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Persistence and Inheritance of Costs of Resistance to Imidacloprid in Colorado Potato Beetle

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ABSTRACT Reduced fitness among resistant versus susceptible individuals slows resistance evolution and makes it easier to manage. A loss of resistance costs could indicate novel adaptations or mutations contributing to resistance. We measured costs of resistance to imidacloprid in a Massachusetts resistant population compared with a Massachusetts susceptible population in 1999 in terms of fecundity, hatching success, egg development time, and sprint speed. Resistance was additive and seemed to be polygenic with high heritability. The fecundity cost appeared overdominant in 1999, and the hatch rate cost was partly recessive in 1999, but neither was significantly different from dominant or recessive. In 2004, we repeated our measures of resistance costs in Massachusetts in terms of fecundity and hatching success, and we added a new resistant population from Maine. In 2005, we compared development time of Maine resistant and the laboratory susceptible colony eggs. Significant fecundity costs of resistance were found in both population in both 1999 and 2004, and significant egg developmental time costs were found in 1999 and 2005. However, the hatching success costs of resistance were significant in 1999 and not apparent in 2004, suggesting some modification or replacement of the resistance genes in the intervening time.

KEY WORDS *Leptinotarsa decemlineata*, dominance, life history, insecticide resistance management

Insecticide resistance is a major problem facing most pest control programs (Denholm and Rowland 1992, Lenormand and Raymond 1998). The most commonly proposed strategy to delay the evolution of resistance is to provide untreated plots within or among fields (McGaughey and Whalon 1992; Tabashnik 1994; Caprio 1998, Carrière and Tabashnik 2001). Susceptible populations persist within these plots and mate with any newly resistant genotypes that arise in adjacent treated areas. However, a number of assumptions need to be met for this refuge-based strategy to succeed. First, resistant alleles should be recessive, so that insecticides kill heterozygotes. Second, in the absence of pesticide, resistant alleles should be associated with the decreased fitness of resistant individuals, so that they are at a selective disadvantage. Preferably, such fitness costs are themselves genetically dominant, so that selection acts efficiently against both hetero- and homozygotes (Carrière and Tabashnik 2001). Finally, there should be sufficient gene flow between resistant and susceptible populations, curtailing production of resistant homozygotes.

If all three conditions are true, refuges are in theory expected to maintain pest populations susceptible to insecticides (Bauer 1995, Carrière and Tabashnik 2001). However, it is important to remember that resistance development is, by definition, a dynamic evolutionary process that could be likened to an arms race between humans coming up with new toxins and ways of their deployment and pests developing new adaptations (Denholm and Rowland 1992). Modification of fitness costs and their inheritance, changes in life history, gene flow between geographically isolated populations, and competition between resistance genes may present additional obstacles to otherwise sound resistance management strategies (McKenzie and O'Farrell 1993, Charpentier and Fournier 2001, Lenormand and Raymond 1998, Raymond et al. 2001). Therefore, sustainable use of chemical control depends on a good understanding of resistance development as an evolutionary process, not just on characterization of resistant individuals and their populations.

Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a major pest in most potato (*Solanum tuberosum* L.)-growing areas of the world. This species has a truly remarkable ability to develop resistance to a variety of insecticides. The first case of resistance was noted for DDT in 1952 (Quinton 1955). Since then, the beetle has lost susceptibility to a wide range of chem-

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icals, including the arsenicals, organochlorines, carbamates, organophosphates, and pyrethroids (Forgash 1985; Ioannidis et al. 1991; Stewart et al. 1997). Some of them failed after 1 yr (e.g., endrin) or even during the first year of use (e.g., oxamyl) (Forgash 1985).

In-furrow systemic applications of imidacloprid are widely used for the Colorado potato beetle control. Such applications maximize plant coverage and significantly increase insecticide persistence in potato foliage. Unfortunately, they also create strong selection pressure on beetle populations toward developing resistance to this compound (Olson et al. 2000, Zhao et al. 2000a). Although imidacloprid is still highly efficient against Colorado potato beetles in most places, isolated cases of its failure have been reported from several commercial farms in northeastern United States (Alyokhin et al. 2006, 2007; Mota-Sanchez et al. 2006).

