Survival, Weight Gain, and Oviposition of Resistant and Susceptible *Plutella xylostella* (Lepidoptera: Plutellidae) on Broccoli Expressing Cry1Ac Toxin of *Bacillus thuringiensis*

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ABSTRACT Leaf bioassays and oviposition choice tests were used to investigate the effects of transgenic broccoli expressing Cry1Ac toxin of *Bacillus thuringiensis* (Berliner) on susceptible and resistant *Plutella xylostella* (L.) larvae. Survival of susceptible 2nd instars on Cry1Ac-expressing broccoli declined from 99.1 to 19.2% at 24 and 72 h, respectively, and larvae exhibited an average weight loss of 0.2 mg/10 larvae at 24 h. Larvae that evolved resistance to foliar sprays of *B. thuringiensis* subsp. *kurstaki* in the field, however, showed no debilitating effects from the Cry1Ac broccoli. Survival of resistant larvae at 24 and 72 h was 98.6 and 90.8%, respectively, and weight gain at 24 h was 1.7 mg/10 larvae, none of which was significantly different from survival or weight gain on control plants. In oviposition choice tests, both susceptible and resistant females were unable to discriminate between Cry1Ac and normal broccoli, laying ≈38–41 eggs per plant per 2 females. Comparing mortality of susceptible larvae on 2 lines of transgenic broccoli (J1R and K20) derived from independent transformation events, we found that the majority of the variance (43.2%) in toxin expression was caused by transformation. Depending upon the transformation, plant could be a significant source of variation but toxin expression within plant was always fairly uniform. Our data indicate that resistance to sprays of *B. thuringiensis* can confer resistance to plants when similar toxins are involved.

KEY WORDS *Plutella xylostella*, *Bacillus thuringiensis*, transgenic plant, resistance, Cry1Ac toxin

ALTHOUGH *Bacillus thuringiensis* sprays have generally claimed only ≈1% of the global insecticide market (Marrone and MacIntosh 1993), projected estimates are that $2.7 billion of the $8.1 billion spent annually on insecticides worldwide could be replaced by *B. thuringiensis* transgenic crops alone (Krattiger 1997). Recommended strategies focus on deploying transgenic plants that express *B. thuringiensis* toxins at levels that are as high as technologically feasible, and relying on separately planted refuges as a means to preserve susceptible alleles and slow the evolution of resistance (Mallet and Porter 1992, Roush 1994, Tabashnik 1994, Alstad and Andow 1995, Liu and Tabashnik 1997, Roush 1997).

Although there are numerous studies that report on the lethal effects of plants expressing *B. thuringiensis* toxin on laboratory susceptible or wild insects (Schuler et al. 1998), there are few studies that have looked at survival and growth of resistant insects on hosts that have been transformed to express *B. thuringiensis* toxin. In the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), Wierenga et al. (1996) found that 2nd instars, exhibiting >1,900-fold resistance ratio for CryIIIA in a leaf dip bioassay, showed higher survival on transgenic foliage than susceptible larvae. Resistant larvae, however, still exhibited much higher mortality on transgenic foliage (46% mortality after 4 d) than on normal foliage (5% mortality). Weight gain of resistant 2nd instars on transgenic potato foliage also was depressed compared with larvae that were fed normal foliage (Wierenga et al. 1996). In the tobacco budworm, *Heliothis virescens* (F.), >19 selections with Cry1Ac toxin produced up to 10,000-fold resistance and larvae proved to be cross resistant to Cry1Aa, Cry1Ab, and Cry1F (Gould et al. 1995). When resistant and susceptible neonates were fed for 5 or 7 d on leaf disks from tobacco expressing Cry1Ab, resistant larvae showed significantly higher survival than susceptible larvae (Gould et al. 1995). Authors stated that survival of resistant larvae on transgenic foliage, however, was not always as high as survival on normal foliage, and larvae on transgenic foliage tended to be smaller than larvae on control foliage (Gould et al. 1995). Data from both these studies suggest that selection with toxins applied to foliage or artificial diet did not select for high enough levels of resistance for normal survival and growth on

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B. thuringiensis-transgenic plants and that some fitness trade-offs were present.

