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Host-plant effects the expression of resistance to *Bacillus thuringiensis kurstaki* in *Trichoplusia ni* (Hubner): an important factor in resistance evolution

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Abstract

Pathogens are thought to exert strong selection on their hosts leading to increased host resistance. *Bacillus thuringiensis kurstaki (Bkt)* is a ubiquitous entomopathogen that has become the mainstay of nonchemical control of Lepidopteran pests and thus, the potential exists for the evolution of resistance in targeted host insects. We have studied the expression of *Btk* resistance in the cabbage looper, *Trichoplusia ni* (Hubner). For this generalist insect herbivore, three common host plants, tomato, pepper and cucumber, vary in suitability for larval growth and development. Here we show that the host plant also affects the overall toxicity of *Btk*, the relative expression of resistance between a resistant and a susceptible line and their F_1 reciprocal crosses, and importantly, the dominance of the resistance trait. This study demonstrates that tri-trophic interactions involving an insect, host plants and a pathogen have the potential to strongly influence the evolutionary response of an insect host to a pathogen.

Introduction

Entomopathogens can exert strong selection pressures on their insect hosts during an epizootic (see Cory & Myers, 2003). This is particularly the case when an epizootic is created by the use of an entomopathogen as a biological control of an insect pest in a managed system. *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) (*Btk*) is currently the most widely used entomopathogen for insect control, and the evolution of resistance to *Btk* has received considerable attention (see Ferre & van Rie, 2002). Often the possibility of interactions between the environment and the genotype of the host insect is disregarded in studies of disease resistance, and this has been the case for *Btk* resistance. For herbivorous insects, the main environmental component is the host plant and this has the potential to modulate the interaction between the *Btk* pathogen and its host species. The complexity of such tri-trophic interactions are only beginning to be unraveled (see review by Cory & Hoover, 2006), yet they likely play a significant role in resistance evolution.

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Host plants are pivotal to the growth and reproduction of phytophagous insects (Awmack & Leather, 2002). Poor plant quality may limit the resources available to insect herbivores for the development of an adaptative trait, such as resistance to an entomopathogen, and they may constrain the expression of the resistance trait to suboptimal levels. For example, variation in food quality influenced 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 (Talloen *et al.*, 2004). Wing melanization of *P. aegeria* varied with the quality of larval food plants; those fed drought-stressed host grasses developed paler wings than larvae reared on unstressed plants.

The response of an insect population to selection by an entomopathogen will ultimately depend on the relative susceptibility of different genotypes in the insect host population. A large difference in pathogen susceptibility between genotypes could result in rapid evolution of pathogen resistance (Hedrick, 2000). Tritrophic

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interactions thus have the potential to alter the susceptibilities of genotypes via genotype-host plant interactions, and ultimately can influence the rate of evolution of pathogen resistance.

Dominance, or the relative toxicity of the pathogen to an individual that is heterozygous for a major resistance trait, may also affect the rate at which resistance evolves. Dominance is commonly defined in terms of genotypes and it is, therefore, generally assumed that dominance is an inherent property of an allele. However, dominance is determined by the relationship among phenotypes of different genotypes, and is, therefore, not intrinsic to an allele and may vary with the environment (Mayo & Burger, 1997; Bourguet, 1999; Bourguet *et al.*, 2000). For example, dominance of a major gene for insecticide resistance in the mosquito, *Culex pipiens*, declined in unfavourable environments from being almost completely dominant to being completely recessive (Bourguet *et al.*, 1996). In *Drosophila kikkawai*, dominance of an allele controlling abdomen pigmentation increased with decreasing temperature (Gilbert *et al.*, 1999). These studies demonstrate the complex nature of dominance and the importance of genotype-environment interactions in its expression. Similar interactions may also be present in insect-entomopathogen-plant relationships.

