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Effectiveness of Natural and Engineered Host Plant Resistance in Potato to the Colorado Potato Beetle

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Abstract. **The Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is the most serious insect pest of potatoes throughout the eastern and north central United States. Host plant resistance to the Colorado potato beetle has been identified in wild *Solanum* species and *Bt*-transgenic potato lines. Detached-leaf bioassays (72 h) were conducted on insecticide-resistant, first instar Colorado potato beetles to study the effectiveness of individual and combined host plant resistance traits in potato. Potato lines tested include non-transgenic cultivars ('Russet Burbank', 'Lemhi Russet', and 'Spunta'), a line with glandular trichomes (NYL235-4), a line with high foliar leptines (USDA8380-1), and transgenic lines expressing either codon-modified *Bt-cry3A* or *Bt-cry5* (*Bt-cry1Ia1*). *Bt-cry3A* transgenic lines, foliar leptine line, and foliar leptine lines with *Bt-cry5* had reduced feeding compared to non-transgenic cultivars. Glandular trichome lines and glandular trichome lines with *Bt-cry5* did not reduce feeding in this no-choice feeding study. Some *Bt-cry5* transgenic lines, using either the constitutive promoters CaMV35s or $(ocs)_3$mas (Gelvin super promoter), were moderately effective in reducing larval feeding. Feeding on *Bt-cry5* transgenic lines with the tuber-specific *patatin* promoter was not significantly different than or greater than feeding on the susceptible cultivars. Mortality of first instars was highest when fed on the *Bt-cry3A* lines (68% to 70%) and intermediate (38%) on the *Bt-cry5* 'Spunta' line SPG3 where the *gus* reporter gene was not included in the gene construct. Host plant resistance from foliar leptines is a candidate mechanism to pyramid with either *Bt-cry3A* or *Bt-cry5* expression in potato foliage against Colorado potato beetle. Without multiple sources of host plant resistance, long-term sustainability is questionable for a highly adaptable insect like the Colorado potato beetle.**

The Colorado potato beetle is the most serious insect pest of potatoes throughout the eastern and north central United States. Control of the Colorado potato beetle has relied almost entirely on pesticides for over 125 years (Casagrande, 1987). Throughout its history, the Colorado potato beetle has shown the ability to develop resistance to every insecticide used for its control (Forgash, 1985). Currently, it is reported worldwide to be resistant to 37 insecticides, including organophosphates, carbamates, organochlorines, pyrethroids, and hydrogen cyanide (Georgiou and Lagunes-Tejeda, 1991).

Bacillus thuringiensis (*Bt*) is an aerobic, gram-positive, soil bacterium that accumulates high levels of insecticidal proteins during sporulation (Barton and Miller, 1993; McGaughey and Whalon, 1992). These crystalline protein inclusions, or δ-endotoxins, are the principal active ingredients in *Bt* formulations used as commercial and organic insecticides (McGaughey and Whalon, 1992). The *Bt* toxins target specific orders of insects and have no known detrimental effects on mammals or birds (McGaughey and Whalon, 1992). The insecticidal crystal proteins produced by Bt are the product of a single gene, which has been isolated, analyzed, characterized, cloned, and codon-modified to increase expression in plants (Adang et al., 1993; Perlak et al., 1993). Transgenic plants deliver the *Bt* toxin with increased efficacy, reduced application costs, and minimal scouting needs compared to foliar *Bt* applications (Lambert and Peferoen, 1992). Currently, there are 22 major groups of *Bt* endotoxins, designated *Cry1-22* with 67 holotypes (Crickmore, 2000), offering toxicity toward Lepidoptera, Coleoptera, and Diptera. The *Bt-Cry3A* protein is effective against Coleoptera (Adang et al., 1993; Perlak et al., 1993) and *Bt-Cry5* (*Cry1Ia1*) is active against Lepidoptera and Coleoptera (Tailor et al., 1992).

Glandular trichomes and leptine glycoalkaloids are two natural host plant resistance mechanisms that are candidates for pyramiding with *Bt*-transgenic potato. Glandular trichomes have been bred into cultivated potato from *S. berthaultii* and are available as breeding line NYL235-4 (Plaisted et al., 1992). Small-bodied insects exhibit modified behavior to these trichomes, including host avoidance and restlessness, reduced feeding, delayed development, and diminished longevity (Tingey, 1991).

