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The cost of resistance to *Bacillus thuringiensis* varies with the host plant of *Trichoplusia ni*

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Selection for resistance to insecticides, diseases and parasitoids is assumed to be costly and often requires tradeoffs with reproductive fitness. The costs of resistance, however, are often difficult to measure. Cabbage looper, *Trichoplusia ni*, a generalist Lepidopteran herbivore, has become highly resistant following the extensive use of the microbial insecticide, *Bacillus thuringiensis kurstaki* (*Bt*) in vegetable greenhouses. We compared the growth rate, pupal size and survival of resistant, susceptible and hybrid *T. ni* larvae fed on tomato, bell pepper and cucumber. Performance was best on cucumber and worst on pepper, and the magnitude of fitness costs associated with *Bt* resistance increased with declining host plant suitability. This supports the hypothesis that in this system, resistance costs are condition dependent and are greatest in the most stressful environment. Management strategies that rely on the presence of fitness costs to reduce the frequency of resistance genes must consider this variation and should be more successful on crops that are less suitable food plants. In general, condition dependence should be considered in studies designed to measure the costs of resistance.

Keywords: condition-dependent fitness; host plant; cabbage looper; fitness costs

1. INTRODUCTION

Traits conferring resistance to challenging environments such as disease, chemical toxins and climatic changes are generally assumed to be associated with strong deleterious costs (Mcnair 1991; Orr & Coyne 1992; Carrière *et al.* 1994). As a result, resistant individuals may allocate a greater portion of resources towards the resistance trait as opposed to growth and/or reproduction (Carrière *et al.* 1994; Bergelson & Purrington 1996). For example, pesticides in agricultural systems strongly select for resistant mutations that are likely to be controlled by major genes with associated deleterious costs (Lande 1983; McKenzie & Batterham 1994). Costs of resistance to chemicals, parasites or parasitoids may be condition dependent such that the trade-off between defence and reproduction becomes more evident under poor resource conditions (Bergelson & Purrington 1996), during over-wintering, a period of environmental stress (McKenzie 1996; Carrière *et al.* 2001*b*) or under conditions of high intraspecific competition (Kraaijeveld & Godfray 1997).

Host plant quality significantly affects the growth and reproduction of phytophagous insects either through nutritional quality or the effects of plant defensive compounds (Awmack & Leather 2002). Condition dependent costs of resistance can be associated with this variation. 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. Thus, variability in plant quality may in turn alter the production or maintenance of defensive traits expressed by the insect and fitness costs associated with resistance of herbivorous insects may vary with the suitability of the host plant.

Owing to concerns regarding the long-term sustainable use of the microbial insecticide *Bacillus thuringiensis* (*Bt*), resistance to *Bt* has been a subject of extensive research. Despite the successful selection of resistance to *Bt* in multiple laboratory populations, significant resistance outside of the laboratory has been detected only in populations of *Plutella xylostella* (review in Ferré & van Rie 2002) and *Trichoplusia ni* (Janmaat & Myers 2003). 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 (Tabashnik *et al.* 1994; Ferré & van Rie 2002) and this has been documented in several cases (Groeters *et al.* 1993, 1994; Liu *et al.* 1999). Costs of resistance have not always been the case however (Perez & Shelton 1997; Sayyed & Wright 2001). This lack of fitness costs may have been owing to the selection of resistant alleles lacking negative pleiotropic effects (Guillemaud *et al.* 1998) or to fitness modifiers at other loci (Liu *et al.* 1996). An additional explanation is that resistance-associated fitness costs were not apparent in an optimal environment, but may have been expressed in other environments.

Cabbage loopers, *T. 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 resistance to *Bt* in *T. ni* populations owing to the observed rapid decline of *Bt* resistance in laboratory populations and a negative relationship between resistance and pupal weights of field populations (Janmaat & Myers 2003). Furthermore, *T. ni* growth rates differed considerably among the

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Table 1. History of selection of the P_R strain.

laboratory generation	number selected	selective dose ($\mu g\ ml^{-1}$ diet)[a]	survivorship to pupation (%)	LC_{50} ($\mu g\ ml^{-1}$ diet)[a]
1	396	0	100	
2	200	0	100	0.68
3	684	1.25	5.0	
4	679	2.5	8.2	
5	785	2.5	19.6	
6	513	5	29	
7	802	10	36.5	20.9
8	402	10	13.9	
9	480	10	24.8	

[a] 1 mg DiPel WP = 16 000 IU.