Refuge-based strategy may be useful in delaying the onset of imidacloprid resistance in the Colorado potato beetle, but its success cannot be taken for granted. On one hand, decreased relative fitness of resistant genotypes in insecticide-free environments was observed in beetle strains resistant to several insecticides (Argentine et al. 1989b; Trisyono and Whalon 1997; Alyokhin and Ferro 1999a), although it has not yet been documented for imidacloprid-resistant beetles. However, resistance to a variety of insecticides in this pest is inherited as a suite of incompletely dominant traits (Argentine et al. 1989a, 1989b; Ioannidis et al. 1992; Rahardja and Whalon 1995). This includes one population from Long Island, NY, that is resistant to imidacloprid (Zhao et al. 2000a). In this study, we investigate the inheritance of traits associated with resistance to imidacloprid in two resistant populations of the Colorado potato beetle. We use this information to estimate the cost of carrying resistant alleles relative to wild-type, susceptible alleles, as measured by two fitness components, fecundity and egg-hatching success, and whether those costs themselves seem to be evolving. The presence of resistance costs will greatly enhance the probability of success for the high-dose–refuge approach to resistance management.

Materials and Methods

Beetle Populations

Susceptible Populations. Colorado potato beetles susceptible to imidacloprid were taken from a laboratory colony of 150–300 beetles originally collected from an untreated potato field in South Deerfield, MA, in 1999. Though the field used for collection was never treated with neonicotinoids, it was ≈2.6 km (1.6 miles) from a commercial field treated with imidacloprid for the three previous seasons. Colonies were maintained in group cages (65 by 48 by 48 cm) at 25–27°C and a photoperiod of 16:8 (L:D) h throughout the year. Each year, 30–50% of the colony was replaced with field-collected beetles from the least resistance wild population found in western Massachusetts to reduce inbreeding and adaptation to cage conditions in captivity.

Resistant Populations. *1999 Experiments.* The beetles resistant to imidacloprid were originally collected from an imidacloprid-treated field in Hadley, MA, in 1999. Alternating generations of their progeny were subject to selection by topically treating 6.5–8.5 mg of second instars with a 1-μl drop of 1.4×10^{-5} g/ml imidacloprid dissolved in acetone, a dose that corresponded roughly to the LD_{50} of the colony. That maintained the resistant colony 8–20 times as resistant (measured by LD_{50}) as the susceptible colony.

2004 Experiments. The beetles resistant to imidacloprid were collected as eggs from two fields in Hadley, ≈3.5 miles from the Hadley source field from 1999, and four adjoining fields in Fryeburg, ME. The experimental adults were drawn at random from ≈200 adults from each location that had survived exposure as second instars to a 1-μl drop of either 5×10^{-5} or 1×10^{-4} g/ml imidacloprid dissolved in acetone.

2005 Experiment. The beetles resistant to imidacloprid were collected as eggs from the same four fields in Fryeburg as the previous year's study. The adults used were drawn at random from ≈200 adults from each location that had survived resistance assays as for 2004 described above.

Crosses. A series of reciprocal crosses within and between resistant and susceptible populations were conducted to measure inheritance and fitness costs of imidacloprid resistance.

1999 Experiments. Twenty-five males and 25 females were drawn randomly from the resistant and susceptible colonies within 2 d after emerging as adults and isolated by sex to preserve their virginity (Alyokhin and Ferro 1999b). Twelve resistant virgin females were individually paired with five resistant and seven susceptible virgin males, and 13 susceptible virgin females were mated individually to eight resistant and five susceptible males. All survived to produce clutches with at least some fertile eggs. The pairs were maintained in individual vented Nalgene boxes (12.5 by 7 by 5.5 cm) in a Percival-I 36VL incubator at 26 ± 1°C and a photoperiod of 16:8 (L:D) h. The beetles were provided with excised potato foliage placed in floral water pics. The foliage was replenished daily and any eggs were collected. A second generation of crosses was carried out using randomly selected offspring from 22 families from both parental and hybrid lines.

2004 Experiments. Offspring of the Massachusetts and Maine field-collected adults were reared to adulthood and isolated by sex upon emergence as adults. Forty-seven pairs of adults mated within and between each resistant population and the laboratory-susceptible line survived to lay eggs. Clutches were collected for up to 22 d (12–22), depending on when laying commenced.

