Insecticidal Activity of Avidin Combined with Genetically Engineered and Traditional Host Plant Resistance Against Colorado Potato Beetle (Coleoptera: Chrysomelidae) Larvae

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SUSANNAH G. COOPER,[1] DAVID S. DOUCHES,[1] AND EDWARD J. GRAFIUS[2]

ABSTRACT   Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is a destructive pest of potato, *Solanum tuberosum* (L.), in North America. It is renowned for adapting to insecticides. With the arsenal of effective insecticides decreasing, it is important to consider alternative forms of control. Biotin is an essential coenzyme for insect growth and development. Avidin is a protein found in chicken egg that sequesters biotin and has shown insecticidal properties against a range of insect. We assessed the effectiveness of avidin against the Colorado potato beetle neonates in a no-choice detached leaf bioassay at 0, 17, 34, 51, 102, and 204 μg avidin/ml over 12 d. The LC_{50} was 136 μg avidin/ml (108–188 95% CL). The combined effects of avidin (136 μg avidin/ml) with Bt-Cry3A or leptines were evaluated with neonates and third instars over 12 and 6 d, respectively. Three potato lines were used: susceptible line, a line engineered to express Cry3A from *Bacillus thuringiensis*, and a line expressing the natural resistance factor leptines. The addition of avidin at the LC_{50} concentration significantly reduced consumption by neonates, but it did not affect consumption by third instars feeding on the susceptible line and the leptine line. Survival of neonates feeding on the susceptible line with avidin was significantly reduced compared with the susceptible line. Survival of third instars on the Bt-Cry3A with avidin was significantly reduced after 3 d compared with survival on the Bt-Cry3A, suggesting the addition of avidin may increase susceptibility to Bt-Cry3A.

KEY WORDS   avidin, Bt-Cry3A, host plant resistance, leptine, *S. chacoense*

Biotin, also called vitamin H or B_8, is an essential vitamin for all organisms. It is a cofactor that covalently binds to several carboxylases that serve in many important biosynthetic pathways such as the citric acid cycle, lipogenesis, gluconeogensis, and fatty acid and amino acid catabolism (Knowles 1989, Alban et al. 2000). Although biotin is a requirement for all life, biotin synthesis is restricted to plants, many bacteria, and a number of fungi (Alban et al. 2000). Animals, along with many fungi and bacteria, must acquire biotin from outside sources such as diet or environment.

Biotin binding proteins have a strong affinity for biotin, with the strongest noncovalent bond found in nature ($K_d = 10^{-15}$ M) (Izrailev et al. 1997). One of the most well-known biotin binding proteins is avidin from chicken, *Gallus gallus* L. (Green 1990, Stevens 1991). Avidin is produced in egg whites. It is a glycoprotein tetramer (66 kDa) comprised of four nearly identical subunits $\approx$17 kDa. Each subunit of avidin tightly binds to a single molecule of biotin. Avidin protects the chicken embryo from disease-causing organisms by sequestering the essential biotin. Without accessible biotin, harmful microorganisms cannot perform essential processes needed for growth and survival (Stevens 1991).

The insecticidal activities of avidin were first discovered in 1959 when it was added to the artificial diet of the housefly, *Musca domestica* L. (Levinson and Bergmann 1959). A molar excess of avidin in an insect diet causes a deficiency in accessible biotin, resulting in abnormal larval development and even death in a range of insect orders (Morgan et al. 1993, Malone et al. 2002, Markwick et al. 2003). Avidin is an excellent candidate for plant transformation because it is a single gene product with insecticidal activity. The gene coding for avidin production has been cloned and has been inserted into a few crops, including maize, tobacco, and potato, providing resistance to a wide spectrum of insect pests (Kramer et al. 2000, Markwick et al. 2001, Burgess et al. 2002). Avidin is safe for consumers because cooking denatures it.

Colorado potato beetle, *Leptinotarsa decemlineata* (Say), is among the most economically significant pests of potatoes, *Solanum tuberosum* spp. *tuberosum* L., in North America, Europe, and western Asia. As little as 12.5% defoliation significantly reduces potato yields; complete defoliation can lead to crop failure (Hare 1980, Mailloux and Bostanian 1989). Colorado

potato beetle is renowned for development of insecticide resistance with resistance reported to >40 insecticides (Whalon et al. 2005). Therefore, examining novel control strategies may be of great consequence for the management of the Colorado potato beetle.

Compared with many other crops, *Solanum* has immense potential genetic diversity for host plant resistance. Many wild *Solanum* species, including *Solanum berthaultii* Hawkes, *Solanum chacoense* Bitter, *Solanum polyadenium* subsp. *orizabae* Bitter, and *Solanum tarijense* Hawkes, are thought to have genetic traits causing insect resistance (Pelletier et al. 1999). Glycoalkaloids have long been associated with resistance to insects and plant pathogens (Maga 1994). *S. chacoense* produces compounds called leptine glycoalkaloids that confer resistance to Colorado potato beetle resistance (Sinden et al. 1986, Lorenzen et al. 2001). Most glycoalkaloids are distributed throughout the plant, including the tuber. However, high levels of glycoalkaloids in the tuber impart a bitter taste and also may be toxic to humans (Van Gelder 1990). Leptine glycoalkaloids are only expressed in the foliage, conferring insect resistance and also alleviating human health concerns associated with high levels in the tuber (Sinden et al. 1986).