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 and would be greatest for larvae fed the poorest food plant.

2. METHODS

(a) *History of* Trichoplusia 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 (labelled T2c in Janmaat & Myers 2003). The *T. ni* population was resistant at collection and was found to be 113-fold more resistant than a reference-susceptible laboratory colony in the first generation of laboratory culture. Two lines were established on a wheat-germ based diet (Ignoffo 1963) and reared at 26 °C, 16 : 8 (light hours : dark hours) and uncontrolled humidity. One line (P_S) was reared without any *Bt* exposure and exhibited a significant decrease in resistance after seven unselected generations (change in LC_{50} from 16 to 0.26 $\mu g\ ml^{-1}$ diet; Janmaat & Myers 2003). The other line was exposed to *Bt kurstaki* (DiPel WP, Valent Biosciences) during each generation of laboratory culture to maintain resistance.

The resistant line was exposed to *Bt* mixed in artificial diet as groups of 20–25 5-day-old larvae (2nd and 3rd instars) in 175 ml Styrofoam cups. All live larvae were transferred to cups with new artificial 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 laboratory culture, fecundity and fertility of the resistant line had declined. To increase the vigour of the resistant line, it was crossed back to the susceptible colony and the resulting hybrid was exposed to increasing *Bt* doses for seven subsequent generations to produce a new resistant line (subsequently referred to as P_R; table 1). The P_R population was not exposed to *Bt* for one generation prior to the establishment of the *T. ni* lines to reduce potential sub-lethal effects that may be passed on from parents to offspring. At the outset of the experiment, the LC_{50} values of the parental P_R and P_S lines were 7.1 and 0.16 $\mu g\ ml^{-1}$ diet, respectively.

(b) 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 lines (P_R and P_S, 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). Individuals from the resistant line were either reared in the presence ($P_R$sel) or absence (P_R) of *Bt* exposure for one generation. A comparison of the P_R and $P_R$sel lines will highlight any trans-generational effects of *Bt* exposure. 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 five different genotypes. Eggs were harvested from caged adults every 2 days, stored at 4 °C and hatched at 26 °C within 2–4 days of collection.

(c) *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 greenhouse crops (bell pepper, variety 444; beefsteak tomato, variety Rapsodie; and long-English cucumber, variety Ventura) were grown in a greenhouse (ambient light and temperature) at the University of British Columbia, Canada, from May to August, 2003. Plants were used approximately two months after planting and before flowering. Neonates hatched from eggs of each of the genetic lines were placed individually onto leaf pieces (2 cm^2) contained in 30 ml plastic cups that were placed inside plastic-covered seedling flats lined with moistened paper towelling 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 towelling. New leaf pieces were provided every 2 days to early instars and daily to fifth instars in the Styrofoam cups. Fully expanded leaves located within the top third of each plant were chosen for feeding. Frass was removed when new leaf pieces were provided. 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. Larval weights were measured (to 0.1 mg) at 10 days of age. The number of days to pupation was recorded and pupal weights were measured (to 0.1 mg) 2 days following pupation. Pupae were then placed into 30 ml plastic cups until emergence and the sex of the emergent adult was recorded.

The experimental treatments of host plant and genotype were randomly assigned to seven different starting dates to account for daily variation in growth rates and egg viability. Mortality was measured as the number of larvae surviving

Table 2. Wald χ^2 tests of the survival of larvae of the five genotypic classes on the three different host plants including the effect of day.

survival	d.f.	χ^2	p
genotype	4	2.6	0.63
host plant	2	19.5	0.000 1
plant×genotype	8	40.8	<0.000 1
day	6	73.4	<0.000 1

to pupation and was analysed using the nominal logistic procedure in JMPIN 4.0 with genotype, host plant, their interaction and day as factors. Day 10 larval weights, time to pupation and pupal weights were analysed using a general linear model (GLM) in JMPIN 4.0 with host plant, genotype and their interaction defined as main effects. The starting date (day) was included as a random factor in all GLM analyses. Sex was included as a main effect in the analyses of time to pupation and pupal weight. Interactions between sex and host plant and sex and genotype were included in the primary analysis of each life-history trait but were not significant and were, therefore, removed from the final analyses. Pupae that did not successfully emerge into adults were not included in the analysis of pupal weight and time to pupation. Larval weights (in mg) were transformed using the natural logarithm and time to pupation was square-root transformed owing to departures from normality in the raw data (Zar 1996). Treatments within genotype and host plant were compared using Student's *t*-test multiple comparisons on least-square means produced from the full GLM and genotypic effects were further compared within each host plant treatment group using Student's *t*-test multiple comparisons. Least-square means and standard errors derived from the GLM analyses are reported in the results.

