Biological Parameters of Convergent Lady Beetle (Coleoptera: Coccinellidae) Feeding on Aphids (Homoptera: Aphididae) on Transgenic Potato

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ABSTRACT We exposed larval and adult *Hippodamia convergens* (Guérin-Ménéville), convergent lady beetle, to *Myzus persicae* (Sulzer), green peach aphid, reared on potatoes expressing δ-endotoxin of *Bacillus thuringiensis tenebrionis*. Because the toxin may be ingested by the aphid, the beetle may in turn be exposed to the toxin. However, we measured no statistically significant effect on survival, aphid consumption, development, or reproduction in beetles.

KEY WORDS *Hippodamia convergens*, *Myzus persicae*, *Bacillus thuringiensis*-transgenic potatoes

MODERN PLANT BIOTECHNOLOGY research has developed plants resistant to diseases, insects and tolerant to certain herbicides (Dale and Flavell 1988). In the 1980s, the transformation of a plant cell with a foreign gene and the regeneration of a fertile plant from that cell were demonstrated (Peferoen et al. 1990). The transferred genes are identified and isolated as follows: The transfer methods for the plant species are developed according to a suitable vector and tissue culture techniques for production of fertile plants. The expression of the gene in the transferred plant is checked for suitable levels of resistance to the target pest in the desired tissues of the plant. The transformed plant is tested to determine its toxicity to humans, the suitability for human use, and the adverse effects on plant metabolism. The main purpose is to regenerate a new plant resistant to pests. This resistance should be stable and inherited in the offsprings of the transgenic plant (Gatehouse et al. 1991).

Transgenic potatoes, expressing a *Bacillus thuringiensis tenebrionis* δ-endotoxin specific to Coleoptera, are known to be resistant to Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Perlak et al. 1993). Shieh et al. (1994) reported that the feeding and probing behaviors of the nontarget pest, *Myzus persicae* (Sulzer), the green peach aphid, measured electronically are not affected when the aphid feeds on transgenic potatoes. However, this result does not preclude that the toxin may be passed on to the beetle predators of the aphids. Our objective was to determine any possible effects in the population parameters of the convergent lady beetles, *Hippodamia convergens* (Guérin-Ménéville), consuming the green peach aphids that feed on transgenic potatoes. These parameters include the development and aphid consumption of the immature convergent lady beetle, the pupal weight, fecundity and longevity of the adult beetle.

Materials and Methods

Transgenic and nontransgenic Russet Burbank potato plants were grown from tubers produced at Hermiston Agriculture Research and Extension Center, Hermiston, OR, provided by HybriTech Seed International. Tubers were planted individually in pots (10 cm) and grown at 22 ± 2°C, 50–55% RH, and a photoperiod of 16:8 (L:D) h. The plants infested with green peach aphids were selected from both transgenic and nontransgenic potatoes. Twelve potato leaflets were removed randomly from 6 transgenic and nontransgenic plants, making 24 leaflets in total. The potato leaflet petioles were wrapped with cotton and inserted individually through the holes in the lids of plastic containers (40 ml) filled with water. The green peach aphid had been exposed to δ-endotoxin prior to the experiment in the treatment with transgenic potatoes. However, it is not clear that the aphids acquired the *B. t. tenebrionis* toxin.

