Interactions of Transgenic-Bt Potato Resistance to Colorado Potato Beetle with the Fitness and Behavior of the Potato Aphid Macrosiphum euphorbiae

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ABSTRACT

The performance and flight behavior of the potato aphid *Macrosiphum euphorbiae* was studied on the 'Superior-BT' line transgenic for the CryIIA toxin of Bacillus thuringiensis (BT), resistant to the Colorado potato beetle; and non transformed 'Superior' line which served as control. Mortality of the treated aphids was negligible and potato lines did not affect the development time of *M. euphorbiae*, but aphids were largest on 'Superior' and smallest on BT potatoes. This difference was reflected in aphid fecundity, which was lowest on 'Superior-BT', and highest on Superior. Incidence of flight in newly emerged alate *M. euphorbiae* that developed on BT was high compared to control. The results illustrate that the performance of a secondary pest of potato can be unpredictably affected by the resistance factor involved in developing specific resistance to a primary pest.

Keywords: Bacillus thuringiensis, Colorado potato beetle, Macrosiphum euphorbiae, Plant-insect interaction, Potato transgenic-BT.

INTRODUCTION

The development of plant resistance as an alternative to chemical control is mostly concerned with primary pests. In contrast, the effects of plant resistance on secondary pests are rarely considered, although altering plant genomes to reduce primary pest damage may strongly influence the fitness and behavior of secondary pests (Teetes, 1985; Wiseman, 1994; Hanzlik et al., 1997). Several studies suggest that the fitness and behavior of secondary pests may change unpredictably when they feed on host plants with improved resistance against primary pest targets (e.g. Shieh et al., 1994; Hanzlik et al., 1997; Girard et al., 1998; Ashouri et al., 2001; Davidson et al., 2006).

The Colorado potato beetle (CPB) Leptinotarsa decemlineata (Say) is the most important cultivated potato pest in the world (Radicke, 1982; Hare, 1990; Holliday, 1996). Chemical insecticide applications can successfully control the CPB but there is growing concern about the mid-term outlook of this approach. It is undesirable from an environmental pollution view point and its long term efficacy is regularly threatened by the evolution of new resistance to previously effective chemicals on beetle populations (Martel, 1987; Hare, 1990; Boiteau et al., 1996).

A variety of secondary insect pests can cause damage to potato crops, including aphids which both cause direct damage by feeding on plant sap, and indirectly damage plants by acting as plant virus vectors (Radicke, 1982; McLeod and Tolman, 1987). An important aphid pest of potato occurring worldwide is the potato aphid *Macrosiphum euphorbiae* (Thomas). *Macrosiphum euphorbiae* is a polyphagous aphid, attacking many different crop and non crop plant spe-
cies (Blackman and Eastop, 1984). It is also a vector of several persistent and nonpersistent viral diseases, and thus can be a serious problem in seed as well as table potato production (Shands et al., 1972; Lange and Broson, 1981; Walker et al., 1984; Boiteau, 1994). Macrosiphum euphorbiae and other aphid pests of potato are of only minor concern in the CPB’s presence, because they may be indirectly controlled by effective chemicals (Boiteau et al., 1996).

Transgenic potatoes containing a Bacillus thuringiensis ssp. tenebrionis (CryIIIA) resistant to CPB are currently being subjected to field trial (Gill et al., 1992; Perlak et al., 1993) and are expected to be generally available to the public soon. Very little is known about the possible effects of this factor of resistance on secondary potato pests (e.g. Boulter et al., 1990; Shieh et al., 1994; Canedo et al., 1998; Ashouri et al., 2001). This resistance mechanism is unlikely to interact with secondary aphid pests as they do with primary defoliators such as the CPB. The objective of this study was to examine the effects of the CPB transgenic-BT resistant line on the potato aphid, M. euphorbiae. We were mostly interested in finding out how this line might affect potato aphid performance, as expressed in its life history characteristics and behavior.

MATERIALS AND METHODS

Host Plants

Two potato lines were used for the treatments: (1) the CPB resistant ‘Superior-BT’ line (Newleaf™), which is transgenic for the CryIIIA toxin of B. thuringiensis tenebrionis (Gill et al., 1992; Perlak et al., 1993) and (2) the ’Superior’ variety which served as the control. For the experiments, we used plantlets at the 4-6 leaves stage.

Insects

Aphids used in the bioassays were obtained from a colony initiated from the potato aphid, Macrosiphum euphorbiae (Thomas), collected from a potato field. We reared aphids on cv. ‘Norland’ potato seedlings in growth chambers at 20±1°C and 65±10% RH under 16L:8D h photoperiod. Plants of each line were caged and maintained under the same conditions as above. Newly emerged 0-12 hour-old adults were used as the test insects.

