Evaluation of a Chemically Inducible Promoter for Developing a Within-Plant Refuge for Resistance Management

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J. Econ. Entomol. 98(6): 2188–2194 (2005)

ABSTRACT Chemically inducible production of *Bacillus thuringiensis* (Bt) toxins in transgenic plants may provide considerable benefits in preventing or delaying the evolution of insect resistance to Bt crops by creating within-plant temporal refuges. We examined the effect of inducible *cry1Ab* expression on survival of different genotypes (RR, RS, and SS) of diamondback moth, *Plutella xylostella* (L.), in transgenic broccoli, *Brassica oleracea* L., plants transformed with a *PR-1a/cry1Ab* expression cassette. Spraying leaves of these plants with the inducer acibenzolar-s-methyl [=benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (ASM) resulted in high levels of Bt toxin, and detached leaves from fully induced plants caused 100% mortality to all instars of *P. xylostella* SS and RS genotypes. When plants infested with larvae were treated with ASM, only a few larvae that were nearing completion of their development were able to survive the induction process. Signal transduction from ASM-treated leaves to new plant tissue also was evaluated using a larval assay. New foliage that emerged after plants were induced remained toxic to ≥80% of RS larvae up to the fourth new leaf. In whole plant tests, however, induced plants remained protected from larval damage for ≥3 wk. Uninduced *PR-1a/cry1Ab* plants seemed to produce low levels of Bt that were undetected by an enzyme-linked immunosorbent assay but that resulted in significant fitness costs for susceptible insects. The suitability of *PR-1a/cry1Ab* broccoli plants for insect resistance management and the requirements of an appropriate inducible promoter are discussed.

KEY WORDS *Bacillus thuringiensis*, *Plutella xylostella*, diamondback moth, PR-1A promoter, resistance management

TRANSGENIC PLANTS EXPRESSING insecticidal toxins from the bacterium *Bacillus thuringiensis* (Bt) provide highly specific and effective pest control with little to no negative impacts on beneficial insects and other nontarget organisms (International Life Sciences Institute 1999). In 2004, Bt crops were grown on >22 million hectares (56 million acres) worldwide, a 25% increase from the previous year (James 2003, 2004). Adoption of Bt technology is expected to continue its growth in response to expanding regulatory approval and the availability of new products that control a wider range of pests (James 2003).

The risk of pest adaptation to Bt toxins continues to cause concern over the use of Bt crops. Although the most effective way to manage resistance to any toxin is to avoid unnecessary exposure (Roush 1989), all currently registered Bt crops produce toxins continuously under the control of constitutive promoters, resulting in strong selection for resistance. The only strategy presently available to manage resistance is to plant a refuge of nontoxic plants to ensure the survival of at least some susceptible insects. Although this tactic seems to have been effective to date (Tabashnik et al. 2003), it has several disadvantages. Refuges frequently sustain severe economic losses and managing insects in the refuge while still maintaining susceptible alleles presents challenges (Shelton et al. 2000). Moreover, there remains considerable uncertainty and debate regarding the most effective size and placement of refuges to ensure that resistant and susceptible insects can mate adequately (Bates et al. 2005).

An alternative resistance management strategy is the use of temporal refuges. Insect resistance genes under the control of chemically inducible promoters (i.e., that are switched on by the application of an otherwise inactive compound) could ensure that Bt toxins are produced only when necessary (Gould 1998). For example, plants could be induced when the pest density exceeds an economic threshold, when vulnerable plant structures or formed, and/or only in heavily infested areas of the field. Temporal refuges increase the scale of the refuge substantially, thereby reducing its dependence on the stringent assumptions of insect dispersal and mating associated with current spatial refuges (Tabashnik 1994). Temporal refuges have been used to successfully manage resistance to

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0022-0493/05/2188–2194$04.00/0 © 2005 Entomological Society of America

conventional insecticides in the past (Roush 1989) and show considerable promise for Bt crops as well.