Numerous studies have examined the inheritance of *Btk* resistance in targeted insect populations (see Ferre & van Rie, 2002). Due to its complex mode of action, there are a number of potential *Btk* resistance mechanisms. *Btk* toxins must be ingested, activated by proteolytic enzymes in the host insect's alkaline midgut, and then bind to the insect's midgut brush border (Faust & Bulla, 1984). This binding causes pores to form in the midgut lining allowing gut contents to leak across the deteriorated membrane and eventually causing the death of the insect (Faust & Bulla, 1984). Resistance mechanisms may occur at each stage of the infection process. Ingestion of a lethal dose of *Btk* during the host insect's larval stage coupled with appropriate gut conditions is required for larval mortality, and therefore host plants are intimately involved in *Btk* toxicity (Kouassi *et al.*, 2001; Cory & Hoover, 2006).

A variety of insect species have been successfully selected for lowered *Btk* susceptibility in the laboratory (Tabashnik, 1994). Despite these demonstrations of the potential for resistance to *Btk*, significant resistance outside of the laboratory has only been detected in populations of *Plutella xylostella* (Ferre & van Rie, 2002) and *Trichoplusia ni* (Hubner) (Janmaat & Myers, 2003). The present study focuses on *Btk* resistance in *T. ni*, or the cabbage looper, which is a polyphagous Lepidopteran indigenous to North America.

Trichoplusia ni are pervasive pests of the major greenhouse vegetable crops grown 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 (Janmaat & Myers, 2005). A previous study has shown that costs of resistance (i.e. reduced growth, pupal weight and survival of the resistant strain) to *Btk* vary among these host plants in the absence of *Btk*, such that fitness costs increase as host plant suitability declines (Janmaat & Myers, 2005). Similar genotype-environment interactions may be prevalent in the presence of the *Btk* pathogen, subsequently affecting the evolution of *Btk* resistance.

We hypothesize that the host plant may modulate the expression of *Btk* resistance in *T. ni*, and may alter the dominance of the resistance trait.

Methods

A *T. ni* colony resistant to *Btk* was initiated from 90 individuals collected from a commercial tomato greenhouse in British Columbia, Canada in 2001 (labeled T2c in Janmaat & Myers, 2003). In the first generation of laboratory culture following collection the *T. ni* population was found to be 113-fold more resistant than a reference susceptible laboratory colony. Two lines were established on a wheat-germ based diet (Ignoffo, 1963) and reared at 26 °C, 16 : 8 h (L : D) and uncontrolled humidity. One line (P_S) was reared without any *Btk* exposure and exhibited a significant decrease in resistance after seven unselected generations (change in LC_{50} from 256 to 4.2 kIU mL^{-1} diet where kIU is equal to 1000 International Units of *Btk* activity) (Janmaat & Myers, 2003). The LC_{50} of the unselected line declined further to a stable value of 2.8 ± 1.0 kIU mL^{-1} diet for more than five generations prior to the current experiment. The other line was exposed to 160 kIU mL^{-1} diet *Btk* (DiPel WP, Valent Biosciences, Libertyville, IL, USA) during each generation of laboratory culture to maintain resistance. In addition, a long-term laboratory colony that had no previous exposure to *Btk* was maintained for genetic analysis and this line is identified as the long-term susceptible population (P_{Lab}).

The resistant line was exposed to *Btk* mixed into artificial diet as groups of 20–25 five-day-old larvae (2nd and 3rd instars) in 175 mL Styrofoam cups. All live larvae were transferred to cups with new artificial diet without *Btk* after 2 days. Surviving pupae were collected and pooled in a mating cage to produce progeny for the next generation. After four generations of laboratory culture, the fecundity and fertility of the resistant line had declined. To increase the vigor of the resistant line, it was crossed back to the susceptible colony and the resulting hybrid was exposed to increasing *Btk* concentrations for seven subsequent generations to produce a new resistant line (subsequently referred to as P_R). The resistant line was exposed to the following *Btk* concentrations: 20, 40, 40, 80, 160, 160 and 160 kIU mL^{-1} diet

from the first to seventh generation of selection respectively.

