# Season-Long Abundance of Generalist Predators in Transgenic versus Nontransgenic Potato Fields<sup>1,2</sup>

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**Abstract** We estimated the effect of deploying Cry3A-transgenic potatoes resistant to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), on the season-long relative abundance of naturally-occurring generalist predators. Low inputs of foliar insecticides were used in the transgenic fields to suppress nontarget pests and in the nontransgenic fields to prevent total defoliation of potato plants by *L. decemlineata*. Dominant plant-foraging heteropteran predators and lady beetles were sampled by sweeping foliage, whereas, ground-foraging carnivorous carabids, ants, and spiders were sampled by trapping in pitfalls. *Orius insidiosus* (Say) was significantly ( $P \le 0.05$ ) more abundant in transgenic treatment fields than in nontransgenic fields in 1994, but not in 1995. None of the coccinellids (3 taxa) were affected by the treatments in either season. The carnivorous carabids (3 taxa) and ants were not affected by either treatment, but spiders were significantly more abundant in the transgenic treatment fields in 1995. We conclude that the deployment of pure stands of Cry3A-transgenic potatoes, with a minimum input of insecticides to suppress non-target pests, will have no deleterious effects on the populations of generalist predators in the potato ecosystem.

Key Words Predators, Orius insidiosus, spiders, transgenic potatoes, Cry3A

Generalist predators are common inhabitants in many agroecosystems in North America. Many species are important in the suppression of potential crop pests and are often thought to prevent outbreaks of some secondary pests (Hagen et al. 1976, Kring et al. 1985). A major deterrent to the increased action of these natural enemies is their often low population densities in commercial fields. Management practices that rely on the use of broad-spectrum pesticides are known to disrupt predator populations. Hence, key predators are often not abundant enough to effect control of target pests.

The use of transgenic plants within IPM potato systems may help ameliorate the deleterious effects of traditional insecticidal sprays on naturally-occurring predators. Experimental studies have demonstrated that Cry3A-transgenic potatoes, which are insecticidal to larvae and adults of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Ferro and Gelernter 1989, Perlak et al. 1993), have allowed for a reduction of the pesticide load in the potato ecosystem (Hoy et al. 1990). At present, some pes-

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ticides may still be necessary to control other key herbivorous pests, such as potato leafhoppers, *Empoasca fabae* (Harris), not affected by Cry3A-endotoxins (see Riddick et al. 1998). Thus, the role of generalist predators in delaying outbreaks of non-target pests will be critical to the successful implementation of transgenic plants, as components of integrated pest management systems. In addition, others have determined that indigenous predators may influence the rate at which herbivorous pests develop resistance to Cry3A-transgenic plants (Gould 1988, 1994, Gould et al. 1991, Arpaia et al. 1997).

The impact of transgenic plants containing  $\delta$ -endotoxins derived from *Bacillus* thuringiensis Berliner on natural enemies has been investigated recently, mainly due to the potential deleterious effects that might be associated with these plants (Jepson et al. 1994). Cry3A-intoxicated prey (neonates of L. decemlineata that were fed transgenic potato foliage) had no significant effect on the development, fecundity, or consumption potential of the lady beetle Coleomegilla maculata (De Geer) (Riddick and Barbosa 1998), a major predator of L. decemlineata (Groden et al. 1990). A seed-mix deployment strategy of Cry3A-transgenic and nontransgenic potato is considered a potential resistance management strategy against L. decemlineata. The use of this approach had no effect on the abundance of C. maculata (Riddick et al. 1998), but the abundance of a specialist carabid, Lebia grandis Hentz, was significantly reduced in seed-mixed fields, in comparison to pure nontransgenic fields. Dogan et al. (1996) demonstrated that the development and fecundity of the convergent lady beetle, Hippodamia convergens Guérin-Meneville were not altered when fed green peach aphids, Myzus persicae (Sulzer), that had fed upon Cry3A-transgenic foliage. In other crop systems, transgenic plants containing  $\delta$ -endotoxins have had minimal (Hilbeck et al. 1998) or no (Orr and Landis 1997, Pilcher et al. 1997) detrimental effects on natural enemies.

