

## Relative Fitness of Colorado Potato Beetle (Coleoptera: Chrysomelidae) Resistant and Susceptible to the *Bacillus thuringiensis* Cry3A Toxin

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**ABSTRACT** Laboratory experiments were conducted to compare relative fitness of strains of Colorado potato beetle resistant and susceptible to *Bacillus thuringiensis* subsp. *tenebrionis* Cry3A toxin. Net replacement rates and intrinsic rates of population increase were calculated for resistant and susceptible populations. During the experiment, susceptible males on average copulated  $13.3 \pm 1.5$  times, whereas resistant males copulated only  $8.0 \pm 1.0$  times. Susceptible females produced an average of  $824.2 \pm 68.1$  eggs and  $590.9 \pm 58.5$  larvae, which was significantly  $>484.6 \pm 48.0$  eggs and  $334.9 \pm 39.7$  larvae produced by an average resistant female. As a result, both net replacement rate and intrinsic rate of increase were reduced for the resistant population. Furthermore, twice as many susceptible beetles as resistant beetles survived overwintering diapause. Our results clearly indicate that relative fitness of resistant individuals is reduced in the absence of *B. thuringiensis* in the environment. Therefore, relaxation of selection pressure through refugia and insecticide rotation will favor decrease in the frequency of resistant alleles in beetle populations.

**KEY WORDS** *Leptinotarsa decemlineata*, *Bacillus thuringiensis*, insecticide resistance

COLORADO POTATO BEETLE, *Leptinotarsa decemlineata* (Say), is the most important insect defoliator of potatoes (Weber and Ferro 1994). This species has a remarkable ability to develop resistance to numerous chemicals that have been used against it. The 1st instance of Colorado potato beetle resistance to synthetic organic pesticides was noted for DDT in 1952 (Quinton 1955). Since then, this insect has become resistant to a wide range of insecticides, including the arsenicals, organochlorines, carbamates, organophosphates, and pyrethroids (Forgash 1985, Ioannidis et al. 1991). Grafius (1997) estimated that annual losses attributable to beetle insecticide resistance in Michigan potato industry range between \$230 and \$270/ha.

Currently, field populations of the Colorado potato beetle can be effectively controlled by the insecticidal materials based on the *Bacillus thuringiensis* subsp. *tenebrionis* Cry3A toxin (Ferro and Gelernter 1989). However, selection of a *B. thuringiensis* resistant strain under laboratory conditions was successful (Whalon et al. 1993; Rahardja and Whalon 1995). A potential threat of the failure of the *B. thuringiensis*-based materials, similar to the failure of synthetic insecticides exists. The probability of rapid resistance development will increase dramatically with increased persistence and coverage by these materials; a condition typical for transgenic plants (Ferro 1993). Gould (1988) and Ferro (1993) presented theoretical evidence that the Colorado potato beetle is likely to develop resistance to transgenic plants expressing high levels of the delta-endotoxin within 6 generations if the use of these transgenic plants is mismanaged.

Strategies proposed to delay the evolution of resistance include provision of temporal and spatial refugia from exposure to toxins, thus minimizing insecticidal pressure on pest populations (McGaughey and Whalon 1992, Tabashnik 1994). If resistance development is associated with the decreased fitness of resistant individuals, such an approach will promote reversion of the pest populations back to susceptibility (Bauer 1995).

Reduced relative fitness of resistant genotypes in insecticide-free environments is characteristic for many insect species. It has been widely documented both for the strains resistant to synthetic insecticides (Ferrari and Georghiou 1981, Roush and Plapp 1982, Beeman and Nanis 1986), as well as for the strains resistant to *B. thuringiensis* (Georghiou 1981, Groeters et al. 1994). In the Colorado potato beetle, a fitness value (intrinsic rate of increase) for an azinphosmethyl-resistant strain was equal to 83% of a fitness value for a susceptible strain (Argentine et al. 1989). Furthermore, a fitness value for a strain resistant both to azinphosmethyl and permethrin was only 76% of a fitness value for the susceptible strain. Similarly, Colorado potato beetles resistant to *B. thuringiensis* had reduced fecundity and shorter ovipositional period, as well as prolonged 3rd larval stadium and lower weight gains during the larval stage (Trisyono and Whalon 1997).