Before inducible Bt plants can be considered as a resistance management strategy, their protein expression patterns and effectiveness as a pest management tool must be determined. Because resistance evolution is driven primarily by the survival of larvae heterozygous for resistance (Roush 1997a, b), it is particularly important to determine whether Bt expression in inducible plants is sufficient to control these larvae. In addition, production of Bt toxins in induced plants must reach a lethal level relatively rapidly, so that the amount of time insects are exposed to selection by sublethal concentrations of Bt is minimized.

To establish "proof of concept" for inducible Bt technology, we produced transgenic broccoli plants carrying a *cry1Ab* gene under the control of the pathogenesis responsive *PR-1a* promoter from tobacco (Williams et al. 1992, Cao et al. 2001). *PR-1a* is induced by the accumulation of endogenous salicylic acid (SA) after pathogen infection or artificially through the application of an SA analog such as acibenzolar-s-methyl [=benzo (1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (ASM) (Jepson et al. 1998). Here, we examine the response of different genotypes (RR, RS, and SS) of the diamondback moth, *Plutella xylostella* (L.), to inducible Bt broccoli plants both during and after treatment with ASM.

Materials and Methods

Plants. Homozygous selfed T2 progeny of transgenic broccoli, *Brassica oleracea* L. spp. *italica* 'Green Comet', line T73-3, were used in the experiments. This line carried one insertion of the *PR-1a/cry1Ab* expression cassette and was inducible by ASM (Cao et al. 2001). Plants were grown in a greenhouse maintained at 21–30°C and a photoperiod of 16:8 (L:D) h.

Insects. Susceptible diamondback moth larvae (SS) were obtained from the previously described Geneva 88 colony (Zhao et al. 2002). The colony was reared on wheat-casein diet (Shelton et al. 1991) and maintained in an environmental chamber at 27 ± 1°C, 50 ± 2% RH, and a photoperiod of 16:8 (L:D) h. Homozygous resistant larvae (RR) were obtained by crossing Geneva 88 moths with a field-collected Cry1Ac-resistant strain (Zhao et al. 2002) and then backcrossed three to four times to G88 and selected after each cross with 2000 ppm Cry1Ac (MVP, 10%) in a diet overlay (Zhao et al. 2002). The resulting diet-adapted resistant strain shared ≥94% genetic similarity with Geneva 88 and was cross-resistant to Cry1Ab (Tang et al. 1997). Resistant insects were able to survive on both constitutive, high-expressing Cry1Ac plants and fully induced PR1A/*cry1Ab* plants. Larvae heterozygous for resistance (RS) were obtained by crossing SS and RR adults to produce RS F_1 larvae. All larvae were hatched and raised on an artificial diet before used for bioassay.

Induction of Plants. *PR-1a/cry1Ab* broccoli plants (Cao et al. 2001) were induced using Actigard, a 50% wettable powder formulation of ASM, at a rate of 62.5 mg/100 ml. Bond sticker-spreader was added at a rate of 100 μl/100 ml. Whole plants were sprayed with ≈25 ml of the Actigard/Bond solution by using a hand-held mister, with care taken to ensure that all existing leaves were thoroughly covered. Ablated leaves were induced by dipping them directly in a solution of ASM and Bond.

Experiment 1: Survival of Larvae on Fully Induced Tissue. To determine whether transgenic foliage was toxic to all stages of SS and RS larvae after induction, mortality of different instars was assessed on fully induced foliage. Ten 11-wk-old transgenic plants (measured from the time of sowing) were induced with ASM, whereas 10 untransformed plants served as a nontransgenic control. After 4–8 d, leaf sections were removed randomly from the newest four leaves and placed in 30-ml plastic cups (Polar Plastics, Winston Salem, NC). Five first, second, third, or fourth instars were transferred from diet onto leaf sections in separate cups. Each cup served as one replicate. Mortality was assessed after 3 d. Surviving larvae were transferred to either fresh induced foliage or nontransgenic foliage, and mortality was reassessed after an additional 3 d.