Glycoalkaloids are the most common form of antibiosis in potato (Sinden et al., 1986). Steroidal glycoalkaloids (solanine and chaconine) are present in all potato tubers and processed products and may cause human health concerns if present at high levels (Sinden, 1987). Glycoalkaloid levels below 20 mg/100 g fresh weight (mg%) are considered safe for human consumption. *Solanum tuberosum* generally contains only 2% to 10 mg% glycoalkaloids. Wild *Solanum* species, such as *S. chacoense* and *S. commersonii*, can have concentrations of 230 and 500 mg% glycoalkaloids, respectively (Sinden, 1987). Leptines are the most active form of glycoalkaloids present in potato for insect control (Lawson et al., 1993) and demonstrate antifeedant properties against Colorado potato beetle at concentrations as low as 100 mg% (Sanford et al., 1996). Leptines and other acetylated glycoalkaloids are reported to be synthesized by only a few accessions of *S. chacoense* (Sanford et al., 1996), including selection USDA8380-1 from *S. chacoense* P.I. 458310. Human health concerns about high glycoalkaloids are not an issue as leptines are synthesized and present only in the leaves and not the tubers (Sanford et al., 1996).

Colorado potato beetle is known to be capable of developing resistance to *Bt* in the lab (Whalon et al., 1993). These natural host plant resistance mechanisms, if combined with engineered *Bt*-transgenic resistance, could increase the effective life of host plant resistance to the Colorado potato beetle. The objectives of this study were to collect baseline data on both the efficacy of several *Bt*-transgenic potato lines and lines with natural resistance mechanisms and to evaluate whether these combinations of engineered and natural resistance mechanisms are more effective than either acting alone.

Materials and Methods

The Colorado potato beetle strain used in this study was collected in 1997 from an insecticide-resistant population at the Montcalm Research Farm, Entrican, Mich. This population, like most populations from commercial potatoes in Michigan, is resistant to most insecticides, including organochlorines, organophosphates, carbamates, and pyrethroids.

Non-transgenic potato clones included: USDA8380-1 (*S. chacoense* P.I. 458310) with high foliar leptines (provided by Dr. L. Sanford, USDA–ARS, Beltsville, Md.), NYL235-4 with glandular trichomes (Plaisted et al., 1992), 'Russet Burbank', 'Lemhi Russet', and 'Spunta'. Two *Bt* genes were tested: *Bt-cry3A* (coleopteran specific) and *Bt-cry5* or *Bt-cry1Ia1* (coleopteran and lepidopteran specific). Potato lines used in this study include *Bt-cry3A*-'Russet Burbank' (RBN15 and RBN20) provided by Dr. J. Kemp at New Mexico State Univ., and *Bt-cry5* transgenic lines of 'Lemhi Russet', 'Spunta', NYL235-4, and USDA8380-1 developed in our lab via *Agrobacterium tumefaciens* transformation as described by Douches et al. (1998) (Table 1). The codon-modified *Bt-cry5* gene construct

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was provided by Zeneca (Tailor et al., 1992). All clones were maintained and micropropagated in tissue culture. For the detached-leaf bioassays, tissue culture plantlets were transplanted to the greenhouse, then grown in a pesticide-free environment for 4–5 weeks until leaves could be sampled.

Effectiveness of host plant resistance in the potato clones to Colorado potato beetle was measured using a laboratory detached-leaf bioassay. The petiole of a single leaf or leaflet was inserted into a 1-dram vial filled with distilled water and sealed with cotton to prevent leakage. Leaves were selected to provide approximately equal surface area among samples being tested. Ten Colorado potato beetle first instars were placed on the leaf using a fine paintbrush. Leaf samples with beetle larvae were placed in a covered petri dish (100 × 15 mm) under continuous fluorescent light (25 μEs^{-1}) at room temperature (22 ± 2 °C). Larval feeding and mortality was read after 72 h. Feeding was estimated visually as the percentage of foliage consumed on 'Russet Burbank'. Larvae demonstrating no visible movement upon removal from the leaf were considered dead. Percent mortality was calculated as number dead larvae/10 × 100%. Because of limited availability of larvae at a single time, not all clones could be tested together. Each assay included three replications of the clones being tested and three replications of 'Russet Burbank' as a control.

Data were analyzed as a completely randomized design using SAS general linear model procedure for one-way analysis of variance (SAS, 1998). Mean comparisons were conducted using pair-wise *t* tests of adjusted least-squares means (LSMEANS). Percent mortality data were arcsine transformed before statistical analysis and re-transformed for presentation of results. Feeding was recorded as percentage of 'Russet Burbank' and sometimes was >100%; therefore, percent defoliation data were not arcsine transformed. Correlation coefficient for feeding (percent defoliation) vs. percent mortality was determined by the Pearson correlation procedure (SAS, 1998).