Except for the findings by Trisyono and Whalon (1997), little is known about other effects resistant alleles may have on the overall fitness of resistant Colorado potato beetle genotypes. Based on the evi-

dence accumulated by a number of authors, we suggest that this fitness will be determined by several major factors. First, resistant alleles may affect male ability to secure mating with a female (Rowland 1991, Groeters et al. 1993), especially because intermale competition in the Colorado potato beetle is fairly severe and often involves fierce male fighting over females (Szentesi 1985). Second, growth potential of a resistant population could be negatively affected by reduced fecundity, survivorship, and time of development of resistant individuals (Tabashnik 1994). A good insight into this issue has been provided by Trisyono and Whalon (1997), but their experimental design did not allow for calculating intrinsic rates of increase of resistant and susceptible populations (Price 1975). Finally, resistant individuals need to survive inclement winter conditions (Goss and McKenzie 1996), which can kill as much as 60% of the diapausing Colorado potato beetle adults in the field (Milner et al. 1992).

In the current study, we investigated relative fitness of Colorado potato beetles resistant to *B. thuringiensis* based on the suggested criteria, and discuss possible implications for the resistance management in commercial potato fields.

### Materials and Methods

**Beetle Strains.** Colorado potato beetles resistant and susceptible to the *B. thuringiensis* subsp. *tenebrionis* Cry3A toxin were obtained from the laboratory strains maintained at Michigan State University (Whalon et al. 1993). Because of their availability at the time of the experiment, susceptible beetles used for comparing male sexual competitiveness were obtained from the colony currently maintained in our laboratory. Genetic variability among the U.S. Colorado potato beetle population is low (Jacobson and Hsiao 1983, Zehnder et al. 1992), and the colonies in Michigan and Massachusetts were maintained under very similar conditions. The beetles from the Michigan strains were shipped as eggs and reared to adults on potted potato plants in wooden frame cages (75 by 50 by 65 cm) in a rearing room maintained at  $23 \pm 3^\circ\text{C}$ . All potato plants, *Solanum tuberosum* L., used in the current study were nontransgenic and belonged to 'Atlantic'. Unless stated otherwise, photoperiod in the rearing room and environmental chambers was maintained at 16:8 (L:D) h.

**Sexual Competitiveness of Resistant Males.** Eight 1-wk-old unmated resistant females were placed into separate petri dishes (14 by 2.5 cm) lined with moistened paper towels. One resistant and 1 susceptible male were introduced into each dish. Both males were marked using the technique described by Unruh and Chauvin (1993). The beetles were provided with excised potato foliage (1 medium-sized compound leaf per dish) and kept at  $23 \pm 3^\circ\text{C}$ . Mating behavior was composed of 3 major elements: male mounting on a female before copulation, copulation (adeagus intromission), and postcopulatory female guarding (male remaining mounted on a female after adeagus with-

drawal). Number of behavioral elements performed by resistant and susceptible males was recorded continuously during the 16-h light period. Experiment was replicated 3 times, with a total of 24 beetles from each strain tested.

**Fecundity and Mortality.** Thirty pairs of virgin teneral adults were taken from the resistant population and another 30 pairs were taken from the susceptible population. The beetles were placed into ventilated plastic containers (12 by 7 by 5.5 cm). One male and 1 female were placed into each container and provided with excised potato trifoliates placed in floral pics filled with tap water. Potato foliage was replaced daily. The number of beetles laying eggs, beetle age at 1st oviposition, number of eggs laid by each beetle, number of larvae that hatched from the laid eggs, and beetle mortality were recorded daily for 30 d. During the experiment, all the beetles were kept in an environmental chamber maintained at  $+27^\circ\text{C}$ .