The research herein determines whether generalist predators (plant and groundforaging) will persist in fields containing Cry3A-transgenic potato. Our main objective was to estimate the relative abundance of dominant heteropteran and coccinellid predators on plants and carnivorous carabids, ants and spiders on the ground. We also assessed the abundance of aphids (1° *M. persicae*) on plants and on the ground, and *L. decemlineata* larvae on the ground. We predict that the pure transgenic fields will harbor more plant and ground-foraging generalists for several reasons. First, the reduced inputs of insecticides would allow, at least theoretically, the persistence of generalist predators in the crop. Also, in the absence of significant defoliation of transgenic potato plants, more harborage becomes available on the plant, in comparison to nontransgenic plants that can become heavily defoliated by *L. decemlineata* late in the season. As a consequence, those generalist species that seek shelter under the cover of plant foliage might find the transgenic fields favorable, and subsequently spend more time in these fields.

# Materials and Methods

**Description of experimental fields.** Field sites were located on three experimental farms of the Central Maryland Research and Education Center, University of Maryland. These farms were the Upper Marlboro Facility, Upper Marlboro, Prince Georges Co.; the Beltsville Facility, Beltsville, Prince Georges Co.; and the Clarksville Facility, Ellicott City, Howard Co. On each farm, isolated treatment fields of 100% transgenic and 100% nontransgenic 'Russet Burbank' potato were planted in 1994 and 1995. Each isolated field on all farms was  $\approx 0.05$  ha and consisted of 24 rows, each 23 m long, with seed pieces spaced 0.3 m apart. The distance between treatment fields on each farm was  $\approx 0.50$  km. The three farms served as replicates in space to test treatment effects. During both years, all fields were planted in early to mid-April and received the same crop management practices. Each field was assigned the same treatment for both years.

**Experimental design.** Colorado potato beetle populations were established at these sites using individuals collected from commercial potato farms, with no previous history of *B. thuringiensis* subsp. *tenebrionis* use. Before the initiation of our experiments, beetles were distributed equally, at a density of 1 beetle per plant cluster (defined as a group of plants originating from the same seed piece), in the nontransgenic field at each farm in 1992 and 1993. This allowed time for the resident populations to become acclimated to each site. To further equalize densities, newly-emerged beetles were collected in May 1994 from the nontransgenic field and distributed to the transgenic field at a density of 1 beetle per plant cluster. In 1995, this re-allocation method was not repeated; we assumed that emerging adults, which had overwintered in or near the nontransgenic fields (in fall 1994) would be capable of finding the transgenic fields in May 1995.

Insecticides were administered in the treatment fields in both seasons using a standard, tractor-driven boom sprayer. Esfenvalerate (Asana, E. I. Dupont de NeMours, Palo Alto, CA) was applied twice during the season at a rate of 0.025 kg (AI)/ha in the nontransgenic field on each farm to prevent total defoliation by *L. decemlineata*. Dimethoate (Cygon 400, American Cyanimid, Havre de Grace, MD), was applied once at a rate of 0.58 liters (AI)/ha to suppress potato leafhopper, *Empoasca fabae* (Harris), in both treatment fields. Both insecticides are toxic to predatory insects (Hurej and Dutcher 1994, Jepson et al. 1995, Duffield et al. 1996, Çilgi et al. 1996, Cho et al. 1997, Hamilton and Lashomb 1997). Sampling ceased for several days after a given field had been sprayed with either insecticide.