To examine dominance of *Btk* resistance, two separate experiments were conducted as described below. In each experiment, *T. ni* were mated to produce four lines: resistant and susceptible parental lines (P_R and P_S or P_{Lab} respectively) and two reciprocal hybrid lines F_1f and F_1m (F_1f: the resistant parent is the female and F_1m: the resistant parent is the male). In each experiment, the P_R population was not exposed to *Btk* for one generation prior to crossing to reduce potential sublethal effects that may be passed on from parents to offspring. Eggs were harvested from caged adults every 2 days, stored at 4 °C, and hatched at 26 °C within 2–4 days of collection.

Artificial diet experiment

In the first experiment, the resistant line (P_R) was crossed with the long-term susceptible colony (P_{Lab}) and exposed to *Btk*-artificial diet mixtures. The inheritance of resistance was examined in reciprocal single-pair crosses between the P_R line and the P_{Lab} colony. Pupae were paired with P_R to produce resistant progeny and were paired with P_{Lab} pupae to produce reciprocal F_1 hybrids. There were a total of seven pairs for each hybrid cross and nine pairs for the resistant cross. Pupae from the P_{Lab} colony were mass crossed (200 individuals total) to produce susceptible progeny. Progeny of each of the crosses were reared on artificial diet for 5 days prior to transfer to the concentrations of *Btk* mixed in wheatgerm diet (methods in Janmaat & Myers, 2003). All assays were performed with a minimum of 5–7 concentrations ranging from 1.25 to 160 kIU mL^{-1} diet depending on the expected resistance level and a control. Twenty to forty larvae were assayed per concentration for each bioassay. Larval mortality was observed 3 days following transfer to the *Btk*-diet mixture. Mortality was 0% in the control treatment group for each cross.

Leaf disc experiment

In the second experiment, larvae were exposed to different *Btk* concentrations on leaf discs of three different host plants. In this experiment, the resistant line (P_R) was crossed with the reverted susceptible line (P_S). In previous experiments, the long-term laboratory colony exhibited poor survival on pepper and tomato leaf discs. P_{Lab} may have lost its ability to effectively utilize pepper and tomato leaves as a host plant, due to its long history of growth on artificial diet. From previous experiments it was evident that P_S still retained its ability to grow on tomato and pepper leaves, and therefore the P_S line was used as the susceptible line in the present experiment. The higher survival of the P_S colony on leaf discs was presumably due to its origin from a tomato greenhouse. Due to logistical constraints, moths from the P_R and P_S lines were mass crossed (40 males × 40 females) to create each of the four lines for the leaf disc experiment. Progeny of the second set of crosses were reared on artificial diet for 3 days and were then transferred to *Btk*-treated leaf discs. Larval mortality was assessed 3 days after transfer to the leaf discs.

Three different host plants were grown for the second experiment: pepper (444, Enza Zaden, Enkhuizen, the Netherlands), tomato (Rapsodie, Syngenta, Boise, ID, USA) and cucumber (Ventura, Rijk Zwaan, De Lier, the Netherlands). Leaf discs (3.5 cm in diameter) cut from new fully expanded leaves from each host plant were dipped in one of six different concentrations of *Btk* serial diluted in distilled water (0, 5, 10, 20, 40, 80 kIU mL^{-1} water). Excess droplets were removed through gentle agitation and leaf discs were air dried on wire mesh racks at room temperature. Dry leaf discs were placed into individual 59.2 mL plastic soufflé cups (Solo Cup Company, Toronto, ON, Canada). Five larvae were transferred to each leaf disc for a total of 50 larvae per treatment. Host plant, *Btk* concentration and population were randomly assigned to seven different experimental dates to account for daily variation in mortality.

