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

D.S. Douches1, T.J. Kisha1, J.J. Coombs1, W. Li1, W.L. Pett1, and E.J. Grafius2

Michigan State University, East Lansing, MI 48824

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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)_mas (Velgin 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 pyramidal 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 (Forgan, 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; McGaughy and Whalon, 1992). These crystalline protein inclusions, or δ-endotoxins, are the principal active ingredients in Bt formulations used as commercial and organic insecticides (McGaughy and Whalon, 1992). The Bt toxins target specific orders of insects and have no known detrimental effects on mammals or birds (McGaughy 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 Peerooen, 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 lep(r)ine 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 (Piaisted et al., 1992). Small-bodied insects exhibit modified behavior to these trichomes, including host avoidance and restless, reduced feeding, delayed development, and diminished longevity (Tingey, 1991).

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1Dept. of Crop and Soil Sciences.
2Dept. of Entomology.

Materials and Methods

The Colorado potato beetle strain used in this study was collected in 1997 from an insecticide-resistant population at the Montcalf Research Farm, Entrick, 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 (Piaisted 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’ (RB15 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

Glycoalkaloids are the most common form of antibiotic 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.

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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 µE·m⁻²·s⁻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).

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, SP5, 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’.

Bt-cry5 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. Thelepine-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 leafy plants, 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 $\times$ 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.
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, L1R1, L1R4, 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 NYL1235-4, 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 GTx-Bt-cry5 lines (GT13 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 <10⁻⁵), 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 × 10⁻⁴ (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 name 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 lepites, and the combination of Bt-cry5 and foliar lepites are effective in reducing feeding and both Bt genes cause significant mortality. Foliar lepites 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 temporally, 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.

### Literature Cited