Experiment 2: Survival of Larvae during Induction (Leaf Assay). To determine the relationship between larval age and survival during the induction process, mortality of different aged SS or RS larvae was assessed on foliage immediately after treatment with ASM. The three youngest, fully expanded leaves were removed from 10-wk-old transgenic plants and dipped in ASM and Bond, or Bond only. Leaves were allowed to air dry for 10–15 min and then placed in 500-ml plastic cups (Fabri-Kal Corp., Kalamazoo, MI). Larvae of four different ages were transferred from diet onto leaves: second instars (2 d old), third instars (3 d old), early fourth instars (5 d old), and late fourth instars (6 d old). Nine replicates (leaves) were tested for each age group. Mortality was assessed after 3 d. To determine whether larvae that were still alive after 3 d had obtained a lethal dose of Bt, larvae that were still alive after 3 d were transferred onto uninduced foliage and monitored for continued survival.

Experiment 3: Survival of Larvae during Induction on Whole Plants. Survival of different aged larvae feeding on intact plants at the time of induction also was evaluated. Ten-week-old uninduced plants were placed in a 1.8 by 0.9 by 1.7-m cage constructed of nylon netting in a greenhouse maintained at 25–33°C and a photoperiod of 16:8 (L:D) h. Thirty SS adult females and 30 SS adult males were released in the cage and allowed to oviposit for 3 d. Plants were then sprayed with ASM 1, 3, or 7 d after the 3-d oviposition period. Nontransgenic Green Comet broccoli plants also were infested and sprayed with ASM in a similar manner. Each plant was transferred to a 0.62 by 0.47 by 0.62-m mesh cage immediately after being induced. There were three replicates (plants) for each time of induction and genotype. The number of larvae on each plant was counted 7 d after induction. The same procedure was repeated using SS females and RR males to obtain RS eggs.

Table 1. Survival of SS and RS diamondback moth larvae on fully induced and nontransgenic broccoli leaves

Genotype	Instar	Foliage type	Mortality after 3 d (%)	
SS	First	Induced	100 ± 0*	$t = 57.70$, df = 28, $P < 0.0001$
		Nontransgenic	4.7 ± 1.7	
	Second	Induced	100 ± 0*	$t = 65.43$, df = 28, $P < 0.0001$
		Nontransgenic	3.3 ± 1.5	
	Third	Induced	98.9 ± 1.1*	$t = 45.51$, df = 28, $P < 0.0001$
		Nontransgenic	3.3 ± 1.8	
	Fourth	Induced	72.0 ± 4.7*	$t = 14.5$, df = 28, $P < 0.0001$
		Nontransgenic	1.3 ± 1.3	
RS	First	Induced	100.0 ± 0*	$t = 27.84$, df = 28, $P < 0.0001$
		Nontransgenic	14.7 ± 3.1	
	Second	Induced	100.0 ± 0*	$t = 86.59$, df = 28, $P < 0.0001$
		Nontransgenic	1.7 ± 1.1	
	Third	Induced	98.9 ± 1.1*	$t = 13.15$, df = 28, $P < 0.0001$
		Nontransgenic	10.0 ± 6.7	
	Fourth	Induced	70.7 ± 4.3*	$t = 12.85$, df = 28, $P < 0.0001$
		Nontransgenic	4.0 ± 2.9	

Each treatment was replicated 15 times, and each replicate consisted of five larvae. Asterisks indicate significant differences between pairs of data, Student's t-test, $P < 0.05$.

Experiment 4: Signal Transduction and Survival of Larvae (Leaf Assay). New foliage that is produced after treatment with ASM produces decreasing amounts of Bt, and survival of SS larva increases as the ASM signal attenuates over time and/or distance from treated plant parts (J.C., unpublished data). To determine whether RS larvae have greater survival than SS larval on potentially low-expressing plant tissue, survival of RS larvae on foliage formed after induction was assessed. Seven-week-old plants were induced with ASM. After 4 wk, all new foliage formed subsequent to treatment with ASM (seven or eight leaves per plant) was removed and the position of each leaf on the plant was noted. Each leaf was divided in two, and half was placed in a 30-ml plastic cup with five RS second instars. Five RR second instars were added to the other half of each leaf as a control. Percentage of mortality was recorded after 3 d and corrected for control mortality using Abbott's formula (Abbott 1925). Each leaf position was replicated 7–10 times.