To further explore survival of resistant larvae on transgenic hosts, we used as our model the diamondback moth, *Plutella xylostella* (L.), the only species that has evolved resistance to *B. thuringiensis* sprays in the field. Extensive use of sprays of *B. thuringiensis* subsp. *kurstaki* to control *P. xylostella* has led to several cases of resistance in Florida (Shelton et al. 1993), Hawaii (Tabashnik et al. 1990), Japan (Hama et al. 1992), the Philippines (Ferré et al. 1991), Central America (Perez and Shelton 1997), and China (Zhao et al. 1993). Screening resistant larvae to the individual components of *B. thuringiensis* subsp. *kurstaki* demonstrated that larvae were highly resistant to spore, Cry1Aa, Cry1Ab, and Cry1Ac (Tabashnik et al. 1993, Tang et al. 1996). Despite differences in geographical origin, data from the Florida, Hawaii, and Japan colonies suggest that resistance to *B. thuringiensis* subspecies *kurstaki* was controlled primarily by 1 or few loci (Hama et al. 1992, Tabashnik et al. 1992, Tang et al. 1997), and that resistance was correlated with reduced binding of Cry1A toxin to specific gut membrane receptors (Tabashnik et al. 1994, 1997; Tang et al. 1996). Differences in the degree of recessiveness of the trait (Hama et al. 1992, Tabashnik et al. 1992, Tang et al. 1997), the presence (Groeters et al. 1994) or absence of associated reductions in fitness (Tang et al. 1997), and tests for allelism (Tabashnik et al. 1997) suggest that >1 allele for resistance could be involved.

Our previous experiments with *P. xylostella* looked at survival of resistant and susceptible larvae on broccoli expressing Cry1Ac toxin after 7 d (Metz et al. 1995). Except for 2 low expressing plants, all high and very high expressing plants produced 100% mortality of susceptible larvae and ≤10% mortality of resistant larvae (Metz et al. 1995). Information about how rapidly susceptible larvae died or whether resistant larvae gained comparable weight on transgenic versus control foliage was lacking. Therefore, in this article, we describe how survival changed during the period from 1 to 3 d and how weight gain changed 24 h after larvae were placed on transgenic and control foliage. We also examine oviposition preference and uniformity of plant dose because of their potential influence on resistance management.

Materials and Methods

Insects. Our susceptible colony of *P. xylostella* was collected in 1988 from cabbage at the New York State Agricultural Experiment Station, Robbins Farm, Geneva, NY. At the time of these studies, the Geneva colony had been maintained on a wheat germ-casein artificial diet (Shelton et al. 1991) for >120 generations. The colony was kept in an environmental chamber at 27 ± 1°C, 35 ± 2% RH, and photoperiod of 16:8 (L:D) h. Before tests on plants, eggs from the Geneva colony were reared on oilseed rape plants, *Brassica napus* L. subsp. *oleifera* (cultivar Dwarf Essex), in the greenhouse at 26–33°C, uncontrolled relative humidity, and photoperiod of 16:8 (L:D) h.

The resistant colony was collected in 1993 and 1994 from commercial crucifer fields in Loxahatchee, FL. A collection made from this region in 1992 (Loxa A) had shown >1,500-fold levels of resistance to Javelin (wettable granules, 6.4% active ingredient, lot no. 7300960, Sandoz, Des Plaines, IL), a commercial formulation of *B. thuringiensis* subsp. *kurstaki* (Shelton et al. 1993), when mortality was read at 96 h. In 1993 and 1994, collections were made from this same region, and resistance in leaf dip bioassays was determined to be 636-fold (Javelin, lot no. 7300960, mortality read at 72 h) (J.D.T., unpublished data), and 829-fold (Javelin, lot no. 8611442, mortality read at 48 h) (Perez et al. 1997), respectively. Larvae from the 1993 Loxahatchee collection were used for survival and weight gain experiments, and larvae from the 1994 collection were used for oviposition tests. Unless otherwise stated, resistant larvae were reared on rape plants in the greenhouse under the conditions described above.

Plants. Cytoplasmic male sterile broccoli, *Brassica oleracea* L. subsp. *italica*, was transformed (Metz et al. 1995) using *Agrobacterium tumefaciens* strain LBA4404 containing the binary vector pMON10517–1 (Monsanto, St. Louis, MO). The latter carried the neomycin phosphotransferase gene and a full-length, synthetic, *B. thuringiensis cry1Ac*-like gene, derived from HD-73. Selection and analysis of successful transformants are described by Metz et al. (1995). Each transformant represented an independent transformation event, and "line" refers to all progeny descended from a single transformant.