Table 1. Potato lines used in this study grouped by host plant resistance mechanism type.

Host plant resistance mechanism	Construct[z]	Parent line	Potato line
Bt-cry3A	*CaMV35S/cry3A*	Russet Burbank	RBN15
		Russet Burbank	RBN20
Leptine glycoalkaloids	*None-NT*	USDA8380-1	LT
Leptines + *Bt-cry5*	*CaMV35S/cry5/gus*	USDA8380-1	LT9
		USDA8380-1	LT25
Glandular trichomes	*None-NT*	NYL235-4	GT
Glandular trichomes + *Bt-cry5*	*CaMV35S/cry5/gus*	NYL235-4	GT8
		NYL235-4	GT13
Bt-cry5 (various constructs)	*CaMV35S/cry5/gus*	Lemhi Russet	LR1
		Lemhi Russet	LR14
	CaMV35S/cry5	Spunta	SPG2
		Spunta	SPG3
		Spunta	SPG4
	GSP/cry5/gus	Spunta	SPS1
		Spunta	SPS4
	Patatin/cry5/gus	Spunta	SPP2
		Spunta	SPP6
Susceptible control	*None-NT*	Lemhi Russet	LR
		Spunta	SP
		Russet Burbank	RB

[z]Transgene construct information: *CaMV35S* = 35S cauliflower mosaic virus promoter; *GSP* = Gelvin super promoter; *gus* = *beta-galacturonidase* reporter gene; *None-NT* = non-transgenic potato line.

Table 2. One-way analysis of variance for percent defoliation and mortality of first instar Colorado potato beetles on host plant resistance potato lines in a 72-h no-choice detached-leaf bioassay.

Percent defoliation:					
Source	df	Sum of squares	Mean square	F value	*P* > F
Potato line	19	182293.23	9594.38	18.67	0.0001
Error	86	44185.26	513.78		
Total	105	226478.49			
CV	33.09				
Percent mortality:					
Source	df	Sum of squares	Mean square	F value	*P* > F
Potato line	19	32048.05	1686.74	4.42	0.0001
Error	86	32827.01	381.71		
Total	105	64875.06			
CV	114.29				

Results

An initial test of larvae from the insecticide-resistant beetle strain revealed that feeding after a 72-h feeding period averaged about one-third of the total leaf surface on a susceptible cultivar of 'Russet Burbank' (data not shown). For the 20 potato lines tested, significant differences for feeding were observed (Table 2). Greatest overall reduction in feeding was provided by the two *Bt-cry3A*–'Russet Burbank' lines, RBN15 and RBN20 (<2% of the feeding that occurred on 'Russet Burbank') (Fig. 1). Greater feeding was observed on LT, LT9, LT25, SPG2, and SPG3 but was not significantly different from feeding on the *Bt-cry3A*–'Russet Burbank' lines (≈10% to 22% of 'Russet Burbank'). Other *Bt-cry5*-transgenic lines exhibited a range of feeding from ≈40% to 136% of 'Russet Burbank'. SPS4, LR1, and LR14 were the only lines within this grouping that had significantly less feeding than 'Russet Burbank'. The glandular trichome line (GT) and the two GT+*Bt-cry5* lines (GT8 and GT13) had feeding levels similar to 'Russet Burbank'.

Beetle mortality was significantly different among potato lines ($P < 0.0001$) (Table 2). Among susceptible controls, mortality was <5% for 'Russet Burbank', 'Spunta', and 'Lemhi Russet' and not significantly different from zero (Fig 2). The highest mortality (68% to 70%) was observed in the *Bt-cry3A* lines RBN15 and RBN20. The next highest mortality was found in the *Bt-cry5* 'Spunta' line SPG3 (38%). Mortality should have been 100% in some lines if the assay had continued for longer than 72 h (data not shown). Mortality for larvae fed on the rest of the lines in this study was not significantly different from mortality on the susceptible controls. The correlation between first instar feeding and percent mortality was $r = 0.53$ ($P < 0.0001$).