Experiment 5: Signal Transduction and Survival of Larvae (Whole Plants). Survival of SS and RS larvae on new foliage of intact plants also was determined. Nine-week-old plants were sprayed with ASM and placed individually in 0.62 by 0.47 by 0.62-m mesh cages in the greenhouse. Once a week for 5 wk, three pairs of diamondback moth adults were released in each cage (SS female × SS male to obtain SS progeny, or SS female × RR male to obtain RS progeny). A 10% solution of sugar water was provided as a carbohydrate source for adults in each cage. The number of larvae (second instar and older) on each plant was counted weekly. There were five replicates (cages) for each larval genotype (SS or RS). One untransformed Green Comet plant served as a population check.

Experiment 6: Fitness of Larvae on Uninduced Leaf Tissue. The newest, fully expanded leaf from 8-wk-old uninduced transgenic plants and nontransgenic Green Comet plants was cut in half and placed in two 30-ml plastic cups. Five SS or RR neonates were added to each cup and placed in a growth chamber at 27°C, 45% RH, and a photoperiod of 16:8 (L:D) h. There were 25 cups (replicates) for each genotype/foliage combination. Larvae were transferred onto fresh foliage every 3 d. Survival to adulthood, pupal weight and developmental time for each genotype feeding on both types of foliage were recorded. Pupal weight and developmental time were based on 39–62 individuals per treatment.

Statistical Analysis. Survival of each instar on induced and uninduced plant tissue was compared using a t-test. The number of insects on whole plants induced at different times after oviposition was compared between inducible and nontransgenic broccoli at each time period using a t-test. Survival rates of different instars during induction, and survival, developmental time, and pupal weights of larvae feeding on uninduced foliage were analyzed by analysis of variance (ANOVA), and the means were separated by the Tukey–Kramer honestly significant difference (HSD) multiple comparison test (Sall et al. 2001). In all cases, $\alpha = 0.05$.

Results

Experiment 1: Survival of Different Instars on Fully Induced Tissue. Mortality of identically aged SS and RS larvae on induced foliage was very similar (Table 1). No first or second instars of either genotype were alive after 3 d of feeding. Few third and fourth instars were alive after 3 d. However, none of these larvae survived to adulthood, even if they were transferred onto nontransgenic foliage, indicating that surviving larvae obtained a lethal dose of Bt.

Experiments 2 and 3: Survival of Larvae during Induction. Newly molted SS or RS second instars that were transferred onto foliage immediately after treatment with ASM did not survive, whereas only 3 and 11% of SS and RS third instars, respectively, were alive after 3 d (Fig. 1). Survival of late SS fourth instars was significantly higher than early fourth instars ($F = 108.4$; df = 3, 32; $P < 0.001$). Survival of RS fourth instars was higher than second or third instars ($F = 50.3$; df = 3, 32; $P < 0.0001$), but there was no differ-

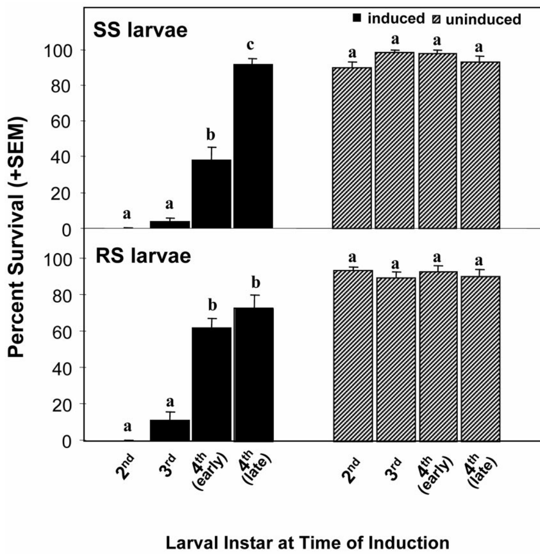

Fig. 1. Survival of SS and RS diamondback moth larvae placed on ablated leaves immediately after induction. Bars with the same letter within each group are not significantly different, Tukey–Kramer HSD test, $P < 0.05$.

ence between early and late fourth instars. Survival of both genotypes on uninduced tissue was >90% (Fig. 1). When all surviving larvae were transferred onto fresh uninduced foliage after 3 d, only eight (out of 37) SS and one (out of 33) RS survivors from induced tissue successfully molted to adults.