Plants chosen for these studies were the progeny of transformants from single T-DNA integration events (Metz et al. 1995). Consequently, only half the progeny produced from pollinating the transformants with 'Green Comet' broccoli were expected to carry a copy of the introduced genes (Metz et al. 1995). To identify which progeny plants expressed the foreign genes, plants were screened with both kanamycin and insect tests 2–3 wk after germination in the greenhouse (Metz et al. 1995). Kanamycin tests were done in greenhouses, and insect tests were done in environmental chambers. Conditions for both areas were as described above. Plants expressing the neomycin phosphotransferase gene did not blanch 1 wk after being sprayed on 4 consecutive days with a solution of 250 mg/liter kanamycin sulfate and 0.05% Triton X-100 (Sigma, St. Louis, MO), and plants expressing Cry1Ac showed little to no damage 7 d after eggs (0–24 h old) from the susceptible colony were placed on the plants. (Neonates hatch after 2–3 d, and are 2nd instars by 7 d on normal plants.) Progeny plants that showed no leaf chlorosis and no insect damage were categorized as 'Bt-expressing,' and plants that showed leaf chlorosis and insect damage or insect damage only were categorized as 'Bt-nonexpressing.' Once categorized, live larvae were removed, plants were transplanted into standard 15.2-cm pots with 14:14:14 Osmocote bead fertilizer (1 teaspoon per pot, Grace Sierra, Milpitas, CA) and returned to the greenhouse. When plants were 1.5–2 mo old, they were used for the experiments described below.

Larval Survival and Weight Gain. Leaf disk bioassays were done to compare survival or weight gain on Bt-expressing and Bt-nonexpressing plants from various transformed lines. A treatment of normal Green Comet broccoli was included for reference but not for statistical comparison. Susceptible larvae were evaluated on 28 Bt-expressing plants (from 5 different transformed lines), 30 (Bt-nonexpressing plants (from 4 transformed lines), and 7 normal plants. Resistant larvae were evaluated on 25 Bt-expressing plants (from 5 transformed lines), 29 Bt -nonexpressing plants (from 3 transformed lines), and 6 normal plants. Other than a few additions, all plants used to evaluate the susceptible larvae were the same as those used to evaluate the resistant larvae. Four leaf disks (32 mm) were cut from 1 mature leaf taken from each plant. Each leaf disk was placed in a 29.6-ml clear plastic cup with a clear plastic lid. Second instars were weighed in groups of 10, and then 5 larvae were placed per leaf disk. Cups were placed in an environmental chamber set at the same conditions as described above. Larvae (in groups of 10) were reweighed at 24 h and returned to the same cup from which they were taken. At 24 h, larval survival was still at or close to 100%, so mortality did not bias weight measurements. Percentage mortality, which was recorded per cup, was evaluated at 24, 48, and 72 h.

Oviposition Choice Tests. The influence of plant type on oviposition behavior was tested by placing *P. xylostella* adults into cages containing 1 normal Green Comet broccoli and 1 Bt-expressing broccoli plant. Preliminary data for these tests showed that the number of eggs laid on the plants was maximized if females 0–24 h old were allowed to mass mate with males of similar age for 24 h before being placed in cages with plants. Once mated, 2 males and 2 females were randomly chosen and placed into each screened cage (47 cm wide, 62 cm deep, 62 cm high) containing plants. There was enough space in the cage so that plants could be positioned without their leaves overlapping. Cages were kept in an environmental chamber (conditions as described above), and after 72 h, the number and location of eggs laid on each plant type in the cage was recorded. Separate oviposition choice tests were done for susceptible and resistant insects.

Plant Variation in Cry1Ac Expression. Variation in Cry1Ac expression, as measured by mortality of susceptible larvae in leaf disk assays, was evaluated for Bt-expressing plants of the J1R and K20 lines. These 2 lines were randomly chosen from several lines developed by Metz et al. (1995) that were designated as high expressors (i.e., the plant expressed enough toxin to kill 100% of susceptible 2nd and 3rd instars in 7 d). Our approach enabled us to estimate the variation in toxin expression between transformations, among transgenic progeny of a given transformed line, among leaves within plants, and among disks within leaves. Leaves from each plant (5 plants from the J1R line and 4 plants from the K20 line) were consecutively numbered from the base of the plant upward and were categorized as being from the lower, middle, or upper portions of each plant. The minimum leaf length taken was ≈8 cm and the average number of leaves taken per plant was 9.1 with a range of 6–12. Leaf disks (32 mm diameter, 2 or 4 per leaf depending upon leaf size) were then cut from every leaf and 5 susceptible 2nd instars were placed onto each disk. As described in the leaf disk assay above, leaf disks which were individually held in plastic 29.6 cups were placed in an environmental chamber and percentage mortality was evaluated at 24, 48, and 72 h.

Data Analysis. Before analysis, plots of mean versus variance were made to determine whether variances were roughly equal. Weight gain data and oviposition data needed no transformation, whereas percentage mortality data showed increasing variance with increasing mean. In these cases, the adjusted arcsine square-root transformation was used (Snedecor and Cochran 1967). Unless specified otherwise, the untransformed data are reported in figures and tables.