Resistance Assays. In all years, larval assays were the primary means for measuring population- and family-level resistance. Second instars weighing 6–8.5 mg were assayed by direct topical application of a 1-μl drop of imidacloprid dissolved in HPLC-grade (0.995)

acetone on the abdomen. Seven concentrations ranging from 1.6×10^{-6} to 1×10^{-4} g/ml plus an acetone control were tested. Larvae were scored 24 h after application, with mortality defined as failure to move a leg for 10 s after the larvae is placed on its back. Dose–mortality curves were analyzed using Polo-PC (LeOra Software 1987). We attempted to assay at least 30 individuals at each dose, but if fewer were available at the correct size, we analyzed the mortality data and included the LD_{50} results if the index of significance for potency estimation, g (Finney 1971), was <0.7 at the 0.95 confidence level.

Baseline resistance was determined separately for field-collected and susceptible populations. Larval assays were carried out as above and LD_{50} values and 95% confidence intervals (CIs) estimated using likelihood ratios in Polo-PC (LeOra Software 1987). In addition, knockdown assays were carried out on the adult beetles used in crosses in 2004 to compare relative resistance of Massachusetts, Maine, and susceptible strains. Adults were given a single dose of 1 μl of imidacloprid dissolved in acetone at a concentration of 1×10^{-3} g/ml. Beetles were considered knocked down if they could not right themselves in 10 min after being flipped over in a petri dish lined with filter paper, and alive if they could right themselves. Beetles were observed every 15 min for 8 h and then one final time after 24 h since application. After 24 h, beetles were scored as alive or knocked down as described above, and dead if they were immobile for 10 min after prodding.

Strain differences in resistance were tested using analysis of variance (ANOVA) of offspring LD_{50} values and planned orthogonal contrasts between each parental line and the means of the hybrid crosses. The degree of dominance (D) of resistance was calculated as in Stone (1968) on a -1 to 1 scale:

$$D = (2RS - RR - SS)/(RR - SS)$$

Standard errors were calculated after Preisler et al. (1990). The 95% confidence interval for D is ± 2 SE. Expected mortalities of the 1999 backcrosses assuming single-locus Mendelian inheritance were compared with observed mortalities using chi-square test, where expected mortality was the average of the hybrid and parental LD_{50} values (but see Preisler et al. 1990). In 1999, the $\ln LD_{50}$ values of the second generation were regressed against the midpoint of the LD_{50} values of the parents' families to measure heritability of resistance. This is a standard midparent-offspring regression, but in the context of our resistance assays, it is actually carried out between LD_{50} values of the progeny of grandparents and parents. Population shifts from 1999 to 2004 were analyzed by ANOVA.

Fecundity, Developmental Time, and Hatching Success

1999 Experiments. All eggs from each day were pooled for that pair and kept in a single 35-mm-diameter plastic petri dish, held in the same incubator at 25°C and a photoperiod of 16:8 (L:D) h. In the first generation, eggs were collected until the female died, from 8 to 42 d of egg laying in this sample. In the second generation, eggs were collected for up to 21 d, so that average rather than total fecundity was analyzed. Clutches were checked for hatching once a day; upon hatching, larvae were removed and the remaining eggs were checked for up to three more days for further hatching. The number of days to hatching for eggs from each clutch for each pair was recorded in the first generation, and the mean, weighted by the number hatching in each clutch, was calculated per pair. In the second generation, fecundity (defined as a total number of eggs laid) but not hatching success was measured. The degree of dominance for fecundity and hatching success was calculated using the same index, D, as was used for the inheritance of resistance. This is mathematically equivalent to the index of heterozygote effect, h, used in other studies of dominance of resistance cost (Roux et al. 2004) except that the scale differs, varying from -1 to 1, rather than from 0 to 1.

2004 Experiments. Virgin adult offspring of resistant, field-collected Maine and Massachusetts beetles were paired within each population or as hybrids with virgin susceptible beetles. Pairs were housed as described above, and eggs were collected daily for up to 21 d. All eggs from each day were pooled by pair and kept in a single 35-mm-diameter plastic petri dish and held in the same incubator at 25°C and a photoperiod of 16:8 (L:D) h. After 20 d, the adults (aged 24–27 d) were assayed using the knockdown trial described above. Hatchlings were counted and removed twice a day to reduce cannibalism. Hatch rates were arcsine transformed to improve normality. Costs of resistance in Massachusetts and Maine populations in 2004 were compared using ANOVA and Student's t-test to look for significant differences between individual means. To compare fecundity costs of resistance within Massachusetts populations among years, a subset of the 1999 clutches were used including only those laid in the first 20 d after pairing, to remove potential confounding effects of including older females' fecundity from 1999. Persistence of the costs of resistance vis-à-vis fecundity and arcsine-transformed hatching success was analyzed using ANOVA of effects of year, parental resistance, and their interaction.