Survival of larvae during induction on intact plants in experiment 3 followed a similar pattern (Fig. 2). When plants were induced 1 or 4 d after oviposition

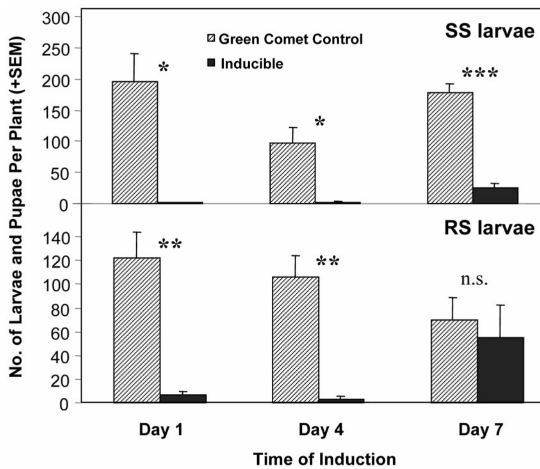

Fig. 2. Number of SS and RS larvae and pupae on plants that were induced 1, 4, or 7 d after oviposition by diamondback moth adults. Larvae and pupae were counted 1 wk after treatment with inducer. Asterisks indicate significant differences between insect counts on inducible and nontransgenic control plants treated with inducer at the same time (t-test). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

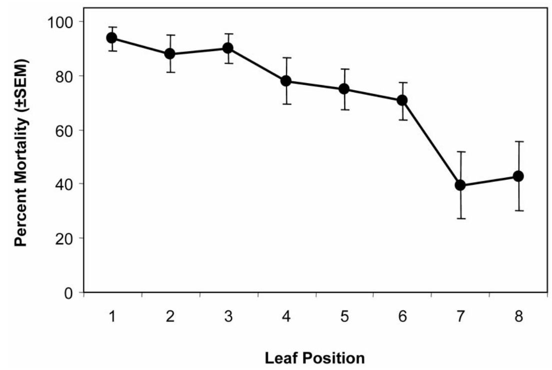

Fig. 3. Mortality of SS and RS diamondback moth larvae on new foliage formed on plants after treatment with inducer.

(when insects were primarily in the egg and second instar, respectively), only a few SS and RS individuals survived compared with larvae feeding on nontransgenic broccoli also treated with inducer. Induction 7 d after oviposition (when larvae were primarily third and fourth instars) resulted in much higher survival. An average of ≈25 SS larvae survived on inducible plants, but this number was still significantly less than control plants. There was no difference in survival between RS larvae feeding on inducible and ASM-treated nontransgenic plants.

Experiments 4 and 5: Signal Transduction and Response of Diamondback Moth. Mortality of RS larvae on leaves formed after induction remained ≥88% for the first three leaves formed subsequent to induction (Fig. 3). Survival of larvae on the fourth new leaf declined to 78% By the time the eighth new leaf was formed, production of Bt was sufficient to cause <45% mortality.

When diamondback moths were released weekly onto induced plants (exp. 5), the number of SS and RS larvae per plant remained <2 until the third week after induction (Fig. 4). Defoliation of plants remained <2% per plant during this time (data not shown).