In addition to insect resistance through traditional breeding, potato is also amenable to genetic engineering. Cry toxin genes have been inserted into potato to impart resistance to several insects (Adang et al. 1993, Perlak et al. 1993, Douches et al. 1998, Coombs et al. 2002). Cry toxins are a class of insecticidal proteins from the soil-borne bacterium *Bacillus thuringiensis* Berliner (Bt) (Sharma et al. 2000). Cry proteins are highly specific in activity. Specificity is often limited to individual insect orders and frequently only a few species within an order are affected. The Bt-Cry3A toxin is active against Colorado potato beetle larvae (Adang et al. 1993, Perlak et al. 1993, Coombs et al. 2002).

The objectives of this study were to 1) assess the potential for using avidin in potato for control of the Colorado potato beetle and 2) examine the combined effects of avidin with the natural host plant resistance, leptines, or the engineered resistance, Bt-Cry3A.

Materials and Methods

Determination of LC_{50}. Colorado potato beetle egg masses were obtained from the New Jersey Department of Agriculture's Phillip Alampi Beneficial Insect Rearing Laboratory, West Trenton, NJ. This strain was originally collected in 1983 from potato and eggplant fields in New Jersey and has been continuously reared without exposure to insecticides. Potatoes ('Yukon Gold') were grown under greenhouse conditions. Fully expanded leaves were collected, and then each petiole was immersed in a water-filled vial sealed with Parafilm. Aqueous solutions of avidin (0, 17, 34, 51, 102, and 204 μg avidin/ml) (Sigma, St. Louis MO) were prepared using distilled water and 0.01% Tween 20 (Sigma). Leaves were dipped and air-dried and then individually placed in petri dishes (125 mm in diam-

eter) lined with filter paper. Ten neonates were placed on each leaf for 12 d in a no-choice test. Leaves were replaced with fresh leaves dipped in the same avidin solution as needed. Mortality was assessed every 4 d. Larvae were considered dead if no movement was observed after being lightly touched with a paintbrush. This procedure was replicated four times (40 larvae per avidin concentration). Percentage of mortality was adjusted with Abbott's formula to correct for mortality on untreated foliage (Abbott 1925). The avidin concentrations were log transformed and analyzed with Probit analysis (PROC PROBIT, SAS Institute 2002). The 50% lethal concentration (LC_{50}) along with 95% fiducial limits (FL) was obtained for avidin.

An identical experiment was conducted (same avidin concentrations and methods) with biotin added to counteract the effects of avidin. Aqueous solutions of avidin (0, 17, 34, 51, 102, and 204 μg avidin/ml) (Sigma) with biotin (0, 0.98, 1.96, 2.94, 5.88, and 11.76 μg biotin/ml) (Sigma) were prepared using distilled water and 0.01% Tween 20 (Sigma). Leaves were dipped and air-dried and then individually placed in petri dishes (125 mm in diameter) lined with filter paper. Percentage of mortality was transformed with the arcsine of the square root to homogenize variance. Data were analyzed design using SAS general linear model procedure for analysis of variance (ANOVA) (SAS Institute 2002). Mean comparisons were conducted using Fisher's least significant difference (LSD) test ($\alpha = 0.05$).

Combined Effects of Avidin. The LC_{50} concentration of avidin was used to determine the combined effects of avidin on a natural and engineered resistant host plants. Three potato clones—Yukon Gold, USDA8380-1 (leptine line), and YGc3.12 (Yukon Gold with Bt-Cry3A)—were evaluated in this study. USDA8380-1 was derived from the wild potato *S. chacoense*, which expresses leptines as a natural host plant resistance factor in the foliar tissue of the plant (Lorenzen et al. 2001). The codon-modified-*cry3A* (coleopteran specific) gene used for transformation of Yukon Gold in this study was obtained from John Kemp (New Mexico State University, Las Cruces, NM) (Sutton et al. 1992). The constitutive ocs_3mas promoter (Ni et al. 1995) was used to promote expression of the *Bt-cry3A* gene. The *Bt-cry3A* transgenic potato line was generated using *Agrobacterium tumefaciens*-mediated transformation (Coombs et al. 2002).

The three potato lines were grown under greenhouse conditions. Leaves were collected, petioles were placed in vials of water, as described above, and dipped into: 0.01% Tween 20 (wt:vol) (Sigma), or 136 μg/ml avidin (Sigma) in 0.01% Tween 20. Leaves were air-dried and then individually placed in petri dishes (125 mm in diameter) lined with filter paper. Neonates (10 per leaf) were placed on the detached leaves in a no-choice test. If leaf quality had degraded significantly or a large area of leaf was consumed, the leaf was replaced. Consumption was visually estimated with square millimeter grid paper and recorded for each group of larvae (Coombs et al. 2002). Consump-
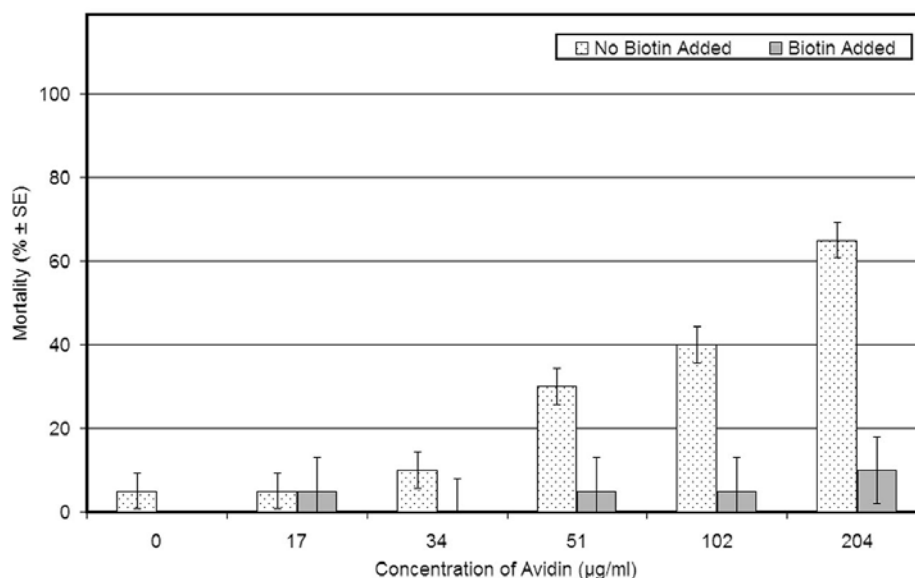
Fig. 1.   Mean percentage of dead Colorado potato beetle neonates fed on Yukon Gold dipped in 0, 17, 34, 51, 102, and 204 μg avidin/ml alone or combined with biotin at 4 times the molar concentration of avidin (0, 1.0, 2.0, 2.9, 5.9, and 11.8 μg biotin/ml). The LC_{50} was determined to be 136 μg avidin/ml (108–188 95% CL). There was no significant difference between avidin combined with biotin at any concentration compared with at 0 μg of avidin/ml ($LSD_{\alpha = 0.05} = 18.6\%$).