The effects of Bt-expressing and Bt -nonexpressing plants on survival or weight gain were compared for each insect type using analysis of variance (ANOVA) in the SAS procedure PROC GLM (SAS Institute 1985). Because there were several nested factors in the experimental design (e.g., leaf disk subsamples nested within plant, plant nested within transformation, and transformation nested within Bt plant type), the model statement used to test the effects of Bt plant type was DEPENDENT VARIABLE = BTTYPE TRANSF(BTTYPE), followed by the test statement H = BTTYPE E = TRANSF(BTTYPE), where BTTYPE was Bt-expressing or Bt -nonexpressing and TRANSF was the transformation line. A PROC SORT by time statement was included so that comparisons were made only within each time examined. For survival data, times examined were 24, 48, and 72 h, and for weight gain, 24 h only. Data from normal plants were not included in our analysis because there was no variation caused by transformation.

Differences in oviposition on Bt-expressing and normal broccoli were evaluated by the PROC *t*-test in SAS (SAS Institute 1985).

For experiments designed to examine the variation in Cry1Ac expression (as measured by the variation in mortality in the J1R and K20 lines), we used the PROC VARCOMP procedure in SAS (SAS Institute 1985). Results from the VARCOMP analysis are reported only for the 72 h data because there was little to no mortality observed at 24 h, and <20% mortality was observed at 48 h. Our nested factors were leaf disk subsamples nested within leaf, leaf nested within plant, and plant nested within transformation. For this analysis, we specified all nested factors in the model statement DEPENDENT VARIABLE = TRANSF PLANT(TRANSF) LEAF(TRANSF PLANT) with the error term being leaf disk subsamples nested within leaf. Results of the VARCOMP analysis estimated the percentage contribution of each factor (i.e., transformation, plant, leaf, and disk) to the total variation in Cry1Ac expression. By sorting by transformation and repeating the VARCOMP procedure (for the levels below transformation), we also were able to determine the percentage contribution of plant, leaf,

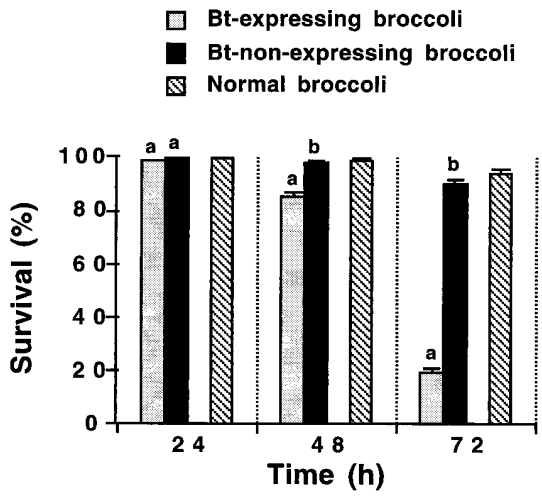

Fig. 1. Survival of susceptible 2nd instars on Bt-expressing and Bt-nonexpressing broccoli in leaf disk bioassays at 24, 48, and 72 h. For each time, different lower case letters indicate significant differences in survival ($P < 0.05$). Survival on normal broccoli is shown for comparative purposes only, and line segments above the bars denote standard errors of the means.

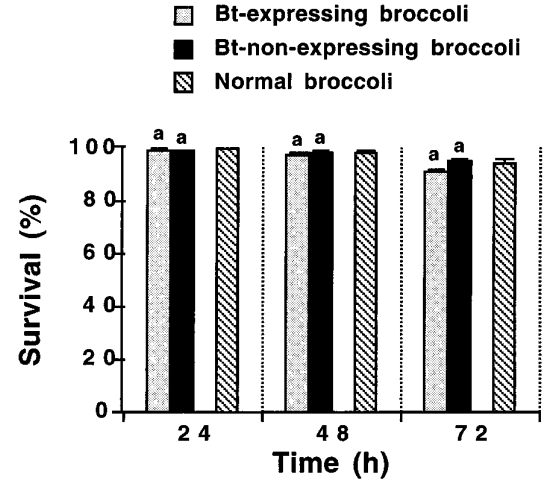

Fig. 2. Survival of resistant 2nd instars on Bt-expressing and Bt-nonexpressing broccoli in leaf disk bioassays at 24, 48, and 72 h. For each time, different lower case letters indicate significant differences in survival ($P < 0.05$). Survival on normal broccoli is shown for comparative purposes only, and line segments above the bars denote standard errors of the means.

and disk to the total variation in toxin expression within each transformation.