2005 Experiment. Egg developmental time was compared between resistant and susceptible populations from Maine. Five females from each population were mated with a single male from the same population. Egg masses were collected two to three times per day, kept in single 35-mm-diameter plastic petri dishes, and they were held in the same incubator at 25°C and a photoperiod of 16:8 (L:D) h. Clutches were observed two to four times per day, all hatchlings removed, and the weighted average of the number of hours to hatch calculated per pair. Development time to hatching was compared using values from resistant and susceptible populations from Massachusetts collected in 1999.

Table 1. Second instar LD_{50} values of field and laboratory populations in micrograms per gram

Yr	Strain	*n*	LD_{50}	Lower	Upper	Slope	χ^2	df	RR
1999	Massachusetts resistant field	184	3.14	2.51	3.98	1.741	2.444	5	6.8
1999	Susceptible	1,347	0.60	0.38	0.84	2.126	9.218	5	1.3
2004	Massachusetts resistant field	1,455	3.65	2.17	6.73	1.837	8.428	6	7.9
2004	Maine resistant field	14,194	9.85	7.75	12.46	2.02	232.20	6	22.9
2004	Susceptible	509	0.51	0.02	1.43	1.446	35.007	7	1.1
2005	Maine resistant field	20,426	6.24	5.89	6.60	1.785	46.249	7	13.6
2005	Susceptible	92	0.47	0.31	0.63	2.59	1.92	5	1.0

RR, resistance ratio.

Sprinting Speed

This study was conducted in 2002. We adapted a sprint-speed assay commonly used as a measure of general physiological condition in vertebrates (Garland 1994) for the Colorado potato beetle. Our method relied on high temperature as an irritant to stimulate locomotion. Individual 5–10-d-old beetles were placed in the center of a paper disc set on a Sybron Thermocline hot-plate. Because experiments using locomotor performance often suffer from difficulty distinguishing behavioral motivation from ability, we conducted preliminary assays to find appropriate stimulus intensity. Below 60°C, some beetles did not walk or waited before walking, and above that temperature some beetles lost coordination to walk; therefore, surface temperature was maintained at 60°C. Beetles were timed as they walked 7 cm from initially crossing a 1-cm-radius circle to an 8-cm-radius circle on the outer edge of the hot plate. One hundred thirty-six resistant and 105 susceptible beetles of both sexes were tested. After $\log_{10}$ transformation, walking speeds were not significantly different from normal (Shapiro–Wilk $W = 0.980$, $P = 0.258$, $N = 266$). Walking data were analyzed using ANOVA for effects of sex and population.

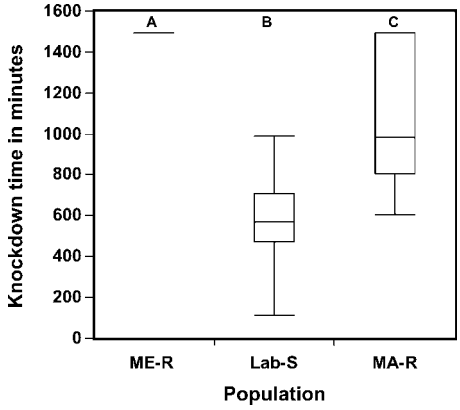

Fig. 1. Knockdown time box plots of Massachusetts, ME, and susceptible laboratory-colony adults used in 2004 crosses. The box encloses the 25–75% quartiles, with the median line drawn within, and the outlier whiskers include the outer points up to 1.5 * interquartile range. There is no box for Maine because all adults were not knocked down at 24 h. Similar letters denote means that are not significantly different at $\alpha = 0.05$ by using Tukey's HSD test.

Results

Baseline Resistance of Tested Populations. LD_{50} values for field-collected beetles were 5.2–21.8 times higher than the corresponding susceptible population (Table 1). Maine field populations were significantly more resistant than the Massachusetts field population in 2004, with no overlap between the 95% CIs of their LD_{50} values. There was also no overlap between 95% CIs of Massachusetts resistant and susceptible beetles in 1999, but there was slight overlap in 2004. There was no overlap between Maine and susceptible-strain CIs of LD_{50} in 2004 or 2005.