**Egg-to-Adult Survivorship and Time of Development.** More than 2,000 eggs laid by resistant and susceptible beetles within a 24-h period were collected (1,045 eggs laid by resistant beetles and 1,080 eggs laid by susceptible beetles). All the eggs were incubated in an environmental chamber at  $+19^\circ\text{C}$  for 6 d. Temperature in the environmental chamber was lower when compared with the previous experiment to delay larval emergence until potted potatoes used in the current experiment reached  $\approx 40$  cm in height (Ferro et al. 1985). On the 7th d, hatching eggs were stapled to potted potato plants. Two to 3 egg masses (a total of  $\approx 75$  eggs) were attached to a single plant. The plants were placed into wooden frame cages (75 by 50 by 65 cm), with a total of 7 plants placed per cage. The larvae were maintained on caged potato plants in a rearing room at  $23 \pm 3^\circ\text{C}$  until they developed into adults. Fafard Growing Mix #2 (a mixture of peat moss, perlite, vermiculite, wetting agent, and liquid starter charge; Conrad Fafard, Agawam, MA) was used as a pupation medium. The number of adults and the time of their emergence from the soil were recorded.

**Intrinsic Rate of Population Increase.** The net replacement rate, or the number of daughters that replace an average female over a course of a generation (Birch 1948), was calculated for resistant and susceptible populations. Mean number of progeny produced by an average female was calculated as mean number of eggs produced by an average female multiplied by a fraction of eggs giving rise to reproductive adults. Because some females did not lay any eggs, mean number of progeny was adjusted by multiplying the mean number of eggs laid by an average egg-laying female by the fraction of egg-laying females. Fraction of eggs giving rise to reproductive adults was calculated as a product of fraction of fertile eggs, fraction of eclosing adults, and fraction of eclosed adults surviving to reproductive maturity. Colorado potato beetles have a 50:50 sex ratio (A.V.A., unpublished data); therefore, to limit our results to female progeny only, the mean number of progeny produced by an average female obtained from the described above calculations was divided by 2. Mathematically, the formula

used for calculating the net replacement rate ( $R_0$ ) in the current study could be written as follows:

$$R_0 = \frac{m \times n \times l_e \times l_a \times l_r}{2},$$

where  $m$  is the fraction of egg-laying females;  $n$  is the mean number of eggs per female;  $l_e$  the fraction of fertile eggs;  $l_a$  the fraction of eclosing adults;  $l_r$  the fraction of eclosed adults surviving to reproductive maturity; and 2 is the sex ratio coefficient.

The net replacement rate values were used to calculate the intrinsic rates of population increase (Birch 1948) as follows:

$$r_m = \frac{\ln R_0}{T},$$

$$T = t_1 + t_2,$$

where  $T$  is the total developmental time;  $t_1$  the mean number of days from egg to adult eclosion; and  $t_2$  the mean number of days from adult eclosion to oviposition initiation.

**Survivorship of Adults During a Diapause.** Resistant and susceptible beetles were reared to adults on potted potato plants in wooden frame cages (75 by 50 by 65 cm) in the rearing room at  $23 \pm 3^\circ\text{C}$ . To induce diapause, photoperiod in the rearing room was set to 8:16 (L:D) h, so that all beetle life stages starting from the 1st instar were exposed to short days. Resistant (105) and susceptible (117) diapausing beetles were sifted from the potted soil and placed into plastic boxes (24 by 14 by 11 cm) filled with heat-sterilized Fafard Growing Mix #2. The beetles were evenly distributed within the Growing Mix and stored for 238 d in a dark cold room at  $+4^\circ\text{C}$ , a temperature falling within the range of 0 to  $+5^\circ\text{C}$  recorded by Milner et al. (1992) for simulating an overwintering habitat under the field conditions. On the 239th d, the boxes were moved to an environmental chamber, where they were maintained at  $+27^\circ\text{C}$  and a photoperiod of 16:8 (L:D) h. Emergence of postdiapause beetles was checked daily, and emerging beetles were removed from the containers. Number of degree-days accumulated by the beetles before emerging from the soil was calculated using  $+10^\circ\text{C}$  as a developmental threshold (de Kort 1990).