Results

Larval Survival and Weight Gain. When evaluating survival on leaf disks cut from Bt-expressing and Bt-nonexpressing broccoli, we found that broccoli type had a significant impact on survival of susceptible larvae (Fig. 1). Survival on Bt-nonexpressing broccoli tended to remain between 90 and 100%, whereas survival on Bt-expressing broccoli declined dramatically after 48 h. At 24 h, mean percentage survival (±SE) of susceptible larvae averaged 99.1 ± 0.5% on Bt-expressing broccoli which was not significantly different from 99.7 ± 0.2% on Bt-nonexpressing broccoli ($F = 0.13$; df = 1, 7; $P > 0.05$). At 48 h, survival on Bt-expressing broccoli dropped to 85.0 ± 1.9%, which was significantly lower than 98 ± 0.6% observed on Bt-nonexpressing broccoli ($F = 14.37$; df = 1, 7; $P < 0.05$). At 72 h, survival on Bt-expressing broccoli showed a precipitous drop to 19.2 ± 2.4% compared with 89.5 ± 2.1% on Bt-nonexpressing broccoli ($F = 191.58$; df = 1, 7; $P < 0.05$). The small drop in survival at 72 h on Bt-nonexpressing broccoli was attributed to 2 plants that were screened as being Bt-nonexpressing but appeared to have some reduced level of toxin expression. If data from these 2 plants were removed from the analysis, survival at 72 h on the control foliage increased to 95 ± 0.9%. Mean percentage survival on normal plants at 24, 48, and 72 h ranged from 94 to 100% and appeared to be comparable to that observed on Bt-nonexpressing broccoli.

Resistant larvae, however, showed little to no mortality in leaf diskl bioassays with the same Cry1Ac-expressing plants. At 24, 48, and 72 h, survival of resistant larvae was between 90 and 100% on all plant types, whether they were Bt-expressing, Bt-nonexpressing, or normal, and no statistical differences between survival on Bt-expressing and Bt-nonexpressing plants were found ($F = 0.26$, 2.43, and 5.48, respectively; df = 1, 6; $P > 0.05$) (Fig. 2). Percentage survival for resistant larvae on Bt-expressing broccoli was 98.6 ± 0.6, 96.6 ± 0.8, and 90.8 ± 1.5% (mean ± SE), respectively. On Bt-nonexpressing broccoli, survival was 99.3 ± 0.3, 97.8 ± 0.6, and 95.1 ± 0.9%, respectively. Mean percentage survival on normal broccoli ranged from 94 to 100%. Furthermore, the average size of resistant larvae after 72 h, whether on Bt-expressing, Bt-nonexpressing, or normal foliage seemed comparable with the size of susceptible larvae on Bt-nonexpressing or normal broccoli. It appeared that resistance mechanism(s) that evolved in response to foliar sprays of *B. thuringiensis* subsp. *kurstaki* in the field were protecting the larvae from the acute toxic effects of the Cry1Ac expressed in the broccoli.

Not surprisingly, Bt-expressing plants also had marked effects on weight gain of susceptible larvae. Unlike mortality, however, these effects were clearly observable at 24 h. Susceptible larvae, reared on disks cut from Bt-expressing plants, showed significantly less weight gain at 24 h than susceptible larvae reared on disks cut from Bt-nonexpressing plants ($F = 293.41$; df = 1,7; $P < 0.05$) (Fig. 3, left side). In fact, larvae on Bt-expressing plants lost 0.2 ± 0.1 mg/10 larvae, whereas larvae on Bt-nonexpressing plants gained 1.8 ± 0.2 mg/10 larvae. The latter weight gain was similar to that observed for susceptible larvae on normal plants (1.8 ± 0.3 mg/10 larvae).

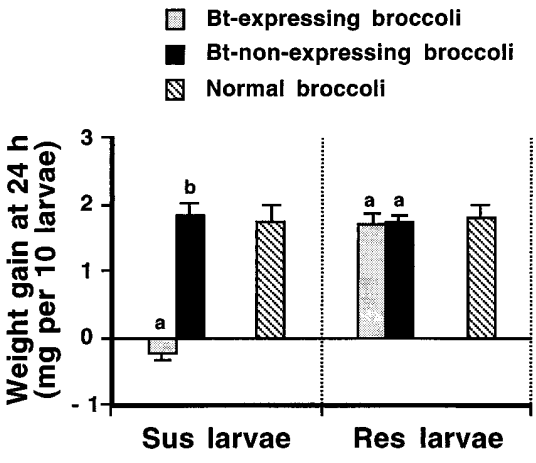

Fig. 3. Weight gain or loss of susceptible (Sus) and resistant (Res) 2nd instars on Bt-expressing and Bt-nonexpressing broccoli in leaf disk bioassays at 24 h. Within each larval phenotype, different lower case letters indicate significantly different values ($P < 0.05$). Weight gain on normal broccoli is shown for comparative purposes only, and line segments above the bars denote standard errors of the means.