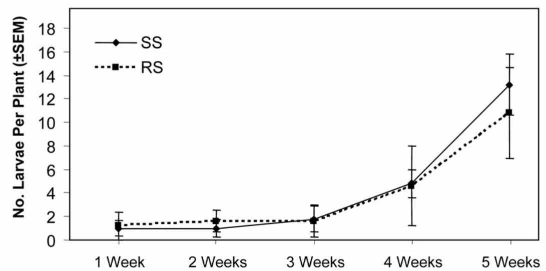

Fig. 4. Number of SS and RS diamondback moth larvae (second instar or older) on intact inducible plants after induction. Three weeks after treatment with inducer, all foliage existing at the time of induction had senesced or was removed manually. The number of larvae on an untransformed Green Comet control plant ranged from 17 to 236 over the course of the experiment.

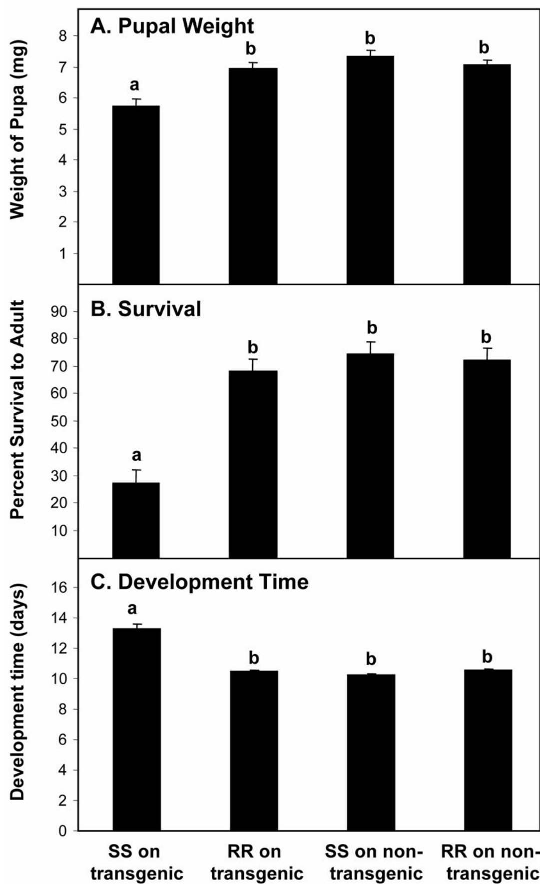

Fig. 5. Fitness of SS and RR diamondback moth larvae feeding on uninduced PR-1A/cry1Ab transgenic plants and nontransgenic control plants. Fitness was measured by weight at pupation (A), survival of larvae to adulthood (B), and length of developmental time (C). Bars with different letters within each subgraph are significantly different, Tukey–Kramer HSD test, $P < 0.05$.

After 3 wk, when old foliage was manually removed from the plant leaving only tissue formed subsequent to induction, the number of larvae began to increase steadily. By week 5 there were >10 SS or RS larvae per plant. Despite this increase, the number of SS and RS larvae per induced plant remained substantially less than the number of SS insects on an untransformed control plant (17–236 over the course of the experiment; data not shown). By week 4, the control plant had suffered severe defoliation and was replaced.

Experiment 6: Fitness of SS Larvae on Uninduced Transgenic Foliage. Weights of SS pupae that developed on uninduced transgenic foliage were significantly lower than RR pupae that developed on the same plant material, and SS and RS pupae that developed on nontransgenic plants (Fig. 5). In addition, survival of SS larvae from neonate to adult was reduced by at least 60% compared with other treatments. Most of the mortality occurred during the early instars (data not shown). Developmental time was also 26% longer for SS larvae on uninduced transgenic foliage compared with all other treatments.

Discussion

An ideal inducible promoter for insect resistance management must meet several criteria: 1) it must quickly activate the expression of insect resistance gene(s); 2) high levels of insecticidal toxins must be produced in treated plant structures; 3) high levels of toxin must be maintained in these structures for the life of the plant (or at least for the duration of exposure to target pests); and 4) signal transduction must occur between treated and untreated plant parts, so that plant tissues formed after treatment with inducer are protected. Results from the current study suggest that chemically inducible *PR1a/cry1Ab* broccoli plants meet some of these criteria, but not all.