Estimating predator abundance. Plant-dwelling heteropteran predators and lady beetles (Coccinellidae) were monitored in the treatment fields during both years using a sweep net technique. This technique has been used previously to monitor populations of foliar predators (Lapchin et al. 1987, Michels et al. 1996, 1997). At approximately weekly intervals, 3 sets of 20 standard 180° sweeps with a 38-cm sweep net were taken in each field. Sampling initiation and termination dates varied among farms because of differences in crop-insect phenology. Also, dates were staggered within a week to distribute the work load. The sampling occurred from 21 June to 9 August 1994, and from 16 May to 20 July 1995. At each collection date, swept insects were stored in plastic bags and brought to the laboratory. A total of 282 sweep samples was taken, 126 samples in 1994 and 156 samples in 1995. Insects were typically identified to the genus and/or species level and counted. A number of predator species were captured and counted. Only the data for the most abundant generalist species of heteropteran and lady beetle predators, which are not significant predators of the target pest (L. decemlineata), are presented herein. The heteropterans were Orius insidiosus (Say) (Anthocoridae), Geocoris punctipes (Say) (Lygaeidae), and Nabis spp. (Nabidae). The lady beetles were Coccinella septempunctata L., Harmonia axyridis Pallas, and H. convergens. These species might be important predators of nontarget pests in the potato ecosystem.

Pitfall traps were used to estimate the abundance of surface-active generalist predators in both treatment fields. Numerous studies have used this standard tech-

nique for monitoring ground-foraging arthropods in agroecosystems (Greenslade 1964, Ericson 1979, Hokkanen and Holopainen 1986, Halsall and Wratten 1988). Traps consisted of plastic cups (473 ml, 9-cm diam opening) sunk into the ground with the rim flush with the soil surface. Leaf litter within 8 to 10 cm of the perimeter of each trap was removed and the soil smoothed to facilitate the movement of carabids around the traps (Greenslade 1964, Powell et al. 1985). Traps were filled to the 1/4 mark with a solution of water and liquid detergent so that captured beetles sank to the bottom of the trap. Three traps were placed within 3 of the 8 plant rows in each treatment field in 1994 and 1995.

Pitfall traps were deployed for 48 h during 1994, but for only 24 h during 1995. In 1994, sampling occurred at intervals of  $\approx$ 7 d from 23 May to 2 August at Upper Marlboro, 23 May to 9 August at Beltsville, and 15 June to 9 August at Clarksville. In 1995, sampling occurred at intervals of  $\approx$ 10 d from 22 May to 18 July at Upper Marlboro, 30 May to 26 July at Beltsville, and 30 May to 27 July at Clarksville. Pitfall collections were taken to the laboratory, and insects were stored in vials of alcohol to be identified and counted at a later date. A number of generalist carabid species were collected in the samples. Only the data for dominant carnivorous species, *Cicindela punctulata punctulata* Olivier, *Poecilus* spp. (*P. chalcites* (Say), *P. lucublandus lucublandus* (Say)), and *Scarites* spp. (*S. subterraneous* F., *S. quadriceps* Chaudoir) are presented herein. We include the tiger beetles in the family Carabidae, after Bousquet and Larochelle (1993).

Also, other arthropods captured in the traps (ants, spiders, Colorado potato beetle larvae, aphids) were counted and analyzed. Direct counts of aphids on the foliage were made by sampling plant clusters, selected at random within treatment fields, and recording the number of aphids (adults and nymphs) observed on the underside of leaves of 6 branch stems of each plant cluster. The number of primary and secondary leaflets, comprising each leaf, on each branch stem ranged from 3 to 15. These aphid counts were made only in 1995 between 7 June and 14 July. Five observation dates were involved at Upper Marlboro, 3 at Beltsville, and 6 at Clarksville. Mostly *M. persicae* were found; although a few other unidentified species were likely present on the plants as well. Voucher specimens are currently held at the R. T. Gast Rearing Laboratory, Mississippi State, MS.

Statistical analyses. Sweep counts of foliar predators and direct counts of aphids on foliage were pooled for the season (1994 and/or 1995) because of the low abundance of both predators and aphids on many of the sampling dates. Similarly, pitfall counts of predators and pests on the ground were pooled. The Mann-Whitney rank sum test (a nonparametric test analogous to the parametric *t*-test) was used to detect significant differences between the treatments