Resistant larvae exhibited no differences in weight gain when tested on the same plants ($F = 0.25$, df = 1,6, $P > 0.05$) (Fig. 3, right graph). Resistant larvae on Bt-expressing and Bt-nonexpressing plants gained 1.7 ± 0.2 mg/10 larvae and 1.7 ± 0.1 mg/10 larvae, respectively. Weight gain on both plant types also was similar to that observed on normal plants (1.8 ± 0.2 mg/10 larvae). These data suggest that resistant larvae, in addition to being able to withstand the acute toxic effects of *B. thuringiensis* toxin, also were immune to sublethal effects that might affect weight gain.

Oviposition. Adult *P. xylostella* moths showed no oviposition preference for plant type. Both resistant and susceptible females exhibited no significant difference in the quantity or placement of eggs laid on Bt-expressing or normal broccoli plants (Tables 1 and 2). In choice tests, susceptible females (2 per cage) laid an average of 41 eggs on the Bt-expressing plant and 40 eggs on the normal plant, which were not significantly different ($F = 1.47$; df = 20, 20; $P > 0.05$). Similarly, resistant females laid 38 eggs on the Bt-expressing plant which did not differ from 40 eggs laid on the normal plant ($F = 1.01$; df = 18, 18; $P > 0.05$). The distribution of eggs laid by susceptible or resistant females did not show any significant differences between Bt-expressing and normal plants. In both experiments, females preferred to lay eggs on the lower stem of the plant, especially in the scar tissue where leaves were once attached; of the eggs laid on the leaves, most were on the upper surface.

Table 1. Oviposition (mean ± SE) of 2 susceptible *P. xylostella* females on Bt-expressing and normal broccoli after 72 h

Plant type	*n*	No. eggs on stem	No. eggs on leaves	Total no. eggs laid
Bt-expressing	21	25.7 ± 5.3a	15.1 ± 4.9a	40.7 ± 7.7a
Normal	21	33.5 ± 5.5a	6.8 ± 3.1a	40.1 ± 6.3a

Within a column, means are not significantly different if followed by the same letter ($P > 0.05$).

Table 2. Oviposition (mean ± SE) of 2 resistant *P. xylostella* females on Bt-expressing and normal broccoli after 72 h

Plant type	*n*	No. eggs on stem	No. eggs on leaves	Total no. eggs laid
Bt-expressing	19	27.1 ± 7.2a	11.0 ± 3.7a	38.1 ± 9.8a
Normal	19	29.8 ± 8.3a	10.5 ± 3.0a	40.4 ± 9.9a

Within a column, means are not significantly different if followed by the same letter ($P > 0.05$).

Plant Variation in Cry1Ac Expression. Using variation in mortality of susceptible larvae at 72 h as our measure of variation in Cry1Ac expression, we found that of the total variation (mean square = 5.13 for plant transformation), 43.2% was caused by transformation, 29.9% by plants within transformation, 3.0% by leaves within plants, and 23.9% by disks within leaves (Table 3). Thus, even though both the J1R and the K20 transformations were classified as high expressors, killing 100% of susceptible 2nd and 3rd instars in 7 d (Metz et al. 1995), the variation between transformations proved to be relatively large. The variation among plants within transformation was roughly similar in magnitude to the variation among disks within leaves, and the variation among leaves within plant was relatively small. Given the latter information, we were not surprised to find that the mean percentage of survival was relatively uniform for leaves arising from the lower, middle, or upper portions of the plant (Fig. 4).

Limiting our VARCOMP analysis to data obtained for the J1R transformation, we found that 11.5% of the total variation was caused by plant, 13.7% by leaf, and the remaining 74.8% by disk (Table 3). For the K20 transformation, 63.6% of the total variation was caused by plant, 4.7% by leaf, and 31.7% by disk (Table 3). Comparing the two, the J1R line exhibited much lower plant-to-plant variation and hence greater uniformity

Table 3. Variance component estimation for mortality in leaf disk assays of susceptible 2nd instars on Bt-expressing broccoli from the J1R and K20 transformations

Variance component	Estimate	% relative contribution
Transformation	0.04478	43.2
Plant	0.03093	29.9
Leaf	0.00314	3.0
Disk (error)	0.02471	23.9
For J1R only		
Plant	0.0030	11.5
Leaf	0.0036	13.7
Disk (error)	0.0196	74.8
For K20 only		
Plant	0.0674	63.6
Leaf	0.004976	4.7
Disk (error)	0.03363	31.7

Variance component estimations (72-h mortality) were based on arcsine-transformed data (SAS Institute 1985).