3. RESULTS

(a) *Host plant effects*

The host plant had a significant effect on all life-history traits measured (tables 2 and 3). Over all genotypes, pepper was the poorest host plant for *T. ni* larvae. The mortality rate was over fivefold higher on pepper leaf pieces compared with cucumber and tomato leaves, and larval growth rate was 67% slower on pepper compared with the other host plants. The longer development time on pepper, however, did not result in a larger pupal size, since pupal weight was 26% smaller in the pepper treatment group compared with the other treatments. 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 between the two host plants. Larvae weighed 110 ± 4 mg at day 10 when feeding on tomato leaves compared with 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 tomato and cucumber environments, respectively. Interestingly, this difference was not reflected in final pupal size of the cucumber and tomato treatment groups, since least-square means were 218.8 ± 5.5 and 216.3 ± 5.4 mg, respectively.

(b) *Genotype–plant interactions*

A significant effect of genotype occurred over all the host plant treatment groups on larval growth, as shown by the significant effect on day 10 larval weight and time to pupation (table 3). No overall genotypic effects were detected for survivorship and pupal weight (tables 2 and 3). A significant interaction between genotype and host plant demonstrated that the presence and magnitude of resistance-associated fitness costs varied with host plant environment (tables 2 and 3). Male pupae were significantly larger than female pupae and the block effect, which accounted for variation between starting dates, was highly significant (table 3).

No differences in larval survivorship occurred among the genotypes when fed cucumber leaves; however, the resistant genotype (P_R) had significantly lower survival than the other genotypes when fed tomato and pepper leaves (figure 1). No larvae of the P_R genotype survived to pupation on pepper. Similarly, survival of larvae of the $P_R$sel genotype was significantly lower on pepper leaves than the P_S, F_1f, and F_1m genotypes. By contrast, survival of $P_R$sel was significantly higher than P_R and equivalent to the other genotypes on tomato. This difference in the performance of the P_R and $P_R$sel genotypes suggests the presence of positive trans-generational effects on tomato that appeared to compensate for poor survival of the P_R genotype on tomato.

Measured larval weights of the P_R-resistant genotype were significantly smaller than those of the susceptible P_S genotype when feeding on pepper (figure 2). On tomato, larval weights of the P_R genotype were smaller than all other genotypes. In comparison, $P_R$sel larval weights did not differ from P_S on both pepper and tomato leaves. In the cucumber treatment group, no differences in larval weights were observed between P_R, $P_R$sel 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 favourable tomato and pepper host plants.

With respect to time to pupation, development of the $P_R$sel genotype was significantly slower than all other genotypes on cucumber. This was not the case for the P_R genotype (table 4). The opposite effect occurred on tomato; the P_R genotype exhibited the longest development time relative to the other genotypes. The development time of $P_R$sel genotype was significantly shorter than P_R and did not differ from P_S and F_1m on tomato. 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 $P_R$sel genotype and vice versa.

The measured life-history traits were affected by the genotype of the maternal parent; however, this effect was not consistent across host plants. F_1f larvae, derived from a cross between P_R females and P_S males, were significantly larger than all other genotypes on tomato, whereas the same genotype was significantly smaller than the reciprocal hybrid F_1m larvae on pepper (figure 2). Therefore, positive maternal effects that increased larval size were observed on tomato and negative effects occurred on pepper. These parental effects extended through development. Development time of the F_1f genotype on tomato was faster relative to the other

Table 3. Analysis of variance tables for larval weight, time to pupation and pupal weight analyses.

	d.f.	SS	*F*	*p*
larval weight				
genotype	4	45.87	10.05	<0.000 1
host plant	2	1459.98	639.52	<0.000 1
plant×genotype	8	68.18	7.47	<0.000 1
day	7	129.07	16.15	<0.000 1
time to pupation (excluding pepper)				
genotype	4	3.86	15.35	<0.000 1
host plant	1	4.07	64.70	<0.000 1
plant×genotype	4	2.00	7.96	<0.000 1
day	16	2.21	5.85	<0.000 1
sex	1	0.31	4.92	0.027
pupal weight[a] (excluding P_R-pepper and $P_R$sel-pepper)				
genotype	4	4311.7	1.2	0.29
host plant	2	128 932.2	74.4	<0.000 1
day	6	32 908.4	6.3	<0.000 1
sex	1	103 978.6	120.1	<0.000 1
pupal weight[a] (excluding pepper)				
genotype	4	4461.46	1.23	0.29
host plant	1	671.03	0.74	0.40
plant×genotype	4	19 146.80	5.30	<0.000 3
day	6	51 191.60	9.44	<0.000 1
sex	1	91 327.37	101.06	<0.000 1