Discussion

The Colorado potato beetle has shown the ability to adapt to many insecticides over the past century (Casagrande, 1987). If this pest is to be managed in potato production, host plant resistance needs to be used along with other integrated pest management (IPM) practices. The *Bt-cry3A* gene (RBN15 and RBN20) was very effective in controlling Colorado potato beetle first instar feeding and also produced mortality. The leptine-based foliar resistance was also effective in reducing feeding of the Colorado potato beetle, but first instar mortality levels were low (18%) on these lines. The low mortality may be partially attributed to the 72-h bioassay. The first instars, when placed on the leptine leaves, tended to delay feeding. Longer term mortality or delayed development may occur. The short bioassay period combined with this avoidance behavior may have led to the low mortality levels observed in this study.

Our data show that the *Bt-cry5* gene is not as effective as the *Bt-cry3A* gene in controlling Colorado potato beetle feeding. The *Bt-cry5* potato lines chosen for this study were selected based on the high potato tuber moth (*Phthorimaea operculella* Zeller) mortality observed in detached leaf and/or tuber bioassays (Mohammed et al., 2000; Westedt et al., 1998). Unlike the *Bt-cry3A* lines, the *Bt-cry5* 'Spunta' lines varied greatly in their effectiveness in preventing feeding by the first instars

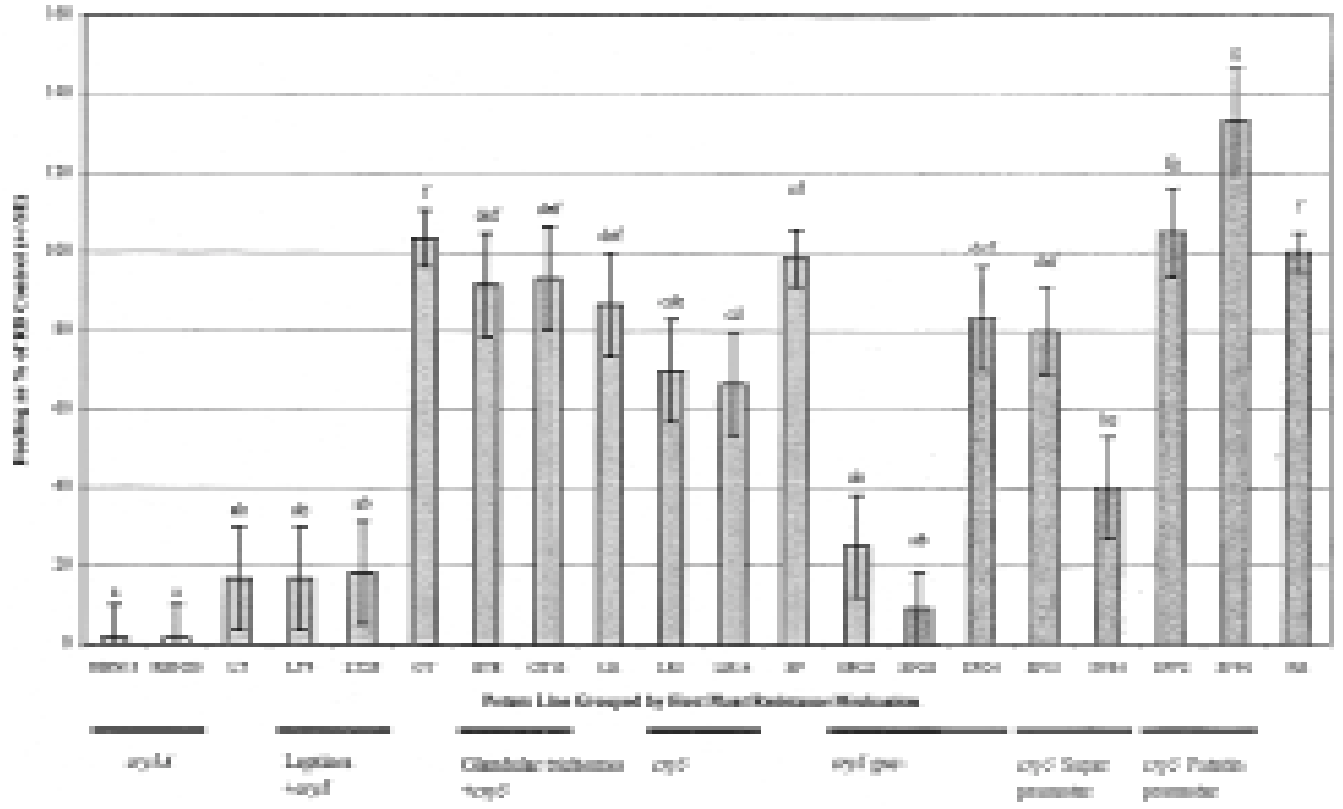

Fig. 1. Feeding of first instar Colorado potato beetles on potato lines with natural, *Bt*-based, and combined host plant resistance mechanisms in no-choice detached-leaf bioassays for 72 h. Feeding is estimated as percentage of the 'Russet Burbank' control. Potato lines are grouped by host plant resistance mechanism. Potato lines with the same letter designation are not significantly different as determined by pair-wise comparisons of least squares means ($\alpha = 0.05$). RB = 'Russet Burbank'; LR = 'Lemhi Russet'; SP = 'Spunta'; GT = L234-5; LT = USDA 8380-1.