Statistical analyses

For experiment one, LC_{50} values of concentration-mortality lines were estimated using the probit analysis procedure in GENSTAT 5 Release 4.1 (Lawes Agricultural Trust, Rothamsted, UK, 1997). Deviance statistics were used to test for differences in mortality over the concentration range between crosses using the accumulated display setting in GENSTAT 5. Approximate Chi-square (χ^2_5) probabilities are shown in the text. In this experiment, no mortality was recorded in the control treatment groups. For both experiments, LC_{50} values were rounded to the nearest hundredth. All LC_{50} values in the text and tables are represented as kIU mL^{-1} diet (Exp. 1) or water (Exp. 2).

For experiment two, the logistic procedure in SAS 9.1 (SAS Institute Inc., Cary, NC, USA, 2002–2003) was used to compare the mortality of larvae in the control treatment group (0 kIU mL^{-1} water) with cross, host plant and set-up date as effects. The PROBIT procedure was used to examine the effects of *Btk* concentration and cross on the larval mortality. Date was included in the PROBIT analysis to account for daily variation. The analysis was conducted separately for each host plant and comparisons between specific crosses within host plant were conducted using a Bonferroni adjusted *P*-value.

For both experiments, 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). Dominance values presented in the text were adjusted to the more traditional range of 0 (completely recessive) to 1 (completely dominant)

according to Liu & Tabashnik (1997) where $D_{LC} = (D + 1)/2$. Dominance variances were calculated according to Preisler *et al.* (1990) and multiplied by 0.5 to account for the dominance adjustment. LC_{50} values were calculated using the PROBIT analysis procedure in GENSTAT 5. Degrees of freedom for each statistical test are noted as a subscript and mean ± SE is shown unless otherwise stated.

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 *Btk* concentration-*T. ni* cross-host 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 randomizations. The number of differences equal to or below 0 was 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 two-tailed *P*-value and by 3 to adjust the *P*-value for the three pair-wise comparisons.

Results

Artificial diet experiment

In the first experiment, hybrid larvae derived from a cross between a resistant *T. ni* line (P_R) and a long-term susceptible laboratory colony (P_{Lab}) and larvae from both parental lines were exposed to a range of *Btk* concentrations mixed in a wheat-germ based diet. No difference was found between the reciprocal hybrid progeny of crosses between P_R and P_{Lab} ($\chi^2_1 = 2.04$, $P = 0.153$) (Fig. 1). Therefore, inheritance of *Btk* resistance was assumed to be autosomal. Progeny of the resistant crosses were 32-fold more resistant (LC_{50} P_R/LC_{50} P_{Lab}) than progeny from the susceptible (P_{Lab}) colony, whereas progeny from the F_1 crosses were only three-fold more resistant (LC_{50} F_1/LC_{50} P_{Lab}). Therefore, responses of the hybrid larvae on artificial diet were similar to that of the susceptible parent (P_{Lab}) indicating that the inheritance of resistance to *Btk* was at least partially recessive (Table 1).

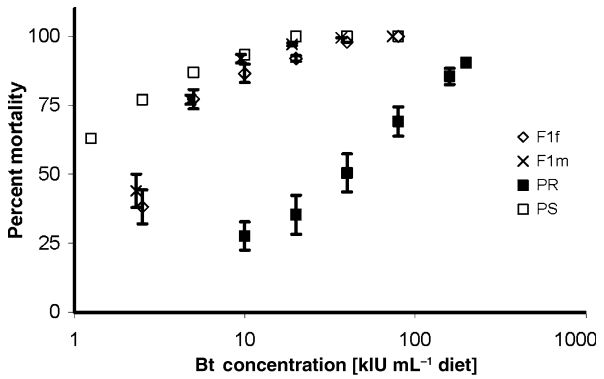

Fig. 1 Mean mortality at each *Bt* concentration of offspring of single-pair crosses within the P_R line, reciprocal crosses between the P_R line and P_{Lab} (F_1f and F_1m), and a mass cross within the P_{Lab} line. Error bars represent standard error.