[a]No resistant (P_R) larvae pupated when fed pepper leaves restricting the analyses on time to pupation and pupal weight. Two analyses were conducted on pupal weight: the first excluded P_R and $P_R$sel genotypes in the pepper treatment groups and the second excluded all genotypes in the pepper treatment group.

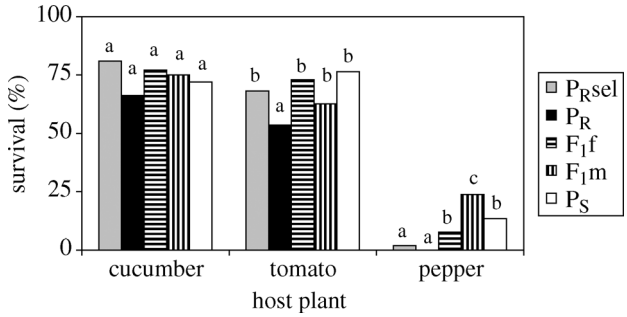

Figure 1. Survivorship to pupation of resistant selected and unselected in the parental generation ($P_R$sel, P_R), hybrid (F_1f, F_1m) and susceptible (P_S) genotypes feeding on cucumber, tomato or pepper. (Significant differences within host plant groups denoted as different letters.)

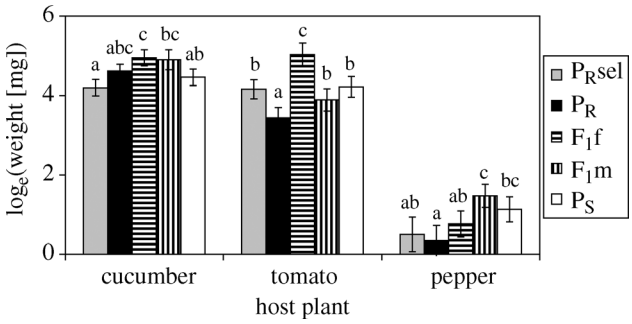

Figure 2. Least-square mean larval weights (±s.e.) of resistant selected and unselected in the parental generation ($P_R$sel, P_R), hybrid (F_1f, F_1m) and susceptible (P_S) genotypes feeding on cucumber, tomato or pepper. (Significant differences within host plant groups denoted as different letters.)

genotypes and slower on pepper (table 4). Furthermore, the reduced survival of F_1m larvae on pepper leaves relative to other genotypes suggests the presence of negative maternal effects on this host plant (figure 1).

Interestingly, pupal weights, the primary correlate of fecundity, did not differ between genotypes in the cucumber treatment group suggesting the absence of sizeable fitness costs in this environment (table 4). The pupal weights of the P_R genotype in the tomato treatment group were smaller than the other genotypes, but only significantly differed from the F_1f and $P_R$sel genotypes. Trans-generational effects owing to *Bt* exposure of the parental $P_R$sel population appeared to increase pupal weight relative to the P_R line in the tomato treatment group. In the pepper treatment, comparisons could be made only between the P_S and hybrid genotypes and no significant differences were found (table 3). A summary table of the host plant and genotypic effects on the magnitude and expression of fitness costs, maternal and trans-generational effects associated with *Bt* resistance is presented in table 5.

4. DISCUSSION

(a) *Host plant effects*

The performance of *T. ni* larvae varied considerably among the three host plants studied with the most rapid growth on cucumber, slower growth on tomato, and the slowest growth on pepper. Pupal size and larval growth rates are frequently correlated with fecundity in phytophagous insects, and lengthened development times can be associated with an increased risk of predation or parasitism (Awmack & Leather 2002). Thus, a trade-off can exist between the advantages of large size at maturity and disadvantages of a long development time (Nylin & Gotthard 1998). However, the extended development

Table 4. Time to pupation (square-root transformed) and pupal weight are shown for the unselected resistant population (P_R), selected resistant population ($P_R$sel), susceptible population (P_S) and the reciprocal F_1f (resistant female) and F_1m (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.)