tion, survival, and biomass of the survivors were measured every 2 d for 12 d. Six replications were performed (60 individuals per potato line × avidin treatment). Percentage of survival was transformed with the arcsine of the square root to homogenize variance. The data sets (consumption, final survivor biomass, arcsine and square root mortality) were analyzed using SAS least-squared means model procedure for a two-factorial design ANOVA, with the factors of potato line and avidin treatment, used to analyze consumption. The means were separated using a pairwise comparison (SAS Institute 2002).

Previous studies have suggested that neonate larvae may be so sensitive to individual resistance factors that combined effects may not be evident; effects of combined resistance strategies may be apparent at the third or fourth instar (Cooper et al. 2004). Therefore, leaf dip bioassays also were performed on third instars to further differentiate resistance strategies. Leaf dip assays were performed similarly to the neonate assays described above. Egg masses were obtained from New Jersey Department of Agriculture and reared in the laboratory on Yukon Gold until the third instar. Five newly molted third instars (within 48 h of molting) were placed on dipped leaves. Leaf tissue was replaced daily. Consumption, survivorship, and biomass of survivors were measured daily for 6 d. Twelve replications were performed (60 individuals per potato line × avidin treatment). The data were analyzed as in the neonate assay (SAS Institute 2002).

Results and Discussion

Determination of LC_{50}. Avidin was toxic to Colorado potato beetle larvae. Colorado potato beetle exhibited a dose–response to avidin in the LC_{50} assay

(Fig. 1). At concentrations higher than 102 μg/ml, larvae did not develop past the third instar. Larvae consuming leaves dipped in concentrations of 51 μg/ml or higher of avidin had significantly higher mortality at 12 d than larvae consuming leaves dipped in 0 μg/ml ($F = 18.26$, df = 5, $P < 0.0001$) (Fig. 1). The LC_{50} values was determined to be 136 μg avidin/ml ($n = 40$, slope = 2.3 ± 0.3, 136 μg avidin/ml (108–188 95% CL), $Pr < \chi^2 = 4.3$) at 12 d. The addition of biotin to the solutions counteracted the negative effects of avidin (Fig. 1). There was no significant difference between mortality at any concentration and mortality at 0 μg avidin/ml + 0 μg biotin/ml ($F = 0.45$, df = 5, $P = 0.8078$). Fisher's LSD value was determined to be 18% mortality, and the highest mean percentage of mortality (10%) was observed for larvae feeding on leaves dipped in 204 μg avidin/ml + 11.76 μg biotin/ml.

The LC_{50} value for avidin and Colorado potato beetle neonates was higher than that previously observed with potato tuberworm, *Phthorimaea operculella* (Zeller) (LC_{50} of 2.3 μg/ml at 9 d); light brown apple moth, *Epiphyas postvittana* (Walker) (LC_{50} of 43.4 μg/ml at 21 d); and *Ctenopseustis obliquana* (Walker) (LC_{50} of 45.7 μg/ml at 21 d) (Markwick et al. 2001). The reported expression levels of transgenic tobacco, *Nicotiana tabacum* L., and transgenic apple, *Malus domestica* Borkh, range from 0 to 24.5 μM (0–416.5 μg/ml) and 1.9–11.2 μM (32.3–190.4 μg/ml), respectively (Murray et al. 2002, Markwick et al. 2003). Although the concentration of the dip solution is comparable with avidin expression in transgenic plants, the actual dose in the dip assay is much lower. The dip was a topical application, whereas the transgenic plants express avidin in each cell of the plant. If transgenic potato plants have comparable expression
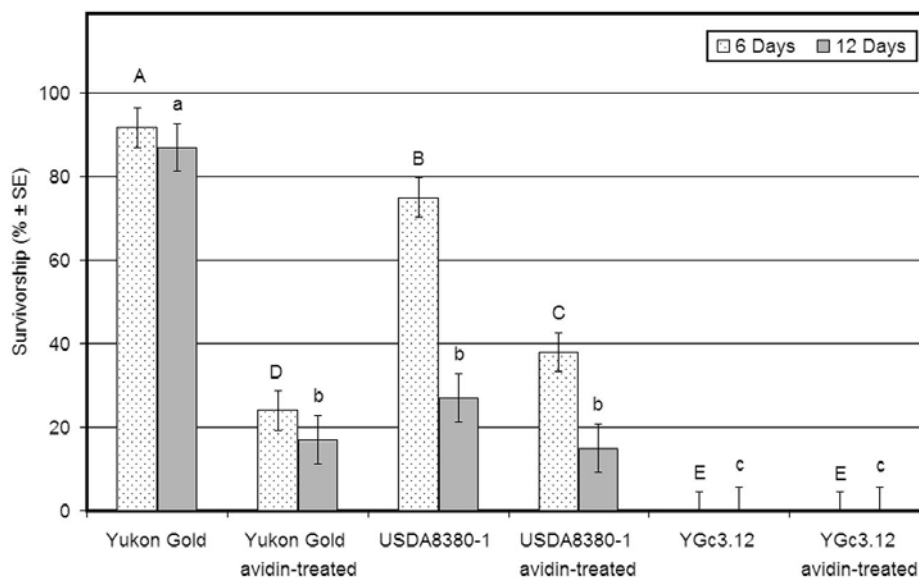
Fig. 2. Mean percentage of Colorado potato beetle neonate survivors fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 6 and 12 d in a no-choice detached leaf bioassay. Means followed by different letters within a date are significantly different at 0.05 level based on analysis of arcsine square-root transformed data; means were separated using a pairwise comparison.