Leaf disc experiment

In the second experiment, *Btk* was presented to *T. ni* larvae of crosses derived from P_R and the reverted susceptible line (P_S) on leaf discs of the three host plants. Unlike experiment one in which no mortality was observed on artificial diet alone, mortality in the control treatment group in experiment two (*Btk* = 0 kIU mL^{-1} water) was significantly affected by the host plant (Plant: $\chi^2_2 = 15.8$, $P < 0.001$). The observed mortality in the control treatment group was 3.3 ± 0.6%, 10.0 ± 3.7% and 9.5 ± 4.3% in the cucumber, tomato and pepper groups respectively. No differences in mortality occurred between the crosses in the control treatment group (cross $\chi^2_2 = 1.32$, $P = 0.51$). Large differences were observed

Table 1 LC_{50} and dominance values for each cross [resistant (P_R), F_1, and susceptible (P_S)], and diet combination (cucumber, tomato and pepper).

Diet	Population	*n*	LC_{50} (95% CI)	D_{50}*
			kIU mL^{-1} water†	
Cucumber	P_R	385	44.3 (30.8–63.7)	0.37 ± 0.25[a]
	F_1	1085	20.7 (16.5–25.9)	
	P_S	450	13.1 (9.1–19.0)	
Tomato	P_R	506	5.0 (2.6–9.6)	0.81 ± 0.95[ab]
	F_1	969	3.8 (2.3–6.1)	
	P_S	435	1.1 (0.2–4.9)	
Pepper	P_R	416	48.0 (37.6–61.3)	−0.29 ± 0.34[b]
	F_1	1026	15.7 (12.2–20.2)	
	P_S	405	20.2 (13.2–30.9)	
			kIU mL^{-1} diet†	
Artificial diet	P_R	2126‡	31.9 (28.6–35.2)	0.34 ± 0.045
	F_1	3196‡	2.9 (2.6–3.2)	
	P_S	180‡	1.0 (0.65–1.4)	

Significant differences in dominance values denoted by superscripts a and b.

*Where D is calculated for a specific mortality level (in this case 50%) according to Stone, 1968 and adjusted to an interval of 0 (completely recessive) to 1 (completely dominant) Liu & Tabashnik, 1997.

†Leaf discs were dipped in concentrations of *Bt* mixed in water in Experiment 2, whereas *Bt* diluted in water was mixed into artificial diet in 1 : 10 ratio (*Bt* solution : diet) in experiment 1.

‡Mean results of single pair crosses are displayed for F_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.

among set-up dates (date $\chi^2_5 = 46.5$; $P = 0.0001$). All host plants were not represented equally on each set-up date and the difference in mortality between crops may have amplified the date effect.

The mortality in response to the *Btk* treatment was lowest over all for the resistant line (P_R) and highest overall for the susceptible (P_S) line demonstrating that resistance to *Btk* differed significantly between these lines (Table 2). In agreement with the first experiment, there were no differences in the concentration-mortality lines of the reciprocal hybrid crosses (cross $\chi^2_1 = 0.10$, $P = 0.74$), suggesting that there are no maternal effects and *Btk* resistance was assumed to be autosomal. Mortality increased as expected with *Btk* concentration (Table 2).

Btk toxicity differed strikingly with larval host plant type (Table 2). The highest mortality over all the crosses occurred when *Btk* was fed to *T. ni* larvae on tomato leaf discs when compared with *Btk*-treated cucumber or pepper leaf discs (Fig. 2). For P_R, the LC_{50} in the cucumber and pepper treatment groups was similar to that obtained in the first experiment on artificial diet, whereas on tomato the LC_{50} was 9-fold lower. Similarly for P_S, *Btk* susceptibility was highest on tomato, and susceptibility declined by 12 to 18-fold in the cucumber and pepper treatments respectively.