Nymphal Mortality and Development Time

The development time and mortality rate from birth to adulthood of 60 aphids per plant treatment were measured. Two adult apterous aphids were caged together on a leaf and, after 12 hours, the adults, and all but one 0-12 hour-old nymph were removed. Infested plants were maintained under a controlled temperature regime fluctuating according to a sine wave pattern between 12 and 22°C (average=17°C), 65±10% RH under 16L:8D h photoperiod. Daily observations were performed in the morning (10 AM) until all the nymphs were either dead or had reached maturity.

Adult Mortality and Fecundity

We determined adult M. euphorbiae and fecundity on the leaves of each potato line, for apterae which had previously developed on the same line. We placed one newly (0-12 hour-old) molted adult aptera on a caged leaf. Twenty plants were used per line. Plants were maintained at 20±1°C and 65±10% RH under 16L:8D h photoperiod. Mortality was checked daily and the nymphs produced were counted and removed. Total fecundity was calculated as the number of nymphs produced by a female over 10 days following adult emergence.

Body Size

Newly emerged 0-12 hour-old adult apte-
rae reared from each line (developed on whole plants under the above conditions) were killed by freezing. Frozen aphids were dried in an oven (Precision Scientific Inc, Chicago, IL, USA) at 60°C for 48 hours. The dry mass of each adult aphid was measured using a microbalance (sensitivity 0.001 mg).

**Flight Tendency**

Twenty-five plants per line were used to compare flight tendency of the young *M. euphorbiae* alatae on the two potato lines under laboratory conditions. Newly emerged 0-24 hour-old adult alatae (developed on leaves of the same line) were released at the upper (abaxial) leaf surface, on the 2 second or third leaf of each plant. We observed the tendency to fly for each individual on a daily basis until they were 10 days old. Each morning following adult emergence, the aphids were individually exposed to a light source until they left the plant or until 60 seconds had elapsed. Counts of aphids taking flight within 60 seconds were used to obtain the age-specific flight incidence for each line.

**Statistical Analysis**

All experiments were arranged as a completely randomized design with two host plants as treatments (Superior-BT and Superior). We used a Pearson’s Chi-square test to detect any effect of host plants on aphid mortality and flight tendency. We used 2-factor analysis of variance (ANOVA) with morph (two levels: alate/apterous), plant treatment (two levels) and their interaction to analyze data on aphid development time. For statistical analyses of adult fecundity and dry weight an independent t-student test was performed.

**RESULTS**

**Mortality**

Nymphal mortality to adult emergence was not significantly ($X^2_1 =1.7; P=0.186$) affected by plant line. Cumulative mortality up to the 8th day of adult life was also not significantly affected by plant line ($X^2_1 = 2.105; P=0.147$; Table 1).

**Development Time**

Two-factor ANOVA indicated no significant interaction between the host plant and aphid morph for aphid development time ($F_{1, 106} =0.095; P=0.759$). For this reason, we examined the effect of host plant line separately for each aphid morph. Apterous aphids developed significantly more rapidly than alate aphids on two lines (Table 1).

**Table 1.** Mortality (%) and development time (day; Mean ± SEM) from birth to adult molt of *Macrosiphum euphorbiae* confined to leaves of two potato lines.

<table>
<thead>
<tr>
<th>Potato line</th>
<th>Mortality</th>
<th>Development time (d)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
</tr>
<tr>
<td>Superior-BT</td>
<td>60</td>
<td>11.67 a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superior</td>
<td>60</td>
<td>5.00 a</td>
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</table>

* number of replicates.
Mean values with the same letter do not differ significantly at the 5% error rate based on Fisher’s PLSD test.
Body Size

Dry weight of *M. euphorbiae* apterous adults was significantly affected by the plant line used during nymphal development ($F_{1, 112} = 178.912; P<0.0001$). Aphids on the Superior-BT line, were smaller than on the non transformed Superior line ($113 \pm 3.7 \, \mu g$ and $205 \pm 6.1 \, \mu g$ respectively; Table 2).

Fecundity

Fecundity in apterous *M. euphorbiae* varied significantly among host plant lines ($F_{38} = 7.77; P<0.0082$). This was lowest aphids on Superior-BT and highest on Superior (on average $41.40 \pm 1.65$ and $47.40 \pm 1.38$ nymphs per 10 days, respectively; Table 2).

Flight Tendency

Aphid flight tendency varied according to age and potato line (Figure 1). In early adulthood, cumulative flight tendency increased rapidly up to day 3, but remained more or less stable after that. On day 10, more than 76% of aphids on the Superior-BT had exhibited the tendency to fly compared to <28% on Superior, hence the cumulative incidence of flight being significantly different between potato lines ($X^2 = 11.54; P<0.0007$; Figure 1).

DISCUSSION

Our results indicated that, unexpectedly, Newleaf™ potatoes expressing the *B. thuringiensis* tenebrionis CryIII A toxin negatively affected adult fitness of *M. euphorbiae* and increased their flight tendency. This and related endotoxins of *B. thuringiensis* are not known to affect aphid feeding or nutrition directly. Our results cannot tell us if the toxin was ingested or if indirect effects of transgene products on aphids are possible. Overexpression of the *B. thuringiensis* toxin in foliage might lower the availability of important aphid nutrients, such as free amino acids in phloem sap (Chu and Henneberry, 1995; Rahbé et al., 1995; Sauvion, et al., 1996; Fischer et al., 1998; Dutta et al., 2005).