	P_R	$P_R$sel	P_S	F_1f	F_1m
cucumber					
time to pupation	3.62±07 ab	3.92±0.07 c	3.56±07 a	3.57±0.07 a	3.71±0.08 b
pupal weight (mg)	217.9±4.6 a	215.2±3.6 a	211.5±3.9 a	209.8±3.3 a	216.9±3.9 a
tomato					
time to pupation	3.97±0.04 a	3.84±0.03 b	3.81±0.04 b	3.70±0.03 c	3.81±0.04 b
pupal weight (mg)	194.4±11.1 a	216.1±10.2 b	207.1±10.5 ab	241.6±11.13 c	204.2±11.7 ab
pepper					
time to pupation[a]	—	—	4.92±0.07 a	5.17±0.08 b	4.79±0.04 a
pupal weight[a] (mg)	—	—	159.8±7.4 a	156.3±9.2 a	166.5±4.4 a

[a]Only the susceptible populations and F_1 hybrids were included in the analysis of pupal weight and time to pupation.

Table 5. Summary of the genotype×host plant effects for each life-history trait.
(Fitness costs were estimated as per cent decreases in survival, larval weight, time to pupation or pupal weight. Fitness costs are identified as 0% if there are no significant differences between the P_R, $P_R$sel and P_S measurements. No P_R pupae were obtained in the pepper environment; NA, not available.)

	survival	larval weight	time to pupation	pupal weight
cucumber				
cost (P_R versus P_S)	0%	0%	0%	0%
maternal effect[a] (F_1f versus F_1m)	0	0	+	0
trans-generational effect[b] (P_R versus $P_R$sel)	0	0	—	0
tomato				
cost (P_R versus P_S)	29%	18%	4%	0%
maternal effect (F_1f versus F_1m)	0	+	+	+
trans-generational effect (P_R versus $P_R$sel)	+	+	+	+
pepper				
cost (P_R versus P_S)	100%	70%	NA	NA
maternal effect (F_1f versus F_1m)	—	—	—	0
trans-generational effect (P_R versus $P_R$sel)	0	0	NA	NA

[a]Maternal effect is positive when the F_1f hybrid measured value is higher or faster than the F_1m hybrid, negative when the F_1f hybrid measured value is lower or slower than the F_1m, and 0 when there is no effect.
[b]The trans-generational effect is positive when the $P_R$sel measured value is higher or faster than that of P_R, negative when the $P_R$sel measured value is lower or slower than that of P_R, and 0 when there is no effect.

time of larvae that were fed pepper leaves relative to those fed other host plants did not translate into larger pupal sizes, demonstrating that pepper is a substandard host for *T. ni*. Pepper was previously shown to be a poor host for *T. ni* (Sutherland 1966) and this could be owing to defensive compounds, such as phenolics (Estiarte *et al.* 1994), or poor nutritional quality. The underlying cause of the slower development of *T. ni* larvae feeding on tomato relative to cucumber is unknown, but is also probably owing to the presence of defensive compounds, such as chlorogenic acid and rutin, both of which negatively affect the growth of *Heliothis zea* (Isman & Duffey 1982*a*,*b*).

(b) *Fitness costs*

The larvae of the resistant genotype grew the slowest on all host plant species relative to the other genotypes and this 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. We are unable to discern whether the fitness cost was owing to direct effects of the resistance trait on life history (i.e. pleiotropy) or linkage to detrimental genes. To distinguish these, a more rigorous genetic protocol with repeated introductions of the resistant gene into the same genetic background would be required (Bergelson & Purrington 1996). Detrimental linkage rather than pleiotropy was the cause of fitness costs associated with herbicide resistance in half of the studies reviewed by Bergelson & Purrington (1996).

When resistance genes are rare, the evolution of resistance depends on the relative fitness of the hybrid compared with the susceptible genotype (Roush & McKenzie 1987). At low frequencies, resistance alleles are primarily present in hybrids, and if fitness costs are dominant, resistance would be slow to increase in the population (Carrière *et al.* 2002). For all life-history traits and in all host plant treatment groups examined here, fitness costs were recessive (i.e. hybrid genotypes did not significantly differ from P_S). Similarly, fitness costs associated with resistance to *Bt*-expressing transgenic cotton in *Pectinophora gossypiella* were recessive (Carrière *et al.* 2001*a*,*b*). By 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). In addition to the variation in the level of dominance of fitness costs among different resistance traits and species, the actions of modifier genes can ameliorate previously dominant fitness cost (McKenzie & O'Farrell 1993; Clarke, 1997; Chevillon *et al.* 1997).