The differences in LC_{50} between the host plant treatment groups for the P_R and P_S lines resulted in a smaller discrimination between the resistant and susceptible lines in the leaf disc experiment when compared with the first experiment (Table 1). Over all host plant groups, progeny of the P_R line were 3.5-fold more resistant to *Btk* than the P_S line, whereas the analagous difference in experiment one between P_R and P_{Lab} was 32-fold when comparing LC_{50} values. This discrepancy was primarily due to an atypical lower level of susceptibility of the P_S line. In the present experiment, the P_S line exhibited a 5- to 7.5-fold increase in LC_{50} (Table 1) on cucumber and pepper relative to previous measures (2.7 ± 1.0 kIU mL^{-1} diet) on artificial diet. The previous measures of P_S susceptibility on artificial diet had remained constant for five generations prior to the leaf disc experiment, and therefore the present results were unexpected.

The resistance of the F_1 hybrid relative to the parental lines (i.e. dominance) differed substantially among host-plant environments (Table 3). The highest dominance value, which corresponds to the hybrids with the highest relative resistance, was estimated for the tomato group (0.81 ± 0.95), however, there was considerable variation in this estimate which does not preclude the possibility that the dominance in this environment is in fact recessive. Interestingly, there was a significant reduction in dominance when *Btk* was ingested on pepper leaves as compared to cucumber ($D_{cucumber}$ vs. D_{pepper}: $P < 0.05$). Resistance was partially recessive when *Btk* was

Table 2 Probit analysis of larval mortality across all host plants with *Btk* concentration (IU), cross and date as factors.

	d.f.	Wald χ^2	*P*
Log(IU)	1	127.7	<0.0001
Host plant	2	86.7	<0.0001
Cross	2	25.6	<0.0001
Date	5	60.2	<0.0001

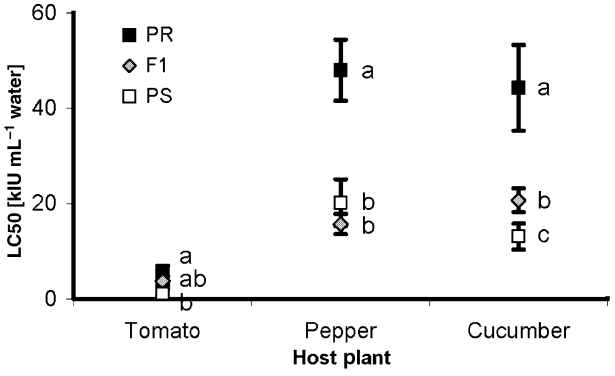

Fig. 2 The LC_{50} for each *Trichoplusia ni* cross host-plant combination where P_R is the resistant phenotype, P_S the susceptible phenotype and F_1 represents the pooled results of the reciprocal hybrid crosses. Error bars represent the 95% confidence intervals and LC_{50} values are considered significantly different if the error bars do not overlap.

Table 3 Probit analysis of larval mortality for each host plant with *Btk* concentration (IU), cross and date as factors.

	d.f.	Wald χ^2	*P*-value
Cucumber			
Log(IU)	1	24.2	<0.0001
Date	5	30.3	<0.0001
Cross	2	18.3	0.0001
P_R vs. F_1	1	5.9	0.015*
P_S vs. F_1	1	7.1	0.008*
P_R vs. P_S	1	18.3	<0.0001*
Tomato			
Log(IU)	1	28.9	<0.0001
Date	5	12.7	0.03
Cross	2	6.1	0.05
P_R vs. F_1	1	2.0	0.15
P_S vs. F_1	1	1.9	0.16
P_R vs. P_S	1	6.1	0.01*
Pepper			
Log(IU)	1	52.7	<0.0001
Date	5	46.8	<0.0001
Cross	2	12.9	0.002
P_R vs. F_1	1	12.7	0.0004*
P_S vs. F_1	1	0.09	0.76
P_R vs. P_S	1	6.7	0.01*

*Comparisons between specific crosses are significant if $P < 0.017$ (Bonferroni adjusted *P*-value).

presented to larvae on cucumber leaf discs with D_{50} (±SE) equal to 0.37 (±0.25). On pepper, *Btk* resistance was completely recessive or possibly underdominant, such that hybrid individuals may be more susceptible than the P_S individuals, ($D = -0.29 \pm 0.34$).