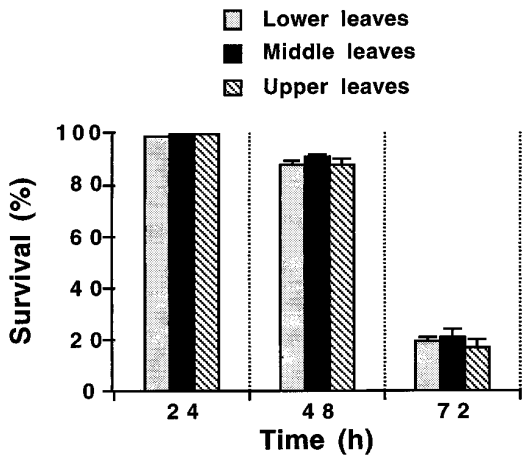

Fig. 4. Effect of leaf position in Bt-expressing plants on survival of susceptible 2nd instars in leaf disk bioassays at 24, 48, and 72 h.

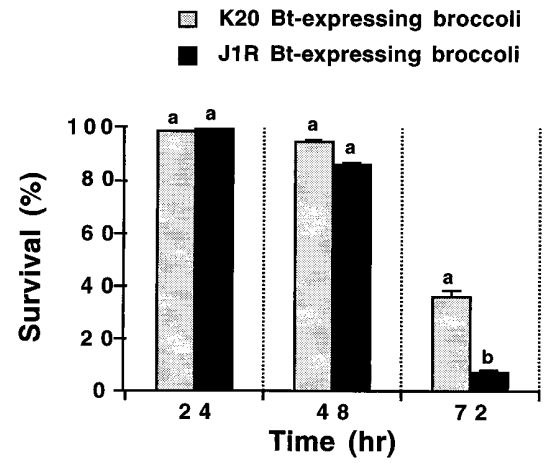

Fig. 5. Comparison of Bt-expressing broccoli from the J1R and K20 transformations on survival of susceptible 2nd instars in leaf disk bioassays at 24, 48, and 72 h. For each time, different lower case letters indicate significantly different values ($P < 0.05$).

in levels of toxin expression than plants in the K20 line. An indication of the range of variation is given in Table 4 where percentage survival is reported for each plant examined from the two lines.

Bt-expressing plants from the J1R line also were more toxic than Bt-expressing plants from the K20 line (Fig. 5). Significant differences in percentage survival, however, were not detected until 72 h. At 48 h, we observed 85.6 ± 1.2% survival on plants from the J1R line, which was not significantly different from 94.8 ± 0.9% survival on the K20 line ($F = 5.42$; df = 1, 7; $P > 0.05$). At 72 h, only 7.1 ± 1.1% survived on J1R plants, which was significantly lower than 36.1 ± 2.5% survival on K20 plants ($F = 5.99$; df = 1, 7; $P < 0.05$). This type of analysis efficiently characterizes both the level of toxicity and the variation in toxicity and should be adopted as one of the standard methods for evaluating toxin expression among different transgenic lines.

Discussion

Our previous studies showed that resistance to foliar sprays containing Cry1A toxins had occurred in larvae of *P. xylostella* populations from Florida (Shelton et al. 1993, Tang et al. 1996). Results presented in this article indicate that these populations also are highly resistant to transgenic broccoli expressing Cry1Ac toxin. Unlike susceptible 2nd instars, resistant larvae displayed similar levels of weight gain and survival on plants expressing *B. thuringiensis* toxin as on plants that did not express toxin. Unfortunately, because the quality of leaf disks could not be maintained for long periods, we were unable to look at survival through pupation. Our data suggest, however, that resistant larvae did not suffer sublethal effects of the Bt-expressing broccoli. More recently, enough transgenic material has been produced to conduct whole-plant studies with our resistant *P. xylostella* larvae, and data have shown that larvae can complete development from egg to adult and cycle for multiple generations on Cry1Ac broccoli just as they would on normal broccoli (J.D.T., unpublished data).

The fact that these larvae never were exposed to *B. thuringiensis* transgenic plants but were already resistant to them forewarns of the problems that could occur when single toxins used for plant expression are not novel but have received widespread use in commercially available sprays. Even if resistance were not yet widespread, modeling studies suggest that the increased selection pressure from commercial releases of transgenic *B. thuringiensis* plants could cause rapid evolution of resistance unless a proactive approach to resistance management is prescribed. Clearly, the need to diversify the types of toxins expressed in plants from *B. thuringiensis* or other sources (e.g., baculovirus enhancins) is paramount because it would give growers more control options. This would be especially important for pests that are notorious for rapidly evolving resistance or for polyphagous pests that attack different host crops. Based on our data, the conclusion drawn by Ramachandran et al. (1998) that transgenic plants expressing *B. thuringiensis* toxin could be used for effective management of *P. xylostella*

Table 4. Survival of susceptible 2nd instars in leaf disk assays of Bt-expressing broccoli from the J1R and K20 transformations

Plant transformation	Plant no.	% survival (72 h), mean ± SE
J1R	1	22.0 ± 5.8
	2	5.0 ± 1.6
	3	9.4 ± 2.3
	4	2.7 ± 1.0
	5	4.4 ± 1.6
KJ20	1	11.4 ± 1.9
	2	18.7 ± 3.0
	3	63.1 ± 3.4
	4	46.9 ± 3.9

populations would, in fact, pose a very high risk for resistance evolution.