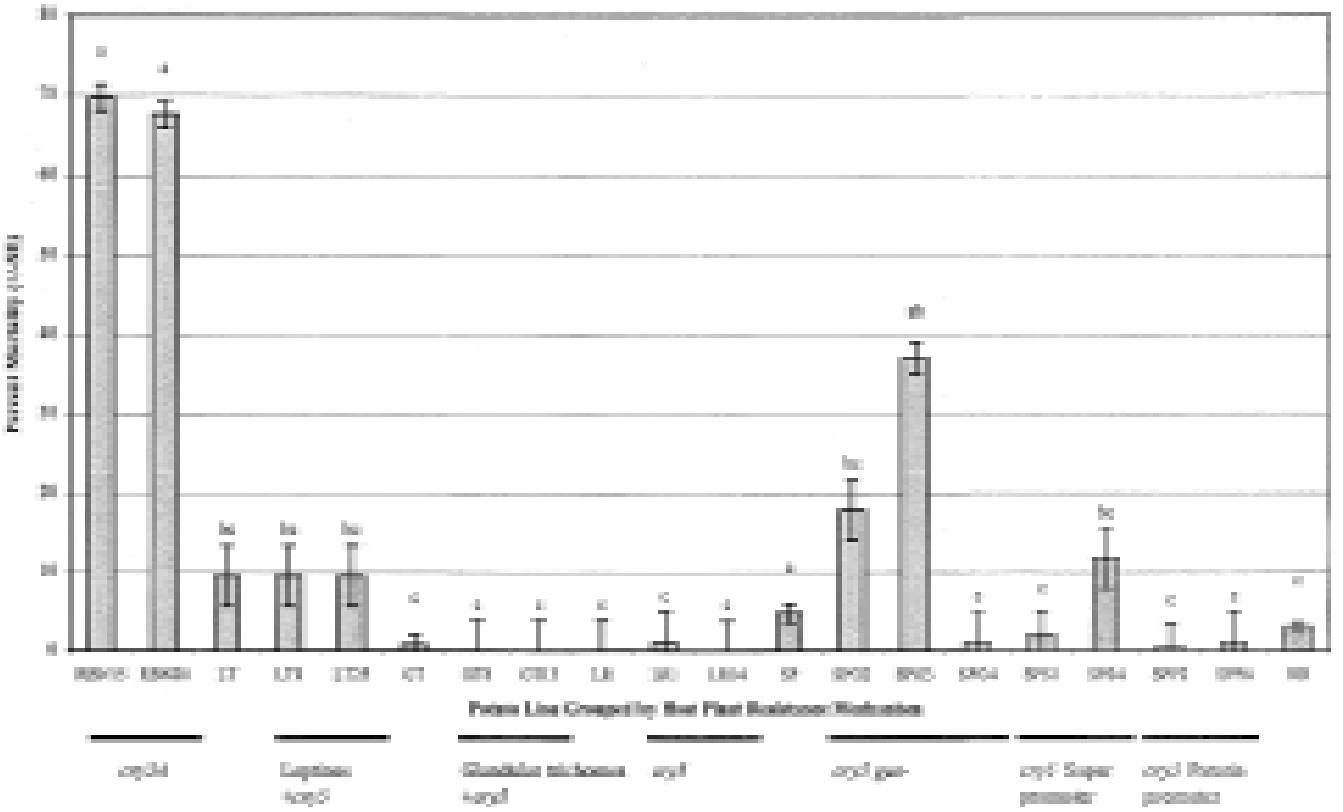

Fig. 2. Percent mortality of first instar Colorado potato beetles on potato lines with natural, *Bt*-based, and combined host plant resistance mechanisms in no-choice detached-leaf bioassays for 72 h. Percent mortality was calculated as number of dead larvae/10 × 100%. Potato lines are grouped by host plant resistance mechanism. Potato lines with the same letter designation are not significantly different as determined by pair-wise comparisons of least-squares means ($\alpha = 0.05$). RB = 'Russet Burbank'; LR = 'Lemhi Russet'; SP = 'Spunta'; GT = L234-5; LT = USDA 8380-1.