Knockdown trials of adults used in 2004 crosses confirmed significant differences in resistance among all three populations (Fig. 1). Mean knockdown time varied among populations (ANOVA: $F = 42.312$; df = 2, 91; $P < 0.0001$). Using Tukey's honestly significant difference (HSD) test, all three populations were significantly different from the other two in knockdown time, with Massachusetts population being intermediate between Maine and susceptible populations.

Inheritance of Resistance. We assayed 4863 second instars from 26 families in the first 1999 generation. Because LD_{50} values of hybrid offspring were not affected by the sex of the resistant parent (using pairwise Student's *t*-test comparisons), they were pooled into a single hybrid cross category. The mean resistance (LD_{50}) for each mating combination in the first generation is presented in Fig. 2. We assayed 5,329 individuals in 21 families in the second 1999 genera-

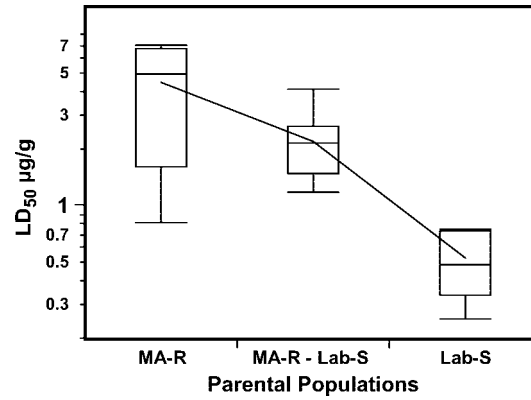

Fig. 2. Box plots of LD_{50} values of 1999 crosses. Similar letters denote means that are not significantly different at $\alpha = 0.05$ by using Tukey's HSD test.

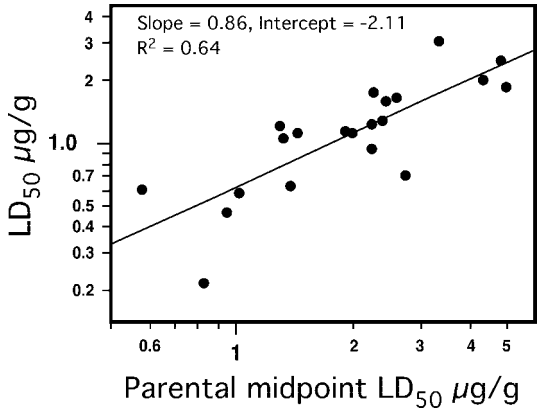

Fig. 3. Midparent-offspring regression of ln-transformed LD_{50} values from 1999. The slope ± SE is 0.82 ± 0.14 and the 95% CI for heritability is 0.55–1.12.

tion. When the observed mortalities of the RS × SS backcross lines were compared with the expected means of R × S and S × S lines for each dose, five of seven single-dose chi-square tests were significant, and the combined test of predicted values from a single major gene was $P(\chi^2_8)<0.0001$ (Table 2). Heritability of resistance estimated as the slope of the regression of offspring with the midparental values was $h^2 = 0.86$ (Fig. 3) ($F = 33.86$; df = 1, 19; $P < 0.0001$).

The mean resistance (LD_{50}) for each mating combination in 2004 is presented in Fig. 4. We assayed 13,202 second instars from 59 families. The ln-transformed LD_{50} values differed significantly among parental populations (ANOVA: $F = 17.68$; df = 6, 27; $P < 0.0001$). Because LD_{50} values of hybrid offspring were not affected by the sex of the resistant parent (using pair wise Student's *t*-test comparisons), they were pooled into a single hybrid cross category for each geographical strain. Resistant pairs' offspring from both Massachusetts and Maine had significantly higher LD_{50} values than the hybrid offspring, and hybrids were significantly more resistant than offspring of susceptible pairs using Tukey–Kramer's HSD test. However, resistant pairs from Maine and Massachusetts were not significantly different from each other, although there was a slight trend for higher resistance in Maine offspring.

Table 2. Test of deviation from expected mortality if resistance is monogenetic

Dose (µg/g)	% response		χ^2	*P*
	Observed	Expected		
0.2	31.7	27.4	1.28	0.258
0.4	54.4	45.9	4.99	0.025
0.9	83.9	57.9	48.43	0.000
1.8	93.7	88.6	4.00	0.046
3.6	97.1	89.0	9.07	0.003
7.1	97.5	97.2	0.05	0.818
14.3	100.0	91.9	6.40	0.011
Total χ^2			74.23	0.000

The chi-square tests the difference of observed and expected response from the F_1 × S backcross.