Additional experiments on these chemically inducible *PR1a/cry1Ab* broccoli plants focused on molecular characterization of protein production in *PR1a/cry1Ab* plants (J.C., unpublished data). Our bioassay results agreed with these protein assays; however, our focus on bioassays with RR, SS, and RS genotypes provides a more comprehensive assessment of how these plants would influence the evolution of resistance

In experiment 1, mortality of SS and RS larvae indicated that inducible plants were capable of producing a "high-dose" of Bt toxin, defined by the U.S. Environmental Protection Agency as one that would kill ≥95% of RS larvae (U.S. EPA 2001). Enzyme-linked immunosorbent assay (ELISA) tests indicated that 3 d after treatment with ASM, Cry1Ab expression levels in *PR1a/cry1Ab* plants were >800 ng/mg of total soluble protein (TSP) (J.C., unpublished data). Unlike constitutively expressing Bt plants, where larvae are most likely to be exposed to Bt toxins as neonates, it was important to demonstrate that all instars of SS and RS genotypes could be killed by fully induced *PR-1A/cry1Ab* plants. Larger larvae may be present on plants at the time of induction, and susceptibility to Bt toxins can decrease with size/age (McGaughey 1978, Van Frankenhuyzen et al. 1997, Huang et al. 1999, Hellmich et al. 2001). However, even large fourth instars eventually suffered 100% mortality when feeding on fully induced tissue.

Although fully induced tissue was capable of delivering a high dose of Bt toxins, maximum levels of Bt are not reached until 48–72 h after induction (Cao et al. 2001; J.C., unpublished data). As a result, it is possible that some larvae may obtain only a sublethal dose, particularly those that are nearing completion of their development at the time of induction. In experiments 2 and 3, some older SS and RS larvae were able to tolerate exposure to sublethal doses during induction, probably because of their reduced sensitivity to Bt. The overall higher survival of RS larvae compared with SS larvae on both intact plants and ablated leaves is most likely due to the advantage conferred by the single resistance allele during exposure to sublethal doses of Bt. However, it is worth noting that when

mortality of survivors in experiment 3 continued to be monitored, only a few RS and SS larvae survived to adulthood.

Differential mortality among different aged larvae on both intact plants and ablated leaves suggests that induction of Bt may need to take place while larvae are in the early stages of development if maximum control is to be achieved. However, from a practical standpoint, survival of older SS larvae may be of little consequence. SS larvae may continue to feed until pupation, but any potential offspring will encounter plant foliage that is fully induced and therefore protected. Of greater concern, from a resistance management perspective, is the apparently higher survival of late RS instars on whole plants in experiment 3. Resistance development is driven primarily by survival of individuals heterozygous for resistance (Roush 1997a, b), and ensuring mortality of RS individuals is key to successful resistance management. Lower mortality of RS larvae could create a selection differential during induction that favors an increase in resistant allele frequency in surviving insects.

ELISA data and results with SS diamondback moth larvae (J.C., unpublished data) indicated that some signal transduction occurred between treated plant parts and new tissue formed subsequent to induction. However, leaves beyond the fourth newly emerged leaf (which generally produced $<$80 ng/mg TSP toxin) (J.C., unpublished data) failed to control RS, a similar result to that obtained using SS larvae (J.C., unpublished data). As a result, insects also may be exposed to sublethal doses of Bt by feeding on tissues formed subsequent to induction that have received only a low level of an inducing signal or by moving between fully induced older tissue and uninduced new tissue on the same plant. Models have indicated that such sublethal exposure can accelerate the rate of resistance development compared with a high dose of toxin (Roush 1997a). Consequently, it may be necessary to make frequent applications of the inducer to maintain high levels of Bt production in growing plants. For example, under the conditions of our greenhouse, application of inducer would be required approximately every 2 to 3 wk to keep Bt levels sufficiently high to control diamondback moth larvae. Repeated applications of the inducer may reduce some of the benefits of growing pesticide-incorporated transgenic plants. However, signal transduction may be sufficient for situations in which insect protection is required for a limited time only at the end of a crop cycle, e.g., during head formation in broccoli when larvae are otherwise difficult to control.

