitness costs, the frequency of resistance genes in the environment is expected to be extremely low (i.e. <10^{-3}) (Georghiou & Taylor 1986). This allelic frequency is predicted to be maintained by the balance between the mutation of new alleles and selection against these new deleterious alleles. However since the deleterious effects of new mutations are often environmentally specific (Kondrashov & Houle 1994), it is predicted that new mutations will persist if they are deleterious only in rare and unfavorable environments and not in the common favorable environment (Hoffman & Merila 1999). Selection will act to remove the new mutations only in the rare unfavorable environments. Therefore, it is possible that mutations that confer resistance to Bt might persist at frequencies higher than expected if their deleterious effects are expressed only in rare environments.
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Chapter VI: Effect of parental host plant and resistance genotype on parental and progeny size

6.1 Introduction

Many polyphagous insect herbivores, such as cabbage loopers, *Trichoplusia ni*, are serious economic pests. *T. ni* feeds on over 160 host plant species (Sutherland & Greene 1984) and is a major pest of cruciferous crops (Shelton, Andaloro, & Barnard 1982). In addition, it is a significant pest of vegetable greenhouse crops (Janmaat & Myers 2003) and is damaging to other field crops such as cotton and soybean (Jost & Pitre 2002) in North America. The ability of *T. ni* to use a wide variety of hosts both concurrently and in succession is key to its pervasiveness as a pest species. However, its performance varies considerably among different host plants. In host acceptance studies, *T. ni* exhibits high fecundity and rapid growth when grown on cruciferous crops but has a longer development time and decreased fecundity on pepper (Sutherland 1966).

The effects of the parental host plant often extend to the next generation via transgenerational effects (Rossiter 1996). Nutritional deficiencies are often transmitted from the parental generation to progeny such that offspring from nutritionally deprived parents are less competitive than progeny from well-fed parents (Fox & Mousseau 1998). Such transgenerational effects can impede or accelerate the response of an insect population to its environment and ultimately determine the long-term persistence of the population (Rossiter 1996). If the parental environment is a reliable indicator of the offspring environment, it may be beneficial for the parental generation to condition progeny for the predicted environment. For example, in many insects exposure of the maternal generation to cooling temperatures or reduced day lengths triggers diapause in the progeny generation (Tauber, Tauber, & Masaki 1986). This cross-generation
conditioning is referred to as transgenerational phenotypic plasticity (Fox & Mousseau 1998). The nutritional experiences of the parental generation may provide a reliable indicator of the progeny’s future food source and transgenerational phenotypic plasticity could be beneficial.

Other environmental stressors, such as disease or chemical toxins, can select for resistance that is often associated with fitness costs (Orr & Coyne 1992; Carrière et al. 1994; Mcnair 1991). This too may influence transgenerational plasticity. Resistant individuals are thought to partition a greater proportion of resources to the maintenance of resistance traits, and this thereby reduces the available resources for growth and/or reproduction (Bergelson & Purrington 1996; Carrière et al. 1994). Constraints on the availability of resources may impact characters associated with reproduction, such as transgenerational plasticity.

The range of suitability of host plants for T. ni development suggests that transgenerational effects may be pervasive in this species. The performance of T. ni varies considerably among the three major vegetable crops grown in commercial greenhouses (cucumber, tomato and pepper). Surveys of progeny from parental T. ni populations grown on different host plants indicated a significant effect of parental host plant on progeny growth. In addition, resistance to the microbial insecticide, Bacillus thuringiensis, has been recently detected in greenhouse populations of T. ni and is associated with significant fitness costs (Janmaat & Myers 2003). Here, I examine the effects of three different host plants on T. ni growth across four T. ni genotypic lines (resistant, susceptible and two reciprocal hybrid crosses) and the effects of the parental host plant on progeny size.
6.2 Methods

*T. ni* larvae were reared on each of three host plants to examine effects on the growth of the parental generation and influences of the parental environment on progeny growth in a common environment. The effects were examined across four genotypes which consisted of two parental strains (P_s and P_R) and their reciprocal hybrids (F_1f, F_1m). In the present chapter, the parental strains were derived from a source population (P5) that differed from the one measured in chapter V (T2c). Therefore, effects of the host plant on the parental generation were remeasured for pupal weight in case there were differences.