Antibiotic plant resistance generally reduces herbivore size (Panda, 1979; Stapl et al., 1997; Souissi and Le Ru, 1998). Smaller adult aphid size could affect population growth in the field through lower or delayed reproduction. Smaller aphids are a potential side-effect of transgenic-BT potatoes which could lead to reduced survival, slower growth, development and their population increase (Ashouri, 2004). Although we found no relation between aphid size variation and potato line, and development time in this study. The reduced availability of nutrients and/or endotoxins of *B. thuringiensis* would negatively affect aphid fitness, and could also enhance flight propensity.

In aphid populations, the proportion of individuals dispersing by flight in search of new host plants usually increases with aphid density, and decreases with host plant quality (Jepson, 1983; Dill et al., 1990). A key life history trait of aphids for surviving systematic variability of host plant quality is the

<table>
<thead>
<tr>
<th>Potato line</th>
<th>Dry weight (µg; Mean ± SEM)</th>
<th>Fecundity / female/ 10 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior-BT</td>
<td>61 113.23 ± 28.58 ***</td>
<td>20 41.40 ± 1.65 **</td>
</tr>
<tr>
<td>Superior</td>
<td>53 205.68 ± 44.45</td>
<td>20 47.40 ± 1.38</td>
</tr>
</tbody>
</table>

$^a$ number of replicate.

** and *** Significant differences using independent t-student test at $P<0.001$ and $P<0.0001$, respectively.
facultative dispersal by newly-emerged alate individuals (Dixon, 1998). Young alatae will usually take flight if the host plant is maturing or deteriorating, but will remain and reproduce locally if the host plant is young and in good conditions (Kennedy and Fosbrooke, 1973; Dixon, 1998).

Despite evidence that the *B. thuringiensis* transgenic potato is not an ideal host for the potato aphid, the aphids matured to become functional alate dispersers that apparently were fully able to express their ability to leave in search of more suitable hosts.

Aphid dispersal and probing behavior are two distinct factors in nonpersistent virus transmission (Boiteau and Singh, 1991; Bailey et al., 1995; Dixon, 1998). Shieh et al. (1994) reported that transgenic potatoes with the *B. thuringiensis* gene did not affect probing behavior of the green peach aphid, *M. persicae*. However, increased flight tendency, as we observed for *M. euphorbiae* alatae on Superior-BT, suggests that more frequent movement by alate aphids may occur in transgenic *B. thuringiensis* potato crops which, in turn, would enhance the risk of spreading viral diseases. The confirmation and characterization of such risk would require additional research.

Our results may also be relevant to understanding and predicting the possible effects of ‘pyramidal’ resistance to potato insect pests. Multi-mechanistic plant resistance development (‘pyramiding’) is seriously considered as an option for herbivorous pest control (Boulter *et al.*, 1990; MacIntosh *et al.*, 1990; Gatehouse and Gatehouse, 1998; Westedt *et al.*, 1998). In theory, such pyramidal resistance could be effective against a significant range of primary and secondary potato pests, including the CPB, other major defoliators such as lepidopteran larvae, and herbivores of the sucking guild including aphids. However, since even the simple monogenic resistance factor can have quite unanticipated effects on non-target pests, making predictions about the outcome of pyramidal plant resistance is very risky at this stage. Much more research is necessary to verify empirically the anticipated effects, and to discover the unanticipated ones, before broad-spectrum plant resistance based on multiple resistance factors in crops such as potatoes can become a reality.

**ACKNOWLEDGMENTS**

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برهمکش گیاه سبز زمینی، میکروبیونیتی Bt مقاوم به سوسک کلرادو با شته

Macrosiphum euphorbiae

1. عاشوری

چکیده

شایستگی و رفتار پروازی شته سبز زمینی Superio-Bt روی رقم Macrosiphum euphorbiae سبز زمینی ترانسزینک با قرار Bacillus thuringiensis با کاربردی CryIIA مقاوم به سوسک کلرادو در مواردی که کمتر از شاهد (شاهد) در شرایط آزمایشگاهی مورد مطالعه قرار گرفت. میزان زادوآوری شتهای شاهد بوته و طول دوره نشیه و نمای زندگی گیاهان نبوده است. میزان زادوآوری شتهای شاهد بوته و طول دوره نشیه و نمای زندگی گیاهان نبوده با مراتب کمتر از شاهد بوته است. میزان پرواز شتههای بالدار جوان پرورش یافته روز گیاهان ترانسزینک در مقایسه با شاهدین بیشتر بوته است. نتایج نشان می دهد عوامل ایجاد مقاومت در بر اثر آفات در جه می تواند بطور غیر قابل پیش‌بینی باشد در جه می تواند بطور غیر قابل پیش‌بینی باشد.