**Statistical Analysis.** Normality of collected data were tested by the Wilks-Shapiro test at the level of significance of  $P \leq 0.05$  (PROC UNIVARIATE, SAS Institute 1989). The data that did not follow the normal distribution were analyzed using MannWhitney rank sum test (PROC NPARIWAY, SAS Institute 1989). Normally distributed data were further tested by the  $F$ -test for the equality of variances at  $P \leq 0.05$ , and then analyzed by an appropriate Student  $t$ -test (PROC  $t$ -test, SAS Institute 1989). Differences between proportions and transition probabilities were estimated using chi-square test (PROC FREQ, SAS Institute 1989).

## Results

**Sexual Competitiveness of Resistant Males.** Ethograms for the mating behaviors of resistant and susceptible beetles are shown on Fig. 1. During a 16-h light period, a single resistant male mounted a female on average  $11.8 \pm 1.4$  times (mean  $\pm$  SE), and a single susceptible male mounted a female on average  $12.58 \pm 1.4$  times. The number of mountings was not significantly different between the strains ( $U = 260.5$ ,  $df = 46$ ,  $P = 0.5777$ ). However, the number of actual copulations was highly reduced for resistant males ( $U = 158.0$ ,  $df = 46$ ,  $P = 0.0076$ ). Resistant males copulated on average only  $8.0 \pm 1.0$  times, whereas susceptible males copulated  $13.3 \pm 1.5$  times. The probability of a successful transition to a copulation was lower both for mounted and guarding resistant males than for susceptible males. The difference was highly significant for mounted beetles ( $\chi^2 = 21.70$ ,  $df = 1$ ,  $P = 0.0001$ ), and significant for guarding beetles ( $\chi^2 = 5.25$ ,  $df = 1$ ,  $P = 0.0219$ ).

**Fecundity.** Ninety percent ( $\pm 5.5$ ) of susceptible and  $86.7 \pm 6.0\%$  of resistant females laid fertile eggs during the experiment. There was no significant difference in percentage of fertile females between resistant and susceptible strains ( $\chi^2 = 0.01$ ,  $df = 1$ ,  $P = 0.9203$ ). Resistant beetles required on average  $5.7 \pm 0.2$  d to pass from the moment of their emergence from the soil to the moment of oviposition initiation, whereas susceptible beetles required on average  $6.6 \pm 0.2$  d (Fig. 2). The difference between the strains was highly significant ( $t = -3.32$ ,  $df = 51$ ,  $P = 0.0017$ ).

Resistant females produced on average  $484.7 \pm 48.0$  eggs and  $334.9 \pm 39.7$  larvae. Susceptible females produced on average  $824.3 \pm 68.1$  eggs and  $590.9 \pm 58.5$  larvae. Both number of eggs ( $t = -4.07$ ,  $df = 46.4$ ,  $P = 0.0002$ ) and number of larvae ( $t = -3.62$ ,  $df = 45.4$ ,  $P = 0.0007$ ) were significantly reduced for resistant females when compared with susceptible females. Proportion of fertile eggs did not differ between the strains ( $\chi^2 = 0.22$ ,  $df = 1$ ,  $P = 0.6418$ ).

**Egg-to-Adult Survivorship and Time of Development.** The process of development from egg to adult took on average  $35.2 \pm 0.13$  d for resistant beetles, and  $33.7 \pm 0.1$  d for susceptible beetles (Fig. 3). The difference was highly significant ( $t = 8.93$ ,  $df = 832$ ,  $P = 0.0001$ ). Proportion of the beetles surviving to adulthood, however, was higher for the resistant beetles ( $42.5 \pm 1.5\%$  versus  $35.3 \pm 1.5\%$ ;  $\chi^2 = 5.12$ ,  $df = 1$ ,  $P = 0.0236$ ). Similar percentage of newly eclosed adults survived to reproductive maturity in resistant ( $8.3 \pm 5.0\%$ ) and susceptible ( $11.7 \pm 5.9\%$ ) strains ( $\chi^2 = 0.37$ ,  $df = 1$ ,  $P = 0.5428$ ).

**Intrinsic Rate of Population Increase.** Net replacement rate was equal to 84.9 for resistant females and 111.3 for susceptible females. Furthermore, resistant beetles developed to reproductive maturity 0.69 d longer when compared with susceptible beetles. As a result, intrinsic rate of increase of the resistant population was equal to 0.1085, whereas intrinsic rate of increase of the susceptible population was equal to 0.1171.