Resistance in 2004 was similar and incompletely dominant in Massachusetts and Maine populations, and between 1999 and 2004 Massachusetts populations. In 1999, the degree of dominance in the Massachusetts population was D ± SE = 0.18 ± 0.35. This value is not significantly different from exactly additive ($D = 0$), and the 95% CI excludes complete dominance or recessiveness. In 2004, the degree of dominance in the Massachusetts population was $D = 0.15 \pm 0.42$, and the degree of dominance in the Maine population was $D = 0.16 \pm 0.35$. Similarly to 1999, the 95% CIs in both Massachusetts and Maine overlap $D = 0$, but exclude complete dominance or recessiveness, but all values tend to slight dominance of LD_{50}.

Fitness Costs of Resistance. *Sprinting Speed.* There was no difference between sexes in walking speed when analyzed within a two-factor ANOVA of walking speed on resistance and sex (Parameter test for sex: $F = 0.0623$; df = 1, 237; $P > 0.8$), nor was there any significant interaction between sex and resistance. Therefore, males and females were pooled. There was a small (1.8 cm/s susceptible versus 1.6 cm/s resistant)

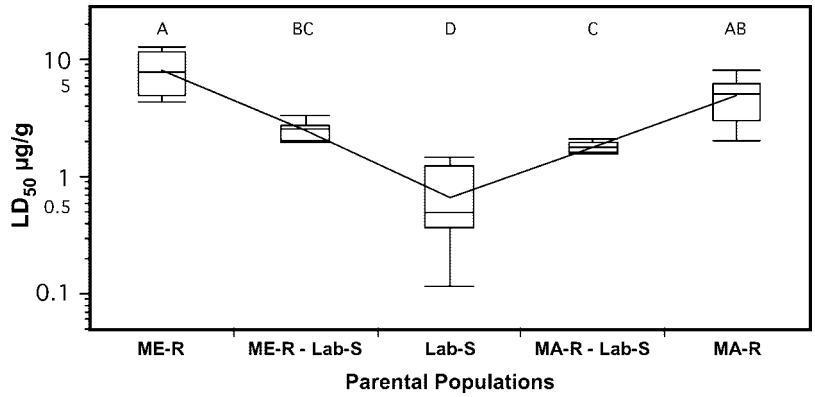

Fig. 4. Box plots of LD_{50} values of 2004 crosses. Similar letters denote means that are not significantly different at $\alpha = 0.05$ by using Tukey's HSD test.

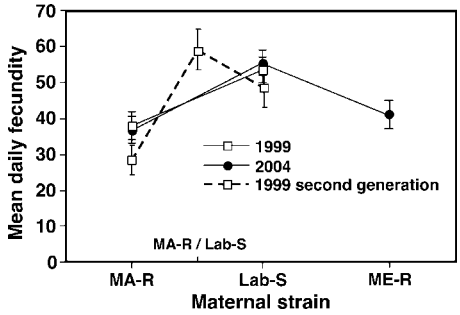

Fig. 5. Least-squares means ± SE of average daily fecundity in 1999 and 2004 in resistant and susceptible females from Massachusetts. This figure combines means from three analyses. The 1999 and 2004 Massachusetts means are from a LS contrast from an ANOVA of fecundity on year, population, and their interaction. The 2004 Maine means and SEs are from an ANOVA comparing Massachusetts resistant, laboratory susceptible, and Maine resistant female fecundity in 2004.

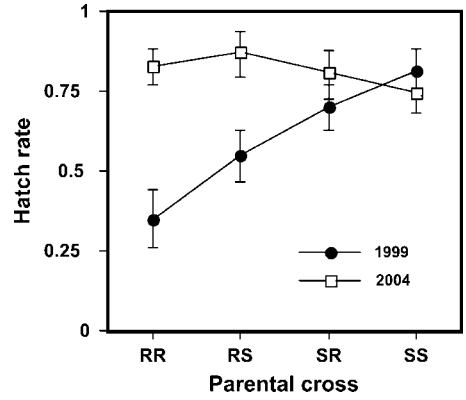

Fig. 6. Mean ± SE of hatching success in Massachusetts resistant, laboratory susceptible, and hybrid crosses in 1999 and 2004.

but significant difference between resistant and susceptible beetles ($F = 6.57$; df = 1,239; $P = 0.011$.