There were 2 treatments. These treatments consisted of lady beetles feeding on aphids that had fed on transgenic and nontransgenic potatoes. Twelve plastic containers were used in each treatment as the experimental units. This experiment was replicated 3 times. Lady beetles were collected initially from potato and peppermint fields at Hermiston and Corvallis, OR, respectively, and reared in an insectary.
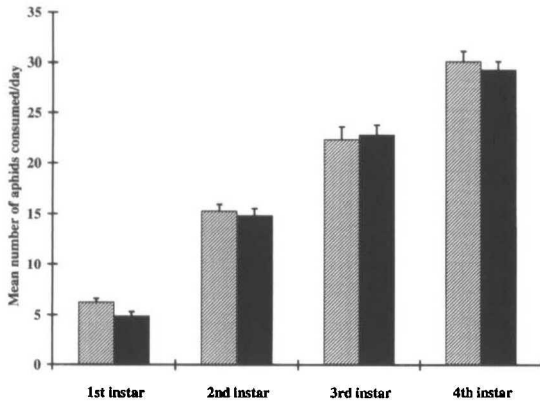
Fig. 1. Mean ± SEM aphid consumption per day in transgenic treated (solid) and control (hatched) convergent lady beetle larvae (n = 36 per treatment).
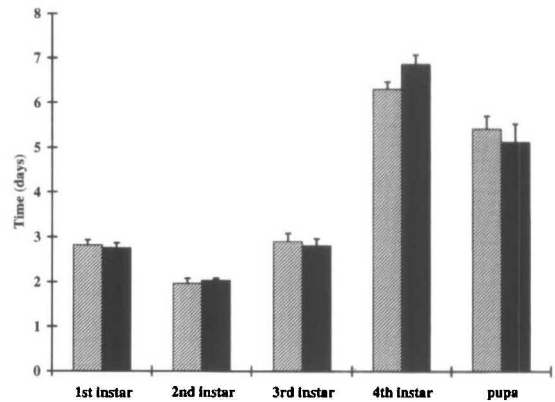

Fig. 2. Mean ± SEM development time of immature convergent lady beetle in transgenic (solid) and control (hatched) treatments (n = 36 per treatment).

A larger cup was inverted over the container to keep the beetle larva and aphid prey inside. One 1st-instar lady beetle larva was placed on each leaf. The leaves were changed every week. For the 1st instar, 10 aphids were supplied daily, 20 aphids were given to the 2nd instar, 30 aphids to the 3rd instar, and 40 aphids to the 4th instar. The number of consumed aphids was determined and uneaten aphids were removed every 24 h. The amount of time between molts was recorded for each stage of larval development. Pupae were weighed immediately after the pupation. Following adult emergence, beetles were sexed and allowed to mate. Mated females were placed individually for oviposition in 40-ml plastic cups with cardboard lids. Eggs were counted and removed.

All information concerning the biological parameters of convergent lady beetles was collected and statistical analyses were done using multifactor analysis of variance (ANOVA) on StatGraphics 5.0 (MathSoft 1989). The response parameters were aphid consumption, time between molts, pupal weight, number of eggs laid, and longevity. For a given response we fitted the following ANOVA. The source of variation: trial (3 replicates), treatment, trial * treatment, and residual.

When an interaction term was found to be insignificant, the interaction sums of squares were pooled with residual sums of squares to get a new residual error term for the F test.

Results

The difference between aphid consumption of lady beetles in the transgenic and nontransgenic treatments was not statistically significant (Fig. 1). The F ratio and P value in the 1st, 2nd, 3rd, and the 4th instars were calculated as 2.586, 0.08; 11.114, 0.002; 1.418, 0.24, and 8.435, 0.0005, respectively. The sample size (n) was 36 per treatment. The small P values in the 2nd and 4th instars were the result of the blocking effect.

The difference between the development times of the lady beetle larvae and pupae was not significant in two treatments (Fig. 2). The F ratio and P value in the 1st, 2nd, 3rd, and the 4th instars and in the pupae were calculated as 0.458, 0.63; 1.349, 0.26; 2.975, 0.057; 13.39, 0.0001, and 0.056, 0.941, respectively. The sample size was (n) 36 per treatment. The low P value in the 4th instar was the result of the blocking effect.