Discussion

This study shows that the host plant can affect the overall toxicity of *Btk*, the relative expression of the resistant trait between crosses, and the genetic dominance of resistance. Together these results demonstrate that tri-trophic interactions have the potential to greatly influence the evolutionary response of an insect population to a pathogen.

Firstly, the unanticipated low susceptibility of the P_S population to *Btk* in the leaf disc experiment must be addressed. Prior to the leaf disc experiment, the P_S population had been assayed periodically on artificial diet containing different *Btk* concentrations. For more than five generations, the LC_{50} of P_S (2.7 ± 1.0 kIU mL^{-1}) was similar to that observed for the P_{Lab} colony. Therefore, the LC_{50} in the present experiment on the cucumber and pepper leaf discs was atypical. The higher tolerance of the P_S line may have been due to random fluctuations in resistance, or due to induced tolerance in response to the diet switch. Tolerance to *Bt* endotoxins has been induced in a susceptible *Ephestia kuehniella* strain by preexposure to a sublethal *Bt* concentration (Rahman *et al.*, 2004). In addition, the change in protocol from the *Btk*-artificial diet-mixtures to the leaf discs may have contributed to the result. This is less likely however as P_R would have been expected to display a similar decrease in *Btk* susceptibility.

The most striking result in the present study was the large increase in *Btk* toxicity on tomato leaf discs relative to the other leaf types. The three host plants examined in the experiment differ considerably in plant chemistry which may have contributed to the differences observed in *Btk* toxicity. Similarly, chlorogenic acid, a constitutive defensive compound of tomato, in combination with peroxidase, an inducible defensive compound of tomato, increased *Btk* toxicity towards *H. zea* (Ludlum *et al.*, 1991). The response of an insect population to selection with an entomopathogen is ultimately dependent on the strength of the selective force. Given the present results selection for *Btk* resistance would be considerably higher on tomato leaves relative to the other host plants. Therefore it is not surprising that of greenhouses surveyed, tomato greenhouses harboured *T. ni* populations with the highest *Btk* resistance levels (Janmaat & Myers, 2003).

A surprising result was the significant differences in genetic dominance of *Btk* resistance between experiments and among host plants. On artificial diet, resistance of the *T. ni* line was inherited as a partially recessive trait. This result was consistent with two other studies on a second *Btk*-resistant *T. ni* strain collected from commercial greenhouses in British Columbia (Janmaat *et al.*, 2004; Kain *et al.*, 2004). Both of these studies were conducted on artificial diet and the inheritance of resistance to *Btk* and an individual *Btk* toxin (Cry1Ac) was found to be partially recessive. However, in the second experiment, resistance varied from complete recessivity, and possible under-dominance, on pepper to partial recessivity on cucumber.

In a previous study using the same *T. ni* strains as in the present leaf disc experiment, the performance of *T. ni* varied considerably among the host plants (Janmaat & Myers, 2005). *T. ni* larvae performed best on cucumber and worst on pepper and the magnitude of resistance associated fitness costs, in the absence of *Btk*, increased with declining host plant suitability. Therefore, fitness costs, in the absence of *Btk,* were inversely proportional to host plant suitability. In the presence of *Btk* and on the best host plant, hybrid individuals were significantly more resistant than the susceptible population, whereas on the worst host plant, hybrid individuals were equivalent in susceptibility, or more susceptible, relative to the susceptible parent population (Table 1). Interestingly, in the study of insecticide resistance in *C. pipiens*, dominance declined in unfavourable environments. This is consistent with a resistance-associated fitness cost (Bourguet *et al.*, 1996). Thus, dominance of costly resistance traits may be condition-dependent such that the strength of genetic dominance declines in unfavourable environments.