levels to that of transgenic tobacco or apple, mortality of Colorado potato beetle would likely be higher than observed in the leaf dip assay because of higher avidin exposure and continuous expression.

Combined Effects of Avidin. *Neonate Assay.* Both potato line ($F_{6\ d}$ = 77.23, df = 2, P < 0.0001; and $F_{12\ d}$ = 30.80, df = 2, P < 0.0001) and avidin treatment ($F_{6\ d}$ = 45.49, df = 1, P < 0.0001; and $F_{12\ d}$ = 18.64, df = 1, P = 0.0002) significantly affected survival at 6 and 12 d. There was a significant interaction between potato line and avidin treatment on larval survival at 6 d (F = 17.17, df = 2, P < 0.0001) and 12 d (F = 14.15, df = 2, P < 0.0001). Larvae consuming avidin-treated Yukon Gold had significantly lower survival compared with larvae feeding on untreated Yukon Gold at 6 and 12 d, suggesting avidin is detrimental to the survival of neonates (Fig. 2). Colorado potato beetle neonates are more sensitive to toxins than later instars because they lack the nutritional and metabolic resources to cope with toxins, and they also are receiving a higher dose per larval mass (Wierenga et al. 1996, Hilton et al. 1998). Consumption rates (milligrams of food per milligram of body weight per day) are highest for neonates. Young larvae also have limited detoxification ability compared with larger larvae or adults contributing to their sensitivity of neonates to toxins (Zhao et al. 2000). Survival of larvae feeding on avidin-treated USDA8380-1 was significantly reduced compared with untreated USDA8380-1 at 6 d. Survival did not significantly differ for larvae feeding on avidin-treated USDA8380-1 compared with untreated USDA8380-1 at 12 d. USD8380-1 produces leptines, which are strong feeding deterrents for Colorado potato beetle (Tingey 1984). The larvae consuming avidin-treated USDA8380-1 may have not received a large enough dose to have detrimental effects on sur-

vival. Survival of larvae did not significantly differ between larvae feeding on avidin-treated Yukon Gold, avidin-treated USDA8380-1, or untreated USDA8380-1 at 12 d, suggesting comparable susceptibility to avidin and leptines in early instars. Survival did not significantly differ between larvae feeding on avidin-treated YGc3.12 compared with larvae feeding on untreated Ygc3.12 at 6 and 12 d. Regardless of the addition of avidin, nearly a 100% of larvae consuming YGc3.12 were dead by 4 d. This was expected because of the strong effect of Bt-Cry3A (Cooper et al. 2004)

Both potato line ($F_{6\ d}$ = 56.49, df = 2, P < 0.0001; and $F_{12\ d}$ = 61.98, df = 2, P < 0.0001) and avidin treatment ($F_{6\ d}$ = 61.87, df = 1, P < 0.0001; and $F_{12\ d}$ = 54.17, df = 1, P < 0.0001) had a significant effect on the amount of feeding at 6 and 12 d. There was a significant interaction between the effects of potato line and avidin treatment on consumption at 6 d (F = 23.70, df = 2, P < 0.0001) and 12 d (F = 25.72, df = 2, P < 0.0001), suggesting the addition of avidin to a host plant resistance factor may decrease larval feeding. Consumption was significantly less on avidin-treated Yukon Gold than on untreated Yukon Gold at 6 and 12 d (Fig. 3). Avidin is antinutritional; it retards the development of larvae, eventually leading to death (Levinson et al. 1992). Smaller larvae consume less foliage than large larvae. The retarded growth of larvae feeding on avidin-treated Yukon Gold likely accounts for the reduced consumption rather than the avidin possessing deterrent properties. The health of larvae feeding on Yukon Gold treated with avidin was severely compromised. The larvae feeding on avidin were often slower than larvae of a similar size.

Consumption was significantly reduced on untreated USDA8380-1 compared with untreated Yukon Gold, likely because of the deterrent properties asso-
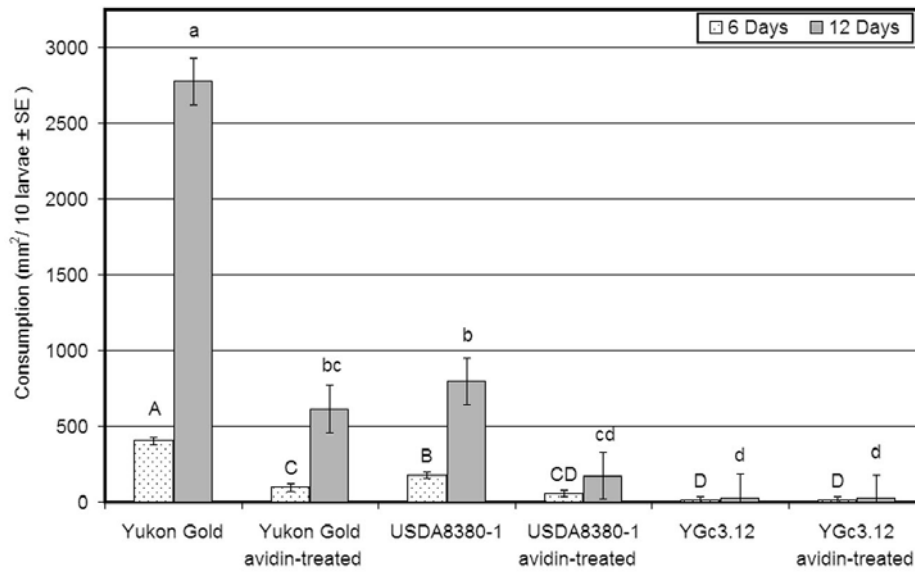
Fig. 3. Mean consumption by Colorado potato beetle neonates on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 6 and 12 d in a no-choice detached leaf bioassay. Means followed by different letters within a date are significantly different at 0.05 level; means were separated using a pairwise comparison.