(Fig. 1). However, RBN15 and RBN20 were selected as the best of a number of *Bt-cry3A* lines. Similarly, most of the *Bt-cry5* potato lines had lower and more variable beetle mortality than the *Bt-cry3A* potato lines, but feeding and mortality on SPG3 were not significantly different from feeding on RBN15 and RBN20 (Fig. 2). For SPP2 and SPP6, the *Bt-cry5* gene expression was targeted to the tuber, and we did not expect any foliar beetle control. In other lines, such as GT8, GT13, LR1, LR14, and SPS1, the lack of control is attributed to the lower *Cry5* protein levels in the leaf from the fusion of the *gus* gene with the *Bt-cry5* gene (*CaMV35S/Bt-cry5/gus*) (Mohammed et al., 2000; Westedt et al., 1998). SPG3, SPG2, and SPG4 differed from our other *Bt-cry5* gene constructs in that the *gus* gene was not fused with the *Bt-cry5* gene in the construct. Our *Bt-cry5* gene constructs (*CaMV35S/Bt-cry5*) without the *gus* gene often cause higher mortality to the potato tuber moth (Mohammed et al., 2000). Position effects of the integrated transgene may have also contributed to the variability observed between the *Bt-cry5* lines. In locations where both potato tuber moth and Colorado potato beetle are insect pests (i.e., Mediterranean region), a *Bt-cry3A* line such as SPG3 may be desirable.

The glandular trichome line NYL235-4, previously noted for its effectiveness against the Colorado potato beetle in the field (Plaisted et al., 1992), was not significantly different from 'Russet Burbank' in our tests. Neither of the GT+*Bt-cry5* lines (GT8 nor GT13) reduced feeding in this no-choice detached-leaf study. Glandular trichomes have demonstrated resistance to a wide range of insect pests of potato (Tingey, 1991). This natural host plant resistance, if combined with *Bt-cry3A* or a highly active *Bt-cry5* for potato beetle control, would still be of value in commercial production because of its control of other small-bodied insects (e.g., aphids and potato leaf hoppers), thus providing broader insect control.

While *Bt*-transgenic plants offer a safe and effective means of insect control, there is concern that the intense selection pressure applied through their widespread use will lead to resistant insect populations and the loss of this valuable tool of IPM (Whalon and Ferro 1998). Currently, *Bt-cry3A* cultivars provide excellent control of the beetle (Perlak et al., 1993); however, the Colorado potato beetle has shown the ability to adapt to *Bt-cry3A* in the lab (Whalon et al., 1993). At present, no beetle populations in the field have shown any adaptation to *Bt-cry3A*, but the potential exists. Resistance management plans for the prevention of evolution of resistant insect populations assume that resistance alleles are rare (gene frequencies $<10^{-6}$), effectively recessive, and that there is a fitness cost associated with resistance (Gould, 1998). However, recent genetic studies of insect populations estimate *Bt*-resistance alleles ranging in frequency from 0.0015 (Gould et al., 1997) to 21% (Tabashnik et al., 1997). For Colorado potato beetle, initial gene frequency for resistance to the insecticide carbofuran was 2×10^{-5} (Ioannidis et al., 1992). Even with this relatively low initial gene frequency, carbofuran resistance increases rapidly with selection because of the intensity of selection (99.9% from a single carbofuran application) and the single dominant gene nature of the carbofuran resistance. Inheritance of *Bt-cry3A* resistance in a Colorado potato beetle laboratory strain was via multiple semidominant genes (Rahardja and Whalon, 1995). These factors suggest that *Bt* resistance in Colorado potato beetle may not meet the resistance management assumptions. Therefore, multiple sources of host plant resistance may be needed.

In this study, we have shown that *Bt-cry3A, Bt-cry5*, foliar leptines, and the combination of *BT-cry5* and foliar leptines are effective in reducing feeding and both *Bt* genes cause significant mortality. Foliar leptines may also cause mortality in longer-term studies. If we pyramid these engineered and natural host plant resistance mechanisms in a potato cultivar or deploy them spatially or temporarily, we may be able to extend the usefulness of these individual host plant resistance mechanisms and provide long-term sustainable management of this highly adaptable pest.

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