(c) *Genotype by environment interactions*

Because the availability of resources to larvae for either defence 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 occurred on the best host plant, cucumber. Intermediate fitness costs occurred with tomato. Therefore, the differentiation among genotypes increased with decreasing host plant suitability and this would increase the selection against resistant genotypes. Similarly, Carrière *et al.* (2004) directly demonstrated that gossypol, a secondary compound of cotton, modifies the costs of resistance to *Bt* as indicated by pupal weight in the pink bollworm, *P. gossypiella*. Resistance-associated fitness costs have also been found to increase during the winter, a potentially stressful period, in *Bt*-resistant pink bollworm, *P. gosypiella* (Carrière *et al.* 2001*b*), 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).

(d) *Trans-generational and maternal effects*

To maintain resistance in the studied *T. ni* population, it was necessary to continually select the population with *Bt* as larvae. A comparison between the resistant P_R line, which was unselected in the parental generation, to the $P_R$sel line may highlight the presence of trans-generational effects resulting from the ingestion of *Bt* in the parental generation. For larvae that were fed tomato leaves, such effects were evident for all life-history traits examined. The surprising result was that exposure of the parental generation to *Bt* appeared to increase fitness of the resistant genotype in the tomato environment. Similarly, larvae from the $P_R$sel population were larger than the P_R population when grown on artificial diet in a separate preliminary experiment (7 day larval weight: 2.44 ± 0.09; 2.05 ± 0.14 square-root transformed least-square means for $P_R$sel and P_R, respectively). By contrast, if P_R experienced a reduction in the frequency of resistance alleles, owing to lack of selection with *Bt* in the parental generation, an increase in the growth rate of P_R relative to $P_R$sel would be expected.

It is possible that a non-genetic effect, such as increased egg provisioning, might have alleviated resistance-associated fitness costs in the $P_R$sel population fed on tomato. Trade-offs in egg provisioning and fecundity are common among Lepidopteran species (Awmack & Leather 2002). On pepper, however, parental exposure to *Bt* did not alleviate fitness costs in the resistant genotype. It is feasible that the proposed non-genetic effect observed on tomato may not have been sufficient to offset fitness costs on the less suitable pepper host plant.

A significant positive maternal effect (ie. $F_1f > F_1m$) was also observed among the genotypes feeding on tomato. Survival, growth rate and pupal size of the F_1f genotype were greater relative to the F_1m genotype in the tomato treatment group, which suggests that the resistant genotype of the mother had a positive effect on the development of larvae. This was not observed on cucumber and was negative on pepper, where survival and growth rates were poorer for the F_1f genotype relative to the F_1m genotype. Non-genetic effects, such as those described above, may also contribute to maternal effects (Mousseau & Fox 1998). Positive or negative maternal and trans-generational effects, such as those described in this study, may impede or accelerate responses to selection and, therefore, warrant further investigation (see Kirkpatrick & Lande 1989).

The trans-generational and maternal effects on tomato might be explained by the fact that these populations originated from a tomato greenhouse. *Trichoplusia ni* populations can have over six generations per year in greenhouses and can survive there in the winter (V. Cervantes, personal communication). Thus, the population used in this study could be well adapted to tomato. In addition, *Bt* remains active in greenhouses for 9 days or more (preliminary experiment) and is often applied multiple times during each growing season, thereby contributing to the intense selection pressure for *Bt* resistance. Adaptation to both *Bt* and tomato might explain the complex maternal and trans-generational effects observed in the tomato treatment group. Host race differentiation in phytophagous insects has been shown to occur in as little as 10 years (Bernays & Graham 1988). Complex genetic interactions have been recognized in both the study of insecticide resistance (McKenzie *et al.* 1982) and in the local adaptation of phytophagous insects to alternative host plants (de Jong & Nielsen 2002).

Managing resistance to insecticides depends on associated fitness costs such that the frequency of resistance alleles will decline when selection is reduced by using insecticide rotations (Ferré & van Rie 2002) or temporal refuges (periods with no *Bt* exposure; Tabashnik *et al.* 1994). In addition, it has been suggested that plant–insect genotype interactions, such as those described in the current study, may be manipulated in conjunction with the high-dose/refuge strategy to minimize the risk of resistance to transgenic *Bt* crops (Carrière *et al.* 2002). Because the magnitude of fitness costs varies with the suitability of the host plant for larval growth and development, we suggest that resistance will be more difficult to control in cucumber greenhouses than in pepper greenhouses.

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