*History of T. ni Colonies*

A *T. ni* colony, resistant to *Bt*, was initiated from 74 individuals collected from a pepper commercial greenhouse in British Columbia, Canada in 2001 (labeled P5 in Janmaat & Myers 2003). Two colonies were initiated in the laboratory from the collected field population and reared on a wheat-germ based artificial diet according to methods described in (Janmaat & Myers 2003). One colony, the susceptible population (P_s), was reared without any *Bt* exposure and exhibited a significant decrease in resistance after 7 unselected generations in the laboratory (from 49.5 to 4.3 kIU/ml artificial diet) (Janmaat & Myers 2003). The other colony, the resistant population (P_R), was selected with *Bt kurstaki* (DiPel WP Valent Biosciences) during each generation of lab culture to maintain resistance (Chapter III).

*T. ni Genotypic Lines*

From the resistant and susceptible populations, four genotypic lines were produced. These genotypic lines were grown on each host plant to examine if the growth of parental
and progeny generations varied across genotypes. One generation prior to initiation of
the experiment, individuals from the resistant colony (PR) were reared on wheat-germ
diet without the addition of Bt to reduce potential transgenerational effects (ie. maternal
effects). For three generations prior to the experiment, the PS population had been reared
on pepper leaves and for 9 earlier generations the PS population had been reared on
wheat-germ diet. In total, the PS population had not been exposed to Bt for 12
generations.

Pupae from each population were sexed and mass crosses of 40 males and 40
females were initiated to produce four genotypic lines: resistant and susceptible (PR and
PS respectively) and two reciprocal hybrids (F1f: the resistant parent is the female and
F1m: the resistant parent is the male). The four genetic lines were assayed on Bt-wheat-
germ diet mixtures to assess resistance level (for methods see Janmaat & Myers 2003).
The LC50 values (95% confidence interval) of the different genotypes were 26.0 (16.9-
39.9), 5.1 (3.4-7.6), 4.3 (2.9-6.3), and 3.5 (2.4-5.0) kIU/ml diet for the PR, F1f, F1m and
PS genotypes respectively.

Host Plants and T. ni Growth Measurements

Each genotypic line was then reared on three different host plants to produce the
parental generation. The different host plants (pepper: variety 444; tomato: variety
Rapsodie; and cucumber: variety Ventura) were grown under greenhouse conditions at
the University of British Columbia, Canada from August to October 2002. Plants were
used ~2 months after planting. Neonates were placed onto leaf pieces contained in 60 ml
plastic cups (5 neonates/cup). The plastic cups were placed inside covered seedling flats
lined with moistened paper toweling to maintain the turgidity of the leaf pieces. All leaf
pieces were rinsed with a dilute hypochlorite solution (15 ml Hypochlorite diluted in 4L of water) to remove pathogens and residues of a whitefly infestation. After 3 days of feeding, surviving larvae were transferred to 175 ml Styrofoam cups and maintained as in Chapter V with the exception that in the present experiment, larvae were reared in groups until they reached the fourth instar.

Sixty neonates from each genotype were placed onto leaf pieces from each host plant treatment group and reared until pupation. Pupae were weighed 2 days after pupation and sexed. Male and female pupae were paired within their respective genotype and host plant treatment group. Pairs were placed into 240 ml paper cups, kept at room temperature, supplied with 10% sugar solution. The cups were lined with black construction paper for oviposition which was changed every three days after adult emergence. The number of eggs was counted for each pair to obtain a fecundity estimate.

Eggs produced by each genotypic pair were hatched and 25 offspring per pair were placed individually onto 1 ml of wheat-germ diet in 30 ml plastic cups that were kept at 26°C. Offspring were weighed after 7 days of growth. Larvae from 2-5 pairs were weighed for each genotype host-plant combination.

Statistical Analyses

Pupal weights, fecundity and offspring size were analyzed using a general linear model (glm) in JMPIN 4.0 with host plant and genotype and their interaction defined as main effects. Sex was included as a factor in the pupal analysis, and the adult pair nested in host plant and genotype was included as a random factor in the offspring size analysis. The cup was included as a variable in the initial analyses and was not found to significantly affect the measured variables and therefore was removed from the final
analyses. Offspring size was analyzed over all genotypes initially without a host-plant x genotype interaction factor due to the absence of offspring from the PR pepper treatment group. Offspring size was then reanalyzed, excluding the PR genotype, to test for the presence of a host-plant x genotype interaction. Offspring size was then reanalyzed, excluding the PR genotype, to test for the presence of host plant x genotype interactions. Genotype and host plant effects were compared using Student’s t multiple comparisons on least square means produced from the full general linear model. Genotypic effects were further compared within each host plant treatment group. Response variables were square root transformed where appropriate to improve normality and homogeneity of variance and transformations are described in the results (Zar 1996).
6.3 Results

The influence of the host plant on growth of both the parental generation and on
the progeny size was measured. First, I report the effects of host plant on the parental
generation followed by a description of the transgenerational effects of the parental host
plant on the progeny generation reared on a common diet. The effects of the parental
host plant on both generations is further separated by resistance genotype.

Parental Generation

Pupal weights of the parental generation significantly differed between host plant
treatment groups demonstrating that the host plants differ in suitability for *T. ni* growth
(Table 6.1). Pupae attained the largest size when fed cucumber leaves, the smallest size
when fed pepper leaves and an intermediate size when fed tomato leaves. The pupal
weights were 187±2 mg, 180±3 mg and 152±3 mg for the cucumber, tomato and pepper
treatment groups respectively (mean±se). Therefore, the pupal weight decreased by 20%
from the best host plant (cucumber) to the worst host plant (pepper). Male pupae were
significantly larger than female pupae (male: 183±2 mg; female: 163±2 mg). These
results were consistent with those observed in chapter V.

Genotype did not have an overall significant effect on pupal weight; its effect
varied, however, with host plant as demonstrated by the significant genotype by host
plant interaction (Table 6.1). The pupal size of the resistant *P* _R_ genotype (175 ±4 mg)
was smaller than the susceptible *P* _S_ (189 ±5 mg) and the *F* _1_ _m_ hybrid (196 ±4 mg)
genotypes when fed cucumber, whereas there were no significant differences between
genotypes on the other two host plants (Genotype Effect within Cucumber: *F*=4.17;
df=3,102; *p*=0.008; Tomato: *F*=0.36; df=3,45; *p*=0.78; Pepper: *F*=2.42; df=3,56; *p*=0.08).
Table 6.1: Analysis of variance tables for pupal weight, fecundity and offspring weight analyses. The effects of host plant and genotype on offspring weight were analyzed both with and without the resistant $P_R$ genotype due to the absence of the $P_R$-pepper treatment group. The response variable fecundity was square root transformed and offspring weight was log transformed in all analyses.

<table>
<thead>
<tr>
<th></th>
<th>DF [num., denom.]</th>
<th>SS</th>
<th>F</th>
<th>p</th>
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<tr>
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<tr>
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<td>7.96</td>
<td>0.001</td>
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<td>0.26</td>
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<td></td>
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<tr>
<td><strong>Offspring Weight</strong> [without $P_R$]</td>
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<td></td>
<td></td>
<td></td>
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<td>0.08</td>
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<tr>
<td>Host Plant</td>
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<td>4.35</td>
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<td>0.97</td>
<td>0.45</td>
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<td>18, 599</td>
<td>112.3</td>
<td>3.96</td>
<td>&lt;0.0001</td>
</tr>
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</table>
In contrast to chapter V, a resistance-associated fitness cost was observed on the
best host (cucumber) rather than on poorer host plants. However, considerably more
individuals survived to pupation on cucumber (53%) as compared to the other treatment
groups (22% and 25% for tomato and pepper respectively) which caused the sample size
in the cucumber group to be 2-fold higher than in the pepper or tomato group. The larger
sample size in the cucumber group may have increased the statistical power and
likelihood that an effect would be detected.

The negative effect of pepper on pupal size was reflected in the fecundity of the
individual pairs (Table 6.1). Pairs that developed on pepper leaves produced 30% fewer
eggs than pairs fed cucumber leaves, and 21% fewer eggs than pairs fed tomato (Figure
6.1). In addition, the genotype also significantly influenced fecundity. The resistant P_R
genotype exhibited a 30% reduction in fecundity relative to the other genotypes (Figure
6.2). This reduction in fecundity was observed despite the non-consistent effect of
genotype on pupal size. Only one P_R pair in the pepper treatment group produced viable
offspring with extremely low fecundity (75 eggs). Therefore, resistance to Bt was
associated with an extreme fitness cost in this population. With respect to fecundity,
there was no interaction between genotype and host plant, in contrast to the effects
observed on pupal size.

Progeny Generation

The weight of offspring, after seven days of growth in on a common diet,
significantly varied among genotypes and host plants of their parents. The largest
offspring were produced by pairs that had developed on pepper leaves, the smallest were
produced by pairs fed cucumber leaves, and intermediate offspring were produced by
pairs fed tomato leaves (Figure 6.1). Therefore, the pepper treatment group, which was associated with the lowest fecundity, was also associated with the largest offspring size. Conversely, the cucumber treatment group was associated with the highest fecundity, and the smallest offspring. The results together suggest that there is a trade-off between egg number and offspring size (Figure 6.1).

The genotype of the parental generation also affected offspring size (Table 6.1). The resistant PR genotype produced the smallest offspring relative to the other treatment groups (Figure 6.2). The PR offspring were 20% smaller than their susceptible counterparts. The hybrid offspring appeared to experience no negative effects, as there were no significant differences in offspring size between the hybrid and susceptible genotypes. The small offspring size of the PR genotype was coupled with reduced fecundity demonstrating the presence of severe resistance-associated fitness costs. These severe costs likely contributed to the lack of viable offspring produced by PR pairs fed pepper leaves.
Figure 6.1: Offspring weight versus fecundity of *T. ni* pairs reared on different host plants.

![Graph showing offspring weight versus fecundity of *T. ni* pairs reared on different host plants.]

Figure 6.2: Offspring weight versus fecundity of different *T. ni* genotypes. $P_R =$ resistant, $F_{1f} =$ hybrid with resistant mother, $F_{1m} =$ hybrid with resistant father, $P_S =$ susceptible.

![Graph showing offspring weight versus fecundity of different *T. ni* genotypes.]

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6.4 Discussion

Variation in the size of progeny both within and among insect populations is common, and this variation can have large effects on individual fitness (Fox & Mousseau 1996). Progeny size and growth rates are often determined by egg size. Typically, progeny from larger eggs survive better, develop faster and emerge as larger adults than progeny from smaller eggs (Fox & Mousseau 1996). However, the advantages of larger egg size are not always demonstrated, and large size appears to be most beneficial in adverse environments (Fox & Mousseau 1996). In the present study and in chapter V, the suitability of the three host plants for T. ni larvae varied from the best host plant, cucumber, to the worst host plant, pepper. In both studies, tomato leaves appeared to provide a host of intermediate quality. Therefore, pepper is an adverse environment for T. ni growth.

Typically, females that experience poor conditions often exhibit reduced fecundity and produce progeny that are less vigorous (Rossiter 1991). Therefore, under poor nutrient conditions it is expected that reduced fecundity would be correlated with reduced progeny size. Interestingly in the present experiment, a negative relationship between fecundity and offspring size was observed across the three host plant treatment groups. Offspring of the most fecund cucumber treatment group were significantly smaller than offspring of the least fecund pepper treatment group. Furthermore, both offspring size and fecundity of pairs fed tomato leaves were intermediate to the other host-plant treatment groups. The cause of the difference in offspring growth is uncertain and may be due to genetic responses to selection in the different host plant environments or due to non-genetic effects of the parental environment on progeny growth. Intense
selection during one generation may have resulted in the survival of only rapidly growing genotypes in the pepper environment. However, these results likely suggest transgenerational plasticity such that progeny size altered in the parental generation, better suits the environmental conditions expected in the next generation (Fox & Mousseau 1998) which is consistent with life-history theory (Sibly & Calow 1983).

Such transgenerational plasticity in progeny size has been well documented in the *Stator limbatus*, seed beetle (Mousseau & Fox 1998). *Cercidium floridum* is a poor quality host for *S. limbatus* as shown by the poor egg-to-adult survivorship and the long development time of *S. limbatus* on *C. floridum* relative to the other hosts, *Acacia greggii* and *A. microphyllum*. The performance of *S. limbatus* on *C. floridum* is enhanced if progeny hatch from large eggs, but offspring from small eggs are sufficient for development on the better quality hosts (Fox & Mousseau 1996). In nature, larger eggs are associated with *C. floridum* (Fox & Mousseau 1996), and *S. limbatus* females have been observed to readjust egg size according to the host plant (Fox, Thakar, & Mousseau 1997). Therefore, *S. limbatus* females adaptively alter egg size to increase progeny fitness. This plasticity comes at a cost due to a trade-off between egg size and number. Thus, females are at a selective advantage if they lay many small eggs in good environments and fewer larger eggs in poor environments.

Transgenerational plasticity is only beneficial, however, if the parental generation is able to predict the environmental conditions of the progeny effectively. *S. limbatus* females are able to adjust egg size even when they are switched to a new host, which suggests that the females can directly assess the oviposition substrate (Fox, Thakar, & Mousseau 1997). In contrast, in the present study *T. ni* progeny size was a product
primarily of the larval feeding experience of the parental generation, because all progeny were reared on artificial diet or on pepper and all T. ni pairs oviposited on the same substrate. Many adult lepidopterans lack the ability to assess directly the quality of the oviposition substrate, but larval feeding experience appears to play a key role in oviposition choice of lepidopterans. In a recent study, T. ni adults that had fed on an oviposition deterrent were more likely to oviposit on leaves treated with the deterrent than inexperienced moths (Akhtar & Isman 2003). Therefore, if the larval feeding substrate of the parental generation is predictive of the progeny environment, due to induction of parental preferences, then altering progeny size accordingly could be beneficial.

Genotype Effects

Resistance to Bt was associated with a significant fitness cost as shown by the reduced fecundity of the resistant P_R genotype and the reduced pupal weight of the P_R genotype in the cucumber treatment group as compared to the other genotypes. The decrease in fecundity of the resistant genotype was coupled with a decrease in offspring size. Therefore, the lower production of eggs was not due to an increase in progeny provisioning as expected from the trade-off between egg size and egg number. The reduced fecundity and progeny size suggests that there were considerable constraints on the reproductive ability of the resistant P_R genotype. This reproductive constraint was likely due to a trade-off between resources allocated to defense rather than reproduction. Similarly, Bt-resistant P. xylostella genotypes have reduced egg fertility (Groeters et al. 1994), which can be caused by decreased egg provisioning.
T. ni is a generalist herbivore, and its ability to effectively utilize a wide variety of host plants may depend on transgenerational plasticity, as appears to be true for the generalist seed beetle S. limbatus (Fox, Nilsson, & Mousseau 1997). For example, egg-size plasticity has been shown to play a role in the expansion of the host range of S. limbatus (Fox, Nilsson, & Mousseau 1997). Progeny of parents reared on the poor host, C. floridum, have increased survivorship on the exotic new host C. ebano, thereby allowing S. limbatus to expand its host range. In heterogenous environments where poor quality hosts cannot be avoided, there is expected to be selection for transgenerational plasticity (Fox & Mousseau 1996). Genotypes that have limited plasticity will be at a disadvantage in these environments. Therefore, the frequency of Bt resistant traits will depend not only on the direct fitness costs associated with resistance but also on the ability of the different genotypes to adjust to different host plants in heterogenous environments.
6.5 Literature Cited


Chapter VII

7.1 Concluding Remarks

The intense use of the microbial insecticide, Bt, for the control of T. ni in commercial vegetable greenhouses has provided optimal conditions for the evolution of Bt resistance. The repeated finding of significant Bt resistance over three consecutive survey years suggests that resistance alleles are being maintained in greenhouse T. ni populations despite the presence of deleterious fitness costs. It is likely that T. ni populations are cycling within greenhouses between growing seasons, thereby maintaining resistance alleles in populations. It was speculated in Chapter II that resistance allele frequencies may be higher in field populations than expected from mutation-selection balance predictions and it is likely that greenhouse T. ni populations act as a source of resistant alleles. T. ni is a significant economic pest across North America and is known to migrate across wide geographic areas (Lingren, Henneberry, & Sparks 1979). We must, therefore, consider the potential impacts of Bt resistance beyond greenhouse crops and across the entire species range.

Selection outside of existing tolerance distributions is predicted to select for a few alleles of major effect that arise due to rare mutations (McKenzie 1996). Consistent with this prediction, Bt resistance in T. ni appeared to be due to two alleles of major effect (Chapter III). However, significant variation remained in the resistant lines suggesting that minor resistant alleles may also be involved in resistance. It is likely that the evolution of Bt resistance in T. ni occurs via a two-step process, as predicted in the introduction, in which major alleles are initially selected for, followed by selection for minor alleles that further increase resistance. The understanding of the genetic basis of
resistance to Bt in the present study will aid in the development of strategies to manage resistance evolution (Gould 1998).

The ability to predict the rate of resistance evolution is central to the development of resistance management strategies and findings of the present study show that the rate of resistance evolution will differ between crops. Differences in the selection differential, as defined as the relative fitness difference between susceptible and resistant individuals, will have a large impact on the rate of resistance evolution such that larger selection differentials will produce more rapid responses to selection. In this study, selection differentials, as measured as resistance ratios, varied between the host plants (Chapter IV). Resistance ratios for the different genotypic lines were equivalent when Bt was applied on pepper or cucumber, however the resistance ratio was 2-fold higher for Bt on tomato leaves. The difference between resistant and susceptible genotypes was magnified in the tomato environment which would increase the rate of resistance evolution. Furthermore, 10-fold lower doses of Bt were required on tomato leaves to achieve 50% mortality as compared to Bt-treated pepper and cucumber leaves. Therefore, tomato provides an favorable environment for the evolution of Bt resistance even at low doses.

It is predicted that higher dominance of resistance alleles will result in more rapid rates of resistance evolution (Georghiou 1972). A common misconception is that dominance is an intrinsic property of an allele and dominance values are commonly ascribed to resistance traits (Bourguet, Genissel, & Raymond 2000). In the present study, the dominance of Bt resistance varied between host plant environments and the largest reduction in dominance was associated with the most severe environment, pepper
(Chapter IV). Therefore, the higher dominance values associated with the better host plant environments (cucumber and tomato) will contribute to more rapid responses to selection in favorable environments.

Combining the observations on the selection differential, $Bt$ toxicity and dominance, suggests that $Bt$ resistance will evolve much more rapidly in tomato greenhouses than in the other greenhouse crops. However, these results are based on the T2c populations which originated from a tomato greenhouse and, therefore, the strains may influence its response to selection in the native environment. Numerous selection experiments have shown that populations often respond most rapidly to selection in an environment similar to that in which they were previously selected (Kearsey & Pooni 1996). However, over the three survey years $T. ni$ populations residing in tomato greenhouses achieved the highest resistance levels suggesting that $Bt$ resistance evolution does occur more rapidly when applied on tomato (Chapter II).

The pervasiveness of $Bt$ resistance alleles in greenhouse and surrounding field populations will depend on the strength of selection against these resistance alleles in the absence of $Bt$ as determined by the associated fitness costs. In chapter II, the rapid decline in resistance to $Bt$ in resistant populations maintained in the laboratory and the negative association observed between mean pupal and progeny size and population resistance levels suggests that $Bt$ resistance in $T. ni$ is associated with a significant fitness cost. However, results in Chapter V clearly show that the magnitude of fitness costs in untreated environments is dependent on host plant for the T2c population. Life-history theory predicts that trade-offs between “expensive” resistance traits and reproduction will be most evident when resources are limiting (Bergelson & Purrington 1996). Consistent
with this prediction, as the suitability of the host plant for \textit{T. ni} growth declined, the magnitude of resistance associated fitness costs in the T2c population increased. It is predicted that the frequency of resistance alleles will decline most rapidly in \textit{T. ni} populations infesting pepper greenhouses as compared to the other crops. In contrast, the minimal fitness costs observed on cucumber leaves suggests that resistance allele frequencies may remain stable in this environment. Many factors can affect the detection of resistance-associated fitness costs and the general likelihood of such costs remains an intensely debated issue (Antonovics & Thrall 1994). However, the present findings do suggest that the inclusion of environmental factors, such as the host plant for phytophagous insects, may help to resolve the issue.

Genetic background is known to play a significant role in the modification of fitness costs (McKenzie, Whitten, & Adena 1982) which suggests that the fitness costs may vary between different resistant populations. In Chapter VI, the relationship between associated fitness costs and host plant was not apparent with the P5 population. For example, the fecundity of the resistant P5 genotype was 30% lower in all host plant environments suggesting that \textit{Bt} resistance was associated with significant fitness costs which were not influenced by host plant. As mentioned previously, the origin of the T2c population was a tomato greenhouse, whereas P5 was collected from a pepper greenhouse. Interestingly, no differences in pupal weight were observed between the genotypic lines of P5 in the pepper treatment group. In contrast, no resistant T2c individuals at all reached pupation on pepper. Therefore, P5 individuals appeared to be better suited for growth on pepper leaves. It is likely that the selection for \textit{Bt} resistance coupled with selection for growth on the host plant has produced co-adapted genotypes.
The role of co-adapted genetic complexes in insecticide resistance (McKenzie, Whitten, & Adena 1982) and in the local adaptation of phytophagous insects to alternative host plants (de Jong & Nielsen 2002) is beginning to gain recognition.

Transgenerational plasticity may also play an important role in the ability of *T. ni* to utilize a wide host range. For example, fewer larger offspring were produced by parents fed the least suitable host plant than parents fed the best host plant. This result suggests that either faster growing genotypes were selected for in pepper environments or that progeny size is altered in the parental generation to suit environmental conditions. If transgenerational plasticity is the cause, it may also provide a mechanism by which *T. ni* can pre-condition progeny in response to other environmental stressors. For example, the exposure of the parental generation to *Bt* significantly increased the survivorship and larval growth of resistant individuals in both the tomato and pepper environments (Chapter V). In fact, fitness costs were almost nonexistent in the tomato environment if the parental T2c generation had been exposed to *Bt*. These results suggest that transgenerational plasticity could be a general response to stress that has contributed to the successfulness of *T. ni* as species.

The next likely step in the evolution of resistance to *Bt* in *T. ni* populations is the emergence of new resistant genotypes that have significantly diminished or absent associated fitness costs. The emergence of such genotypes has been observed in long-term studies of the evolution of resistance to chemical insecticides (McKenzie J.A. & Clarke 1988; Raymond et al. 2001). Since resistance-associated fitness costs vary between host plant environments, selection for new fitter resistant genotypes will not be equal in all environments. For example, there will be minimal selection pressure for new
resistant genotypes in cucumber greenhouses due to the absence of associated costs. Since phenotypic differences between resistant, susceptible and hybrid genotypes were the largest in the most extreme nutritional habitat, selection for fitness modifiers or new resistant alleles will be the most intense in this environment. It is predicted that selection for Bt resistance in the unfavorable pepper environment will produce new fitter resistant genotypes that experience a correlated increase in fitness on other host plants. Thus, extreme nutritional conditions that magnify the expression of resistance-associated fitness costs may result in the appearance of new resistant genotypes that can invade other host plant environments.

In summary, T. ni is the second reported species to have evolved resistance to Bt foliar sprays outside of the laboratory. The inheritance of Bt resistance in T. ni is consistent with a 2-locus genetic model and resistance alleles appear to be associated with deleterious fitness costs. However, the host-plant significantly affected both the dominance of resistance and the magnitude of associated fitness costs. The expression of resistance in hybrid individuals increased with the suitability of the host plant, whereas associated fitness costs were reduced with increasing host plant suitability. Therefore, tritrophic interactions play a significant role in the progression of resistance evolution in T. ni.
7.2 Literature Cited