Fecundity. Total fecundity varied among crosses in 1999 (ANOVA: $F = 3.162$; df = 3, 21; $P = 0.046$) with RR and SS significantly different using Student's *t*-test at $\alpha = 0.05$ (Fig. 5). Massachusetts female parental population had a significant effect on fecundity in both years (1999: ANOVA; $F = 8.764$; df = 1, 23; $P = 0.007$, 2004: ANOVA; $F = 10.709$; df = 2, 38; $P = 0.0002$), and Maine and Massachusetts resistant females did not differ in mean fecundity by using pairwise comparisons using Student's *t*-test. The fecundity cost of resistance was stable within Massachusetts between 1999 and 2004. The ANOVA results of average daily fecundity on year, resistance, and their interaction are presented in Table 3. There were no significant year or interaction effects, suggesting fecundities of resistant beetles were consistently ≈31% lower over that interval.

In the second 1999 generation, female genotype again had a significant effect on fecundity (ANOVA: $F = 10.394$; df = 2, 19; $P = 0.0009$) (Fig. 5). The estimated dominance, *D*, was 2.04, suggesting strong underdominance of the fitness cost. However, the high variance of *D*, 6.02, cannot significantly distinguish among any levels of dominance. Furthermore, pairwise comparisons using Student's *t*-test suggest that the mean fecundities of heterozygotes and susceptible females were not different.

Table 3. Analysis of variance for the effect of year and resistance on average fecundity and hatching success

Source	df	SS	*F* ratio	Prob > *F*
Fecundity				
Yr	1	2.5635	0.0148	0.9038
Cross	1	3754.6721	21.6243	<0.0001
Cross × yr	1	31.3771	0.1807	0.6727
Error	47	8160.724		
Total	50	11969.300		
Hatching success				
Yr	1	0.642	13.783	0.0006
Cross	3	0.282	2.016	0.1260
Cross × yr	3	0.652	44.667	0.0066
Error	43	2.003		
Total	50	3.385		

Hatching Success. Arcsine-transformed hatching success varied significantly among cross types in 1999 (ANOVA: $F = 3.094$; df = 3, 21; $P = 0.023$) (Fig. 6). Resistant clutches had significantly lower hatch rates than susceptible ones, using Tukey's HSD test for pairwise comparison, but hybrid eggs had hatch rates not significantly different from each other or from either pure strain. The LS means for hatch rates, SS = 0.82, *SR* = 0.63, and RR = 0.35, suggest a somewhat recessive hatching cost, with an estimate of $h = 0$.

In 2004, there were no significant differences in arcsine-transformed hatching success among Maine or Massachusetts resistant or susceptible clutches, or hybrid clutches (ANOVA: $F = 0.698$; df = 6, 34; $P = 0.653$). Within Massachusetts, the hatching success cost seems to have been lost between 1999 and 2004. The ANOVA of arcsine transformed hatch rates on year, cross, and their interaction are presented in Table 3, and a plot of the LS means from the ANOVA are presented in Fig. 6. There was no consistent effect of cross type, but significant year and interaction effects occurred, suggesting any effect of resistance on hatching success was lost.

Development to Hatch. Hours to hatch varied significantly among resistant, susceptible, and hybrid crosses in 1999 (ANOVA: $F = 5.082$; df = 2, 22; $P = 0.015$) (Fig. 7). Resistant pairs laid clutches that took 9.7 h longer, on average, to hatch than susceptible pairs, a significant difference using Tukey's HSD test, and hybrids were intermediate in time to hatch.

Discussion

Costs of resistance to imidacloprid are significant in the Colorado potato beetle. The fecundity of resistant beetles was almost one third less than that of susceptible females. The hatching cost, when present, was

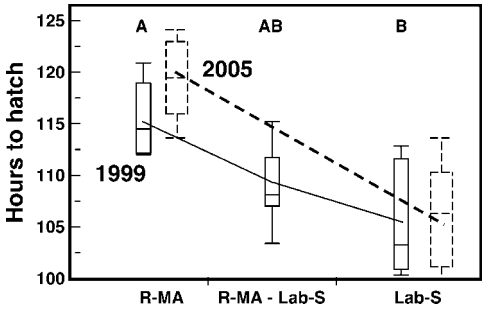

Fig. 7. Box plots of times to hatch in (solid lines) 1999 and (dashed lines) 2005. Similar letters in 1999 denote means that are not significantly different at $\alpha = 0.05$ by using Tukey's HSD test.