ciated with leptines (Fig. 3) (Tingey 1984). Consumption significantly decreased for larvae feeding on avidin-treated USDA8380-1 compared the USDA8380-1 in at 6 and 12 d, but the biomass did not significantly differ (Fig. 3 and 4). The lower consumption is likely the result of fewer surviving larvae eating the avidin-treated USDA8380-1 compared with untreated USDA8380-1 (Fig. 2). Larvae feeding on avidin-treated USDA8380-1 seemed much weaker and had slower movement than larvae feeding on USDA8380-1. Feeding on untreated USDA8380-1 did not significantly differ from avidin-treated Yukon

Gold at 12 d, but the combined resistance of the avidin-treated USDA8380-1 did have significantly less consumption than untreated USDA8380-1 at 12 d.

Potato line significantly affected the biomass of survivors at 6 d ($F = 16.33$, df = 2, $P < 0.0001$) and 12 d ($F = 16.94$, df = 2, $P < 0.0001$), but the addition of avidin did not significantly affect biomass at 6 d ($F = 0.07$, df = 1, $P = 0.7999$) or 12 d ($F = 3.39$, df = 2, $P < 0.0755$) (Fig. 4). Biomass of survivors was significantly reduced for larvae fed on avidin-treated Yukon Gold compared with larvae fed on untreated Yukon Gold at 12 d, demonstrating the negative effect of avidin on
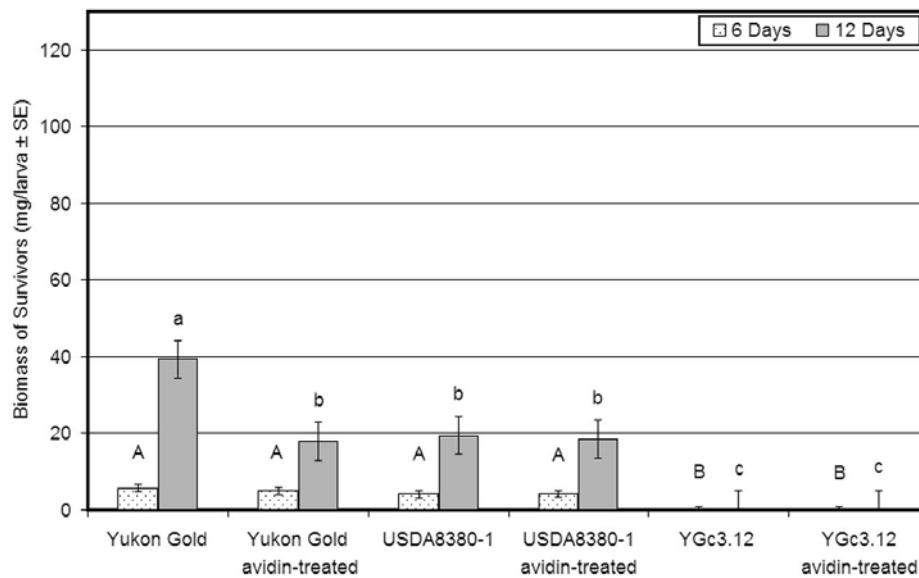

Fig. 4. Mean biomass of surviving Colorado potato beetle neonates fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 6 and 12 d in a no-choice detached leaf bioassay. Means followed by different letters within a date are significantly different at 0.05 level; means were separated using a pairwise comparison.
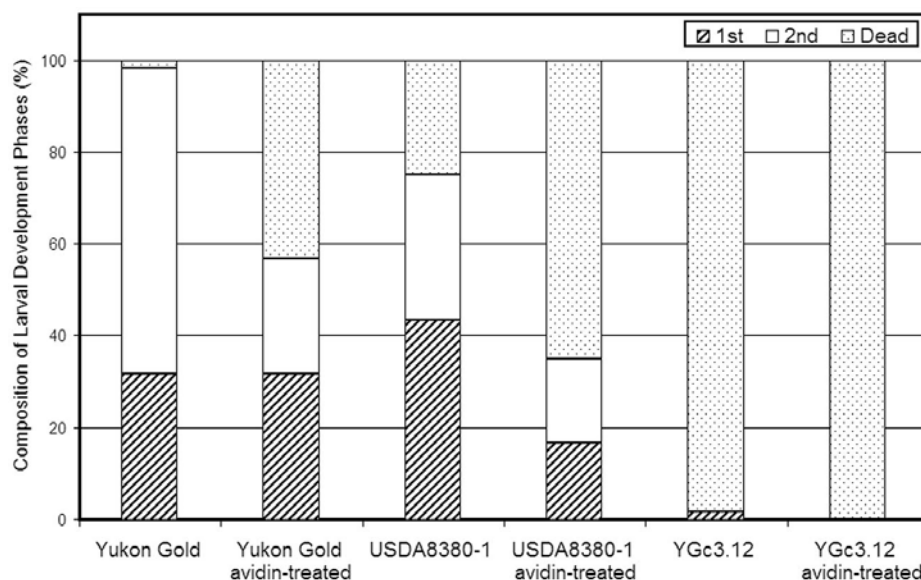
Fig. 5. Developmental stages of Colorado potato beetle neonates fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 6 d in a no-choice detached leaf bioassay.