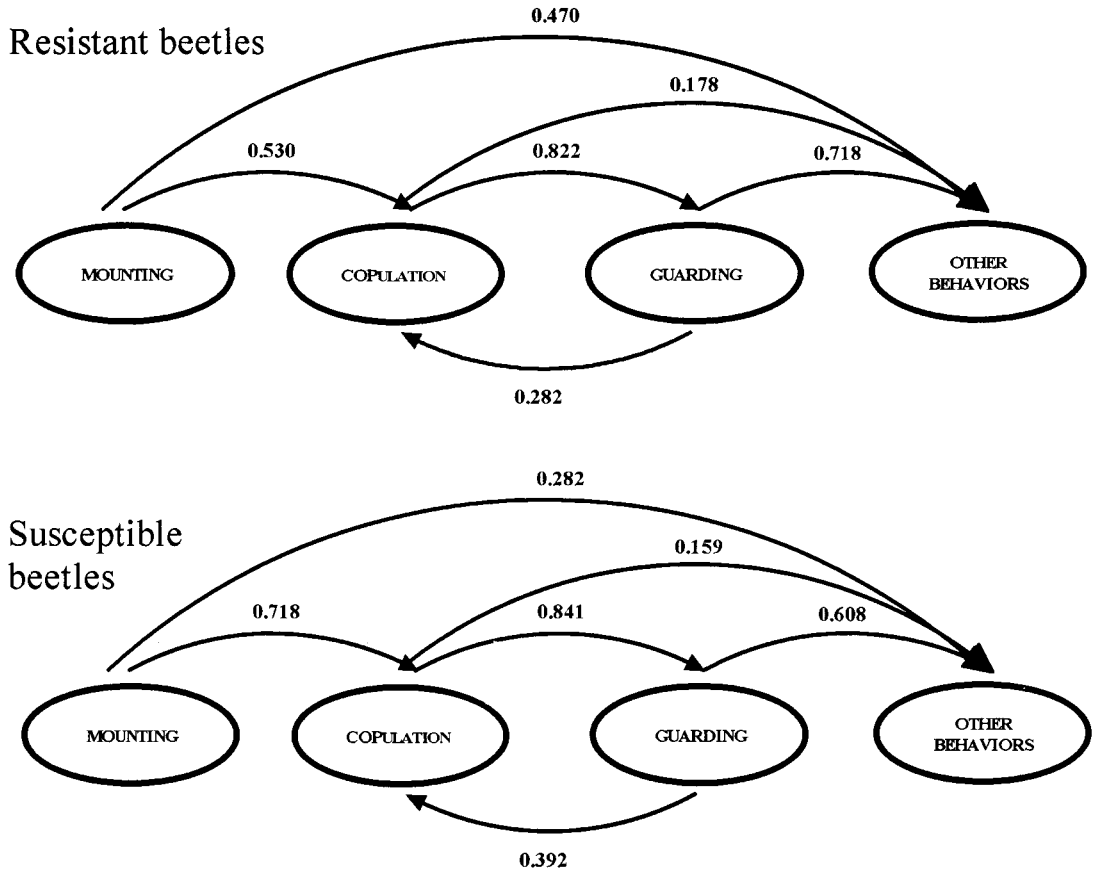


Fig. 1. Ethograms of mating behavior for Colorado potato beetles resistant and susceptible to *B. thuringiensis* subsp. *tenebrionis* Cry3A toxin. Numbers above the arrows show conditional probabilities of transition between behavioral elements.

**Mortality of Reproductive Adults.** Relatively few reproductive adults died during the experiment ( $3.9 \pm 3.7\%$  of resistant beetles and  $4.1 \pm 3.8\%$  of susceptible beetles), with the mortality being very similar for both strains ( $\chi^2 = 0.29$ ,  $df = 1$ ,  $P = 0.5912$ ). However, significantly more resistant beetles ( $76.2 \pm 4.1\%$ ) than susceptible beetles ( $48.7 \pm 4.6\%$ ) died during diapause ( $\chi^2 = 8.05$ ,  $df = 1$ ,  $P = 0.0045$ ). Before emerging from the soil, resistant beetles accumulated on average  $114.92 \pm 6.3$  DD, and susceptible beetles accumulated on average  $114.75 \pm 5.4$  DD. The difference between the strains was not significant ( $U = 814.50$ ,  $df = 83$ ,  $P = 0.5371$ ).

**Discussion**

Reduced sexual competitiveness of males, reduced population growth rate, and increased overwintering mortality clearly indicate that relative fitness of resistant individuals is significantly reduced in the absence of *B. thuringiensis* in the environment. As a result, relaxation of selection pressure is likely to favor population's reversion to susceptibility (Tabashnik 1994, Bauer 1995). Indeed, Rahardja and Whalon (1995)

reported that under laboratory conditions Colorado potato beetle resistance ratio decreased from 200- to 48-fold in 12 generations without selection. Laboratory strains used by Rahardja and Whalon (1995), as well as in the current study, are likely to be genetically different from naturally occurring beetle populations. However, diminished fitness of insecticide-resistant genotypes is common among insects (Crow 1957). Furthermore, Argentine et al. (1989) reported reduction in intrinsic rates of population increase for field-collected azinphosmethyl and azinphosmethyl/permethrin-resistant Colorado potato beetles, similar to the reduction observed in the current study. Therefore, we believe that our data provide an opportunity to have an insight into possible developments under the field conditions before resistance to the Cry3A toxin has become a problem for commercial growers. Based on the laboratory results, resistance management techniques that allow for the existence of a sufficiently large susceptible population, such as refugia and pesticide rotations, should be expected to delay evolution of resistance to the Cry3A toxin in the Colorado potato beetle. Careful monitoring of field beetle populations for signs of resistance develop-

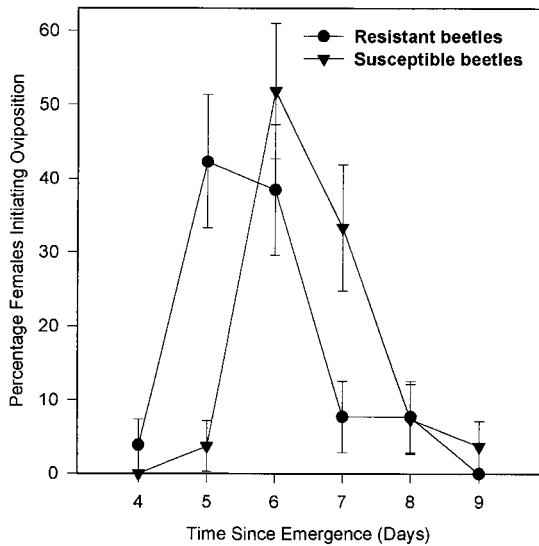


Fig. 2. Preovipositional periods for Colorado potato beetles resistant and susceptible to *B. thuringiensis* subsp. *tenebrionis* Cry3A toxin.

ment, and investigations of relative fitness of field-selected resistant strains will be essential for an implementation of a successful resistance management program.

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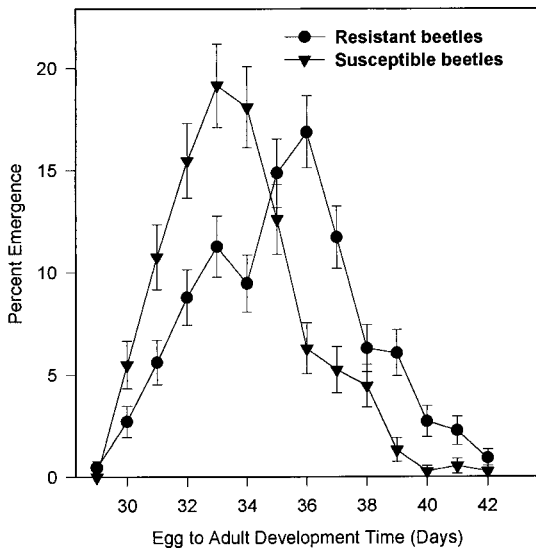


Fig. 3. Time of development from egg to adult for Colorado potato beetles resistant and susceptible to *B. thuringiensis* subsp. *tenebrionis* Cry3A toxin.

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