In experiment 5, survival of diamondback moth larvae on intact plants in the week after induction suggested that the biology and/or behavior of the target pest may influence the length of time that plants are protected. Although Bt production was sufficient to kill larvae only until the fourth new leaf was formed in the leaf assay ($\approx$2 wk) in experiment 4 (Fig. 3), when protein levels were still above 300 ng/mg TSP (J.C., unpublished data), intact induced plants remained protected for at least 3 wk (Fig. 4), at which time leaves that had been directly induced were manually removed from the plant. The majority of diamondback moth eggs are laid at the base of plant stem in broccoli (S.L.B., unpublished data). As a result, most hatching neonates would have encountered plant tissue at the base of the plant that was fully induced and therefore received a lethal dose.

Repeated tests with ELISA indicated that uninduced tissue in plants from line T73-3 used in the experiments contained no detectable amounts of Bt (S.L.B., unpublished data). However, observations of larval fitness and survival on uninduced plants suggested that very low levels of Bt, too low to be detected by ELISA, were being produced. This was confirmed by tests that compared the fitness and survival of SS and RR genotypes on uninduced transgenic tissue (Fig. 5). The lack of detection may have been due to our use of younger leaves (i.e., the three most recently formed, fully expanded leaves) because ELISA tests on older leaves (i.e., 8 wk old) did reveal the presence of low levels of Bt proteins (e.g., $\approx$50 ng/mg TSP) in uninduced *PR1a/cry1Ab* broccoli plants (J.C., unpublished data). When survival of SS neonates across a wider range of transgenic plant material was tested, mortality on foliage from 46 different plants ranged from 0 to 100% after 3 d (data not shown), indicating considerable variation in the noninduced production of the Bt across plants.

Although the low level of basal Bt production had significant negative effects on SS larvae, the highest mortality of larvae occurred on plants treated with ASM. In additional tests, ASM was not observed to cause any direct mortality to *P. xylostella* larvae through contact (by spraying larvae), ingestion (consumption in a diet overlay), or through non-Bt-related changes in plant physiology (feeding on nontransgenic plants treated with ASM) (data not shown). Thus, the higher mortality observed on ASM-treated transgenic plants can be attributed to the induced production of Bt toxin.

The most likely explanation for the observed “leakiness” of the PR-1A promoter is that it was responding to endogenous signaling compounds in the plant. The difficulty in controlling such compounds or external stimuli greatly limits the feasibility of a plant-derived promoter for field use (Jepson et al. 1998), because even low levels of toxin can select for resistance when there is differential selection between susceptible and heterozygous resistant larvae (Onstad and Gould 1998a, b; Roush 1997a, b). In the case of *PR-1A/cry1Ab* broccoli, where the objective was to establish proof-of-concept for an available inducible promoter as a pest management and resistance management tool, even the relatively controlled conditions of the laboratory and greenhouse did not prevent leakage of the PR-1a promoter. As a result, the use of these plants to conduct further tests on the effectiveness of an inducible promoter for resistance management is limited. It might be possible to eliminate the endogenous production of the Cry1Ab protein by developing plants with inducible promoters that are not derived from plant sources, such as ecdysone receptors from

insects (Jepson et al. 1998, Padidam et al. 2003). These promoters are unlikely to be induced by endogenous plant compounds and are readily induced by commercially available IGRs (Padidam et al. 2003). However, the question of signal transduction must be thoroughly addressed for any potential inducible promoter to be used in insect-resistant transgenic crops.

Results from the current study and our previous work (Cao et al. 2001) as well as ongoing research (J.C., unpublished data) suggest that inducible promoters show considerable promise for insect resistance management with respect to the speed of gene induction and levels of protein expression. In other areas, however, such as signal transduction and control of gene expression, some fundamental questions must be addressed before this technology can be considered a viable option for most pest/crop systems.

Acknowledgments

We thank Ciba-Geigy (Crop Protection Division) for kindly providing ASM. This work was supported by USDA–NRI Grant 2002-35302–12502.

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Received 1 April 2005; accepted 14 July 2005.