larval growth. Biomass did not significantly differ between the untreated and avidin-treated USDA8380-1; perhaps neonates did not receive a sufficient dose to retard growth (Fig. 4). USDA8380-1 produces leptines that deters Colorado potato beetle feeding (Tingey 1984). At 12 d, larvae fed on the untreated Yukon Gold consumed almost 3 times as much as larvae fed on the untreated USDA8380-1. The leaves were dipped in avidin solutions; therefore, larvae consuming more tissue ingested more avidin. Larvae feeding on avidin-treated USDA8380-1 likely consumed far less leaf tissue and less avidin than larvae feeding on avidin-treated Yukon Gold. Because of the rapid mortality on YGc3.12, biomass data were only collected at 2 d.

Larvae fed on Ygc3.12 died within 4 d and did not develop past first instar (Fig. 5).

Although the biomass of survivors did not significantly differ for larvae feeding on avidin-treated USDA8380-1 compared with larvae fed on untreated USDA8380-1, the addition of avidin did retard larval development. At 8 d, >40% of the surviving larvae that fed on untreated USDA8380-1 were third instars, whereas <6% of surviving larvae that fed on avidin-treated USDA8380-1 treated were third instars. No larvae survived to second instar feeding on Ygc3.12 regardless of avidin treatment (Figs. 5 and 6).

Colorado potato beetles spend ≈12–17 d as larvae, with ≈7–11 d to reach fourth instar (Walgenbach and
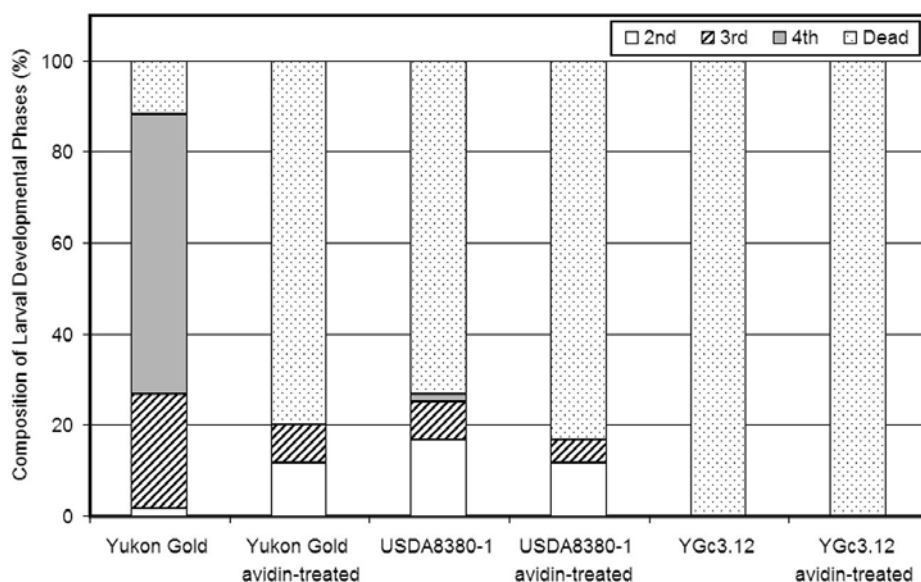

Fig. 6. Developmental stages of Colorado potato beetle neonates fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 12 d in a no-choice detached leaf bioassay.
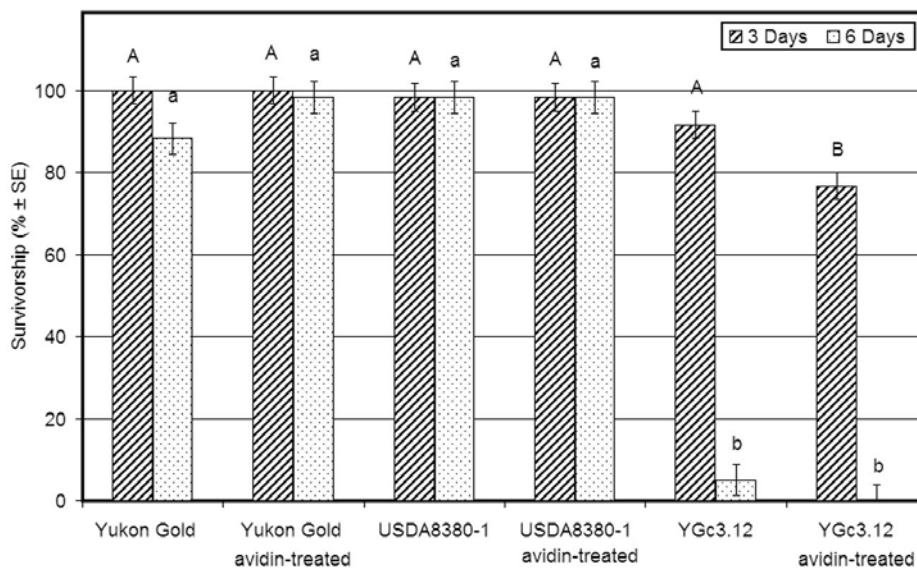
Fig. 7. Mean percentage of Colorado potato beetle third instar survivors fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 3 and 6 d in a no-choice detached leaf bioassay. Means followed by different letters within a date are significantly different at 0.05 level based on analysis of arcsine square-root transformed data; means were separated using a pair wise comparison.