>50%. The longer time to hatch for eggs of resistant beetles will reduce population growth directly, but it is likely to have a greater effect indirectly, through increasing risks of cannibalism or predation by the 13% increase in time to hatch (Hazzard et al. 1991). The combination of reduced fecundity and slower development will reduce intrinsic growth rates of resistant genotypes. The slower sprint speeds observed in resistant beetles also may act indirectly to select against resistance, if it is indicative of reduced dispersal capabilities.

The fecundity cost is similar between the two geographically disjoint populations. One potential pitfall of the present and several past studies of resistance costs is that the resistant and susceptible populations may differ in traits other than resistance, because the sources may be from different geographical areas, or they may have been held in the laboratory for different numbers of generations. We tried to address this by comparing more than one resistant population, and by adding wild beetles to our susceptible colony each summer to reduce inbreeding and adaptation to laboratory conditions. One observation that lends confidence that our results are not an artifact of selection on a laboratory susceptible strain is that there was no difference in fecundity between the laboratory susceptible strains between 1999, the year it was collected entirely from the wild, and 2004, after several years in the laboratory.

Some costs, such as reduced fecundity and increased developmental time, were highly conserved over time. Loss of resistance costs has been ascribed to the presence of modifiers (Lenski 1988, Mason 1998, McKenzie and O'Farrell 1993, Charpentier and Fournier 2001), although in other well studied cases cost reduction seems to have occurred through allele replacement rather than addition of cost modifiers (Zhao et al. 2000b, Raymond et al. 2001). It is possible that an allelic substitution occurred in Massachusetts that expressed higher hatching success in 2004 than 1999, or that modifiers arose to eliminate the difference in hatch rate. It is possible that the modifier or variant was present in 1999, as different fields were used as a source, and the closest field from 2004 was about 3 km from the 1999 source population.

The partial dominance in resistance observed here contrasts slightly with the dominance of −0.23 and −0.11 measured at 3 and 7 d after imidacloprid treatment in adult Colorado potato beetles from the Long Island population (Zhao et al. 2000a). It is not possible to determine whether the difference is significant, as our confidence intervals overlap the measures of D from Zhao et al. 2000a, and theirs did not include confidence intervals. However, all our three measures in 1999 and 2004 all were slightly dominant, whereas both of the earlier measures tended toward recessive inheritance. The earlier study used adults, whereas the current study used larval assays. Dominance often varies among life stages (McKenzie 2001), and it was seen to decline in the Colorado potato beetle larvae exposed to permethrin (Follett et al. 1993). In a single-dose assay the dominance observed depends almost wholly on the dose chosen (Roush and McKenzie 1987). In a dose–response assay, dominance should be more comparable between studies.

Interestingly, both the current study and the study by Zhao et al. (2000a) found evidence of polygenic inheritance of resistance to imidacloprid in the field. Polygenic inheritance has been argued to occur as an artifact of laboratory selection studies, because population sizes in captivity limit the strength of selection that can be applied, resulting in observation of only the genes of small effect already present in the susceptible population (Roush and McKenzie 1987). Because most theoretical models advocating high-dose–refuge approach assumed that resistance is controlled by a single gene, a certain caution might need to be exercised when applying their findings to managing imidacloprid resistance in the Colorado potato beetle.

The recessive cost in fecundity observed in the current study is probably not an artifact of heterosis, because the populations were only in the laboratory for two to four generations before the experiment, and they were collected from fields a few kilometers from each other in an area densely populated with potato fields. Together with partially dominant of resistance, this favors resistance genes when they are found in individuals heterozygous at some or all resistance loci. Thus, there is likely to be a challenge to long-term management using a refuge approach.

Certain caution needs to be exercised when using our results to develop an applied resistance management plan. First, systemic toxin imidacloprid is delivered by ingestion, which may constitute different nuances of selection from the topical applications used in the current study and by Zhao et al. (2000a). Second, concentrations of toxin are likely to vary between different times of year and locations within the potato plant. Finally, there is likely to be genetic variation among Colorado potato beetle populations from different geographic areas. Still, the significant costs found do suggest that resistance management using refuges, or temporal rotation of treatments with non-neonicotinoids, can have an effect on prolonging efficiency of imidacloprid.

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