Another concern is that the mechanism of resistance to *B. thuringiensis* in *P. xylostella* is physiological. The normal weight gain of resistant larvae on Cry1Ac broccoli corroborates our previous investigation implicating a change in the midgut toxin receptor as the underlying mechanism of resistance (Tang et al. 1996). It appears that once toxin was prevented from binding to the gut, gut integrity was not eroded by pore formation (Knowles 1994), and larvae were immune to the lethal effects of the Cry1A toxins. In contrast to possible behavioral mechanisms, such as avoidance (Gould et al. 1991) or abandonment (Harris et al. 1997), physiological resistance is less likely to be overcome with molecular methods that increase toxin expression levels once resistance has reached detectable frequencies in the field (e.g., >1%). Even if increasing the dose could partially overcome resistance, it is likely that other more resistant variants would arise, similar to the multiallelic variants *kdr* and *super-kdr*, which evolved in response to increasing doses of DDT and pyrethroids in house flies (Farnham et al. 1987).

Our data on oviposition of resistant and susceptible adult females support previous reports that female Lepidoptera do not differentiate between *B. thuringiensis* and non-*B. thuringiensis* hosts. Compared with previous reports of *P. xylostella* oviposition, our data are unique because they examine both the quantity and distribution of eggs laid by resistant and susceptible females on *B. thuringiensis* transgenic and non-*B. thuringiensis* host plants. Groeters et al. (1992) found no effects of *B. thuringiensis* in oviposition choice tests of resistant and susceptible females, but they used petri dish assays of *B. thuringiensis* dipped and water-dipped leaf disks. In a more realistic setting, Riggin-Bucci and Gould (1996) tested oviposition of susceptible females and found that similar numbers of eggs were laid on *B. thuringiensis* -sprayed and control-sprayed plants in greenhouse and field tests. The distribution of eggs on the plant, however, was not reported (Riggin-Bucci and Gould 1996) and oviposition of resistant females on plants was still unknown. Females of the European corn borer, *Ostrinia nubilalis* (Hübner) are like *P. xylostella* in that they do not differentiate between *B. thuringiensis* transgenic and non-*B. thuringiensis* host plants (Orr and Landis 1997). In field tests, Orr and Landis (1997) found that corn type had no significant effect on European corn borer egg mass size, density, or distribution in the plant.

If oviposition preferences existed, they could influence the rate at which resistance evolves. For example, given that resistance is a recessive trait and that the *B. thuringiensis* crop is planted with a separate but nearby refuge in the form of an alternate host crop or weed, one would expect resistance to evolve more slowly if resistant and susceptible females preferred to oviposit on refuge plants (Groeters et al. 1992) than if oviposition were random. The lack of oviposition preference, however, will facilitate the integration of other integrated pest management (IPM) practices such as pheromone lures to either disrupt oviposition among resistant adults emerging from the transgenic crop or for attracting susceptible males from refuges into transgenic areas.

Our VARCOMP analysis of the variation in toxin expression among *B. thuringiensis* transgenic plants was presented to underscore the advantages this type of analysis provides. By collecting the data in a structured manner, not only can one compare the toxicity of different plant lines, but one can also determine where the variation in toxin expression occurs (i.e., estimate how much of the variation is caused by transformation event to plants within lines or to leaves within plants). To date, many, if not all publications that evaluate transgenic plant lines fail to characterize fully the variation in toxin expression. Their best estimate of variation is the mean survival ± SE with a range (e.g., Koziel et al. 1993), or the number of replicates with live larvae (Jenkins et al. 1993). Therefore, if the ultimate criteria for developing plants for commercial release are high toxicity and uniform toxin expression, it follows that VARCOMP analysis or similar types of statistical procedures should be more widely adopted.

Our work shows clearly that there is the potential for the interaction of pesticide sprays and transgenic crops with respect to resistance, but not in the usual direction most popularly conceived. Here it was the sprays that selected for resistance to transgenic plants, and not the other way around. Resistance is a risk for both transgenic crops and sprays whenever they are used intensively. Hopefully, with continued research, both *B. thuringiensis* sprays and *B. thuringiensis* crops will become integral components of sustainable agriculture rather than steps on the pesticide treadmill.

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