Wyman 1984). In our 12-d neonate assay, no larvae feeding on avidin-treated leaves progressed to fourth instar, whereas 61.7% of larvae feeding on untreated Yukon Gold progressed to the fourth instar (Fig. 6). The addition of avidin to artificial diets delays insect and mite growth and also compromises reproduction of mites (Levinson et al. 1992, Markwick et al. 2001). With transgenic plants expressing high levels of avidin, Colorado potato beetle larvae may not be able to develop to fourth instar, survive pupation, or efficiently reproduce. Further studies need to be performed to more closely examine effects of avidin on rate of development of neonates to adulthood and on the fecundity of surviving adults.

Third Instar Assay. Potato line significantly affected survival at 3 d ($F = 14.24$, df $= 2$, $P < 0.0001$) and 6 d ($F = 314.33$, df $= 2$, $P < 0.0001$), but the avidin treatment did not significantly affect larval survival at 3 d ($F = 3.44$, df $= 1$, $P = 0.0681$) and 6 d ($F = 0.11$, df $= 1$, $P = 0.7413$). There were significant interactions between effects of potato line and avidin treatment on third instar survival at 3 d ($F = 3.44$, df $= 2$, $P < 0.0379$) but not at 6 d ($F = 2.36$, df $= 2$, $P < 0.1019$). Survival of third instars was not significantly affected by the avidin treatment on Yukon Gold or USDA8380-1 at 3 or 6 d (Fig. 7). The avidin LC$_{50}$ was determined with neonates, which are typically much more susceptible to toxins than later stages such as third instars. Also, the dose/larval mass would be less for the third instars compared with neonates consume at a higher rate (milligrams of food per milligram of body size) than larger larvae.

Third instars fed on avidin-treated YGc3.12 had significantly lower survival compared with third instars fed on untreated Ygc3.12 after 3 d. However, nearly 100% of larvae consuming Ygc3.12 were dead by

6 d regardless of the avidin treatment because of the high toxicity of Bt-Cry3A to Colorado potato beetle larvae (Fig. 7) (Perlak et al. 1993). When combining resistance factors with Bt-Cry3A, the added effects are often masked, especially in early instars (Cooper et al. 2004). Avidin did not seem to reduce survivorship of larvae on Bt-Cry3A in the neonate assay. The addition of avidin may increase the susceptibility of larger Colorado potato beetle larvae to the Bt-Cry3A toxin. *Helicoverpa armigera* (Hübner) larvae had a significantly higher mortality when fed on a transgenic avidin plant painted with Bt-Cry1Ba compared with the transgenic avidin plant or Bt-Cry1Ba-painted plant alone (Burgess et al. 2002). Therefore, insects feeding on plants expressing high levels of a Bt toxin and avidin may have a higher mortality than insects feeding on a plant expressing either resistance factor alone.

Both potato line ($F_{3\,d} = 90.11$, df $= 2$, $P < 0.0001$; and $F_{6\,d} = 399.71$, df $= 2$, $P < 0.0001$) and avidin treatment ($F_{3\,d} = 12.61$, df $= 1$, $P < 0.0001$; and $F_{6\,d} = 4.97$, df $= 1$, $P = 0.0292$) significantly affected the area consumed by third instars at 3 and 6 d. There was a significant interaction between potato line and avidin treatment on consumption by third instars at 3 d ($F = 4.6$, df $= 2$, $P = 0.0135$) but not at 6 d ($F = 0.99$, df $= 2$, $P = 0.3777$). Larvae consumed significantly less avidin-treated Yukon Gold than untreated Yukon Gold at 3 d (Fig. 8). After 6 d, consumption did not significantly differ between untreated Yukon Gold and avidin-treated Yukon Gold. A higher dose of avidin may increase the length of development of third instars. Larvae consumed significantly less of untreated and avidin-treated USDA8380-1 than untreated Yukon Gold; consumption did not significantly differ between untreated and avidin-treated USD8380-1 at 3 or 6 d. Larvae consumed significantly
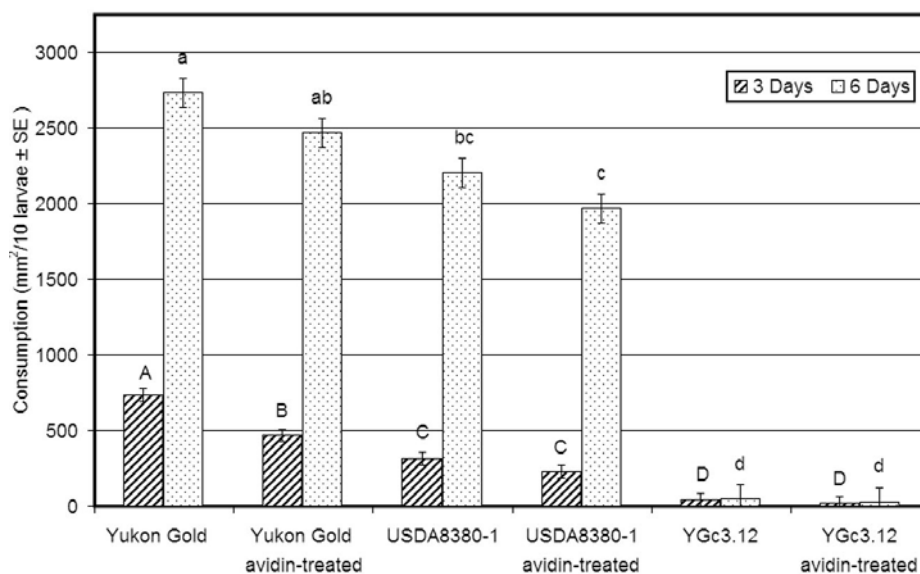
Fig. 8. Mean consumption of Colorado potato beetle third instars on three potato lines (Yukon Gold, USDA8380–1, and YGc3.18) dipped in either 0 μg avidin/ml or 136 μg avidin/ml solution at 3 and 6 d in a no-choice detached leaf bioassay. Means followed by different letters are significantly different at 0.05 level; means were separated using a pairwise comparison.

less YGc3.12 than Yukon Gold or USDA8380-1 regardless of avidin treatment. Consumption did not significantly differ between untreated and avidin-treated YGc3.12 at 3 or 6 d.