A long-standing debate has focused on the general observation of the dominance of wild-type alleles (i.e. *Btk* susceptibility would be the wild-type allele in the present case) (see summaries in Mayo & Burger, 1997; Bourguet, 1999). Wright proposed that dominance of a wild-type allele is a consequence of metabolism, if it is assumed that most deleterious mutations cause a reduction in enzymatic activity. Wright's theory has gained favour and is the rationale generally used to explain the observed phenomenon that mutant phenotypes tend to be recessive (Orr, 1991; Bourguet, 1999).

The 'physiological theory of dominance', however, is not adequate to explain all observations, such as the finding that genetic dominance can be a plastic response (Bagheri & Wagner, 2004). In addition, the physiological theory of dominance, does not extend to noncatalytic gene products (Kacser & Burns, 1981), and, therefore, likely does not extend to *Btk* resistance genes (Bourguet & Raymond, 1998). Alterations of midgut receptor binding sites are the most common form of *Btk* resistance in Lepidoptera (Tabashnik *et al.*, 1998; Ferre & van Rie, 2002). This form of resistance has been reported for four independent resistant strains of *P. xylostella,* one strain of *Heliothis virescens*, the tobacco budworm, and one strain of *Plodia interpunctella*, the Indianmeal moth (Tabashnik *et al.*, 1998), and likely extends to *T. ni*. Therefore, a different physiological model is needed to explain dominance of resistance to *Btk* endotoxins.

As shown by Bourguet & Raymond (1998), dominance of pesticide resistance genes that alter receptors or channels can be explained using relatively simple biochemical terms. For example, DDT and pyrethroid insecticides cause sodium channels to remain irreversibly open, the opening of few such channels results in death (Narahashi, 1992). Genes resistance to DDT or cyclodienes typically code for insensitive target sites (ffrench-Constant *et al.*, 1996). Individuals heterozygous for resistance possess 50% sensitive target sites and are, therefore, vulnerable to the insecticide, rendering resistance recessive (Bourguet & Raymond, 1998). In contrast, cyclodiene insecticides cause GABA-gated chloride channels to remain permanently closed (see Clark *et al.*, 1995). Resistance to cyclodiene target-sites is often semi-dominant, as 50% of the chloride channels remain open in heterozygous individuals allowing them to survive (ffrench-Constant *et al.*, 1996; Bourguet & Raymond, 1998).

With respect to *Btk*, resistance of *T. ni* is likely due to a modification of midgut receptors. Hybrid *T. ni* individuals would then experience a reduced level of mid-gut damage relative to susceptible individuals. If hybrid individuals were able to rapidly repair damaged midgut cells, they may survive exposure to *Btk* toxins (Martinez-Ramirez *et al.*, 1999). Rapid repair of damaged midgut cells has been suggested as a potential *Btk* resistance mechanism in at least one insect species (Forcada *et al.*, 1999). It is possible that in resource rich environments, such as cucumber, hybrid individuals may be able to devote more resources to mid-gut repair. In resource poor environments such as pepper however, hybrid individuals may be less able to repair mid-gut damage, thereby decreasing the dominance of resistance. Thus, the plasticity of dominance of *Btk* resistance can be explained using a similar rationale to that used for the plasticity of fitness costs associated with resistance in the absence of *Btk*.

Implications

The significant effect of host-plant on the expression of *Btk* resistance and on the dominance of resistance in *T. ni* emphasizes a need for developing resistance management strategies that are tailored to specific plant-insect combinations. Similar host-plant effects on *Btk* resistance may extend to other generalist insect herbivores, such as the European corn borer which feeds on over 300 different host plants. Moreover, these results illustrate the complexity of tri-trophic interactions and the potential of host plants to influence the co-evolution between insect populations and their pathogens.

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