No significant difference was observed in pupal weight between the treatments (F = 0.183, P = 0.83, n = 36 per treatment). Fecundity of the lady beetles also did not significantly differ between the 2 treatments. The beetles in the transgenic group laid slightly more eggs than those in the control group. This difference might be considered biologically significant (F = 1.074, P = 0.357, n = 16 in transgenic treatment; n = 14 in control treatment). Finally, adult females emerging from the larvae fed on aphids were reared on either transgenic or nontransgenic potatoes. The longevity of the lady beetles did not differ significantly in 2 treatments. The beetles in the transgenic treatment lived slightly longer than the ones in the control treatment, which may be biologically important (F = 0.806, P = 0.458, n = 16 in transgenic treatment; n = 14 in control treatment).

Discussion

These experiments demonstrate that the effect of transgenic potatoes on the convergent lady beetles is not significant. We found no difference in the tested biological parameters. The potential transfer of toxic material from the transgenic plant through its aphid prey either does not occur or, if it does, is not toxic to the convergent lady beetle.

In the target pests, the sublethal effects of sprayed *B. thuringiensis* include reduced host consumption, reduced egg production, and reduced adult longevity (Flexner et al. 1986). We could not document such effects on convergent lady beetles,

leading us to suggest that they are not present. We did observe differences, but could not achieve an acceptable degree of significance. The possibility therefore exists that effects do occur but at such low levels that much higher samples would be required to distinguish them. The biological relevance of such minor effects would be questionable.

Practical limitations of current microbial pest control agents are high cost of preparations compared with the chemical pesticides, short field stability, and need for multiple applications (Barton and Miller 1993). The need for a less expensive and more stable pest control agent led to the idea of transforming plants to express *B. thuringiensis*. The main advantages of transgenic plants are reduction in the use of chemical and improved residual activity. The disadvantages of engineering *B. thuringiensis* toxin genes are possible resistance development and public acceptance of engineered foods (Holmes 1993, Starnes et al. 1993).

Gene transfer technology in higher plants was developed using solanaceous species. Resistance to insect predation is an important purpose of the process. The most successful strategy is *B. thuringiensis* δ-endotoxin-expressing transgenic plants such as transgenic tobacco toxic to tobacco hornworm, *Manduca sexta* (L.), larvae and transgenic tomatoes resistant to corn earworm, *Heliothis zea* (Boddie) (Fischhoff et al. 1987, and Houck et al. 1993). The relationship between the amount of CryIA(b) (*B. t. kurstaki*) protein expressed in the plant and the level of protection from the pest damage was positive (Warren et al. 1992). Surprisingly, parasitism activity increased on transgenic tobaccos against *Heliothis* larvae, but the difference was not significant (Warren et al. 1992). Transformed plants expressing *B. thuringiensis* toxin contain low levels of toxin, but the presence of this gene caused adverse effects on the plants by causing high levels of mortality to the test insects. Despite the low levels of toxin expression, most of the transformed plants were toxic to *M. sexta*, suggesting very high toxicity which *B. thuringiensis* toxins may cause to susceptible insects (Gatehouse et al. 1991). The CryIII gene, which contains toxic polyhedron to Coleoptera, showed toxicity to *Tenebrio* spp and Colorado potato beetle. The toxicity was highest in the early larval stages (Barton and Miller 1993).

When *B. thuringiensis* is sprayed, a target insect must ingest *B. thuringiensis* crystals to be affected. The protoxin is released from ingested crystals and protoxin is activated to the toxin. The toxin binds to the receptors on the midgut cell membranes. The insect stops feeding and dies (Martin 1994). We do not know whether or not the green peach aphid is exposed to the toxin while feeding on the transgenic potatoes or even whether it is susceptible; therefore, the results are not easy to interpret.

Development time and aphid consumption in our 2 treatments were similar to the results of Chedester (1979), Obrycki and Tauber (1982), and Miller (1992), suggesting that experimental procedures followed in our experiments are well within other published studies and therefore not a source of error.

Overall, our experiments suggest that transgenic potatoes will have little direct effect on nontarget predatory organisms such as convergent lady beetle, although field tests clearly must be done in addition to laboratory experiments. An interesting area of investigation would be to measure the level of exposure of aphids to the toxin and whether or not aphids and lady beetles are susceptible at all.

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