Potato line significantly affected the biomass of survivors at 3 d ($F = 74.47$, df = 2, $P < 0.0001$) and 6 d ($F = 190.82$, df = 2, $P < 0.0001$), but the avidin treatment did not significantly affect the biomass of survivors at 3 d ($F = 0.21$, df = 1, $P = 0.6481$) or 6 d ($F = 0.17$, df = 1, $P < 0.6853$). The LC_{50} was determined using neonates. Neonates are more susceptible to toxins than third instars. If a higher concentration of

avidin was used, avidin may have demonstrated negative effects on third instars such as retarded growth. Surviving larvae fed on avidin-treated and untreated Yukon Gold had the highest biomass, but the two groups did not differ significantly from each other (Fig. 9). The biomass of surviving larvae feeding on either avidin-treated or untreated USDA8380-1 was significantly lower than biomass of larvae fed on Yukon Gold, but similarly it did not significantly differ from each other. The biomass of survivors fed on the avidin-treated and untreated YGc3.12 was significantly lower than the biomass of larvae fed on Yukon
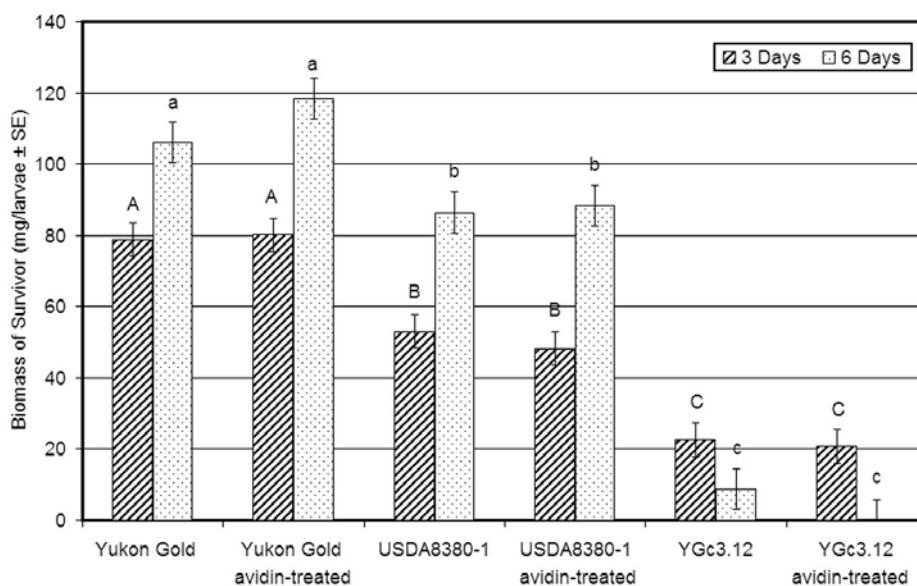

Fig. 9. Mean biomass of surviving Colorado potato beetle third instars fed on three potato lines (Yukon Gold, USDA8380-1, and YGc3.18) dipped in either 0 or 136 μg avidin/ml solution at 3 and 6 d no-choice detached leaf bioassay. Means followed by different letters are significantly different at 0.05 level; means were separated using a pairwise comparison.

Gold or USDA8380-1, but it did not significantly differ from each other.

The current study suggests avidin may be an effective resistance factor against Colorado potato beetle larvae. Avidin is an excellent candidate gene for plant transformation because of its insecticidal properties. Similar to Bt-cry toxins, avidin is a single gene product. If avidin is expressed throughout the plant, it may disturb the biosynthetic pathways of plants, which use carboxylase that requires biotin (Alban et al. 2000). Biotin is located throughout much of the plant cell, including the cytoplasm, mitochondria, and chloroplast, but it is not present in the vacuole of the cell (Baldet et al. 1992). Previous studies have attached a vacuolar targeting leader sequence to the avidin, diverting the produced avidin into vacuole of the cell and reducing or eliminating interference with plant biochemical pathways (Murray et al. 2002).

Currently, most commercial insecticidal transgenic plants rely on *Bt-cry* genes that are designed to combat select groups of pests. With the universal dependence of biotin, avidin may confer broad-spectrum resistance. A broad-spectrum insecticide can kill beneficial insects such as predators or pollinators along with the targeted pest. Using transgenic plants to deploy avidin could decrease the negative effects on beneficial insects. It may be effective against a number of potato insect pests such as wireworms, *Conoderus falli* (Lane), and variegated cutworms, *Peridroma saucia* (Hübner). In addition, avidin may even provide some protection against other noninsect potato pests such as scab, *Streptomyces scabies* (Thaxter), or late blight, *Phytophthora infestans* (Mont.) de Bary.

Combining resistance factors with distinctly different modes of action has been shown to increase insecticidal activity and also may increase the durability of individual toxins (Roush 1998, Zhao et al. 2005). Avidin has antinutritional activity and may not have the "quick kill" effect like Cry toxins. Combining avidin with stronger toxins such as Bt-Cry or natural host plant resistance, may increase the both the effectiveness and longevity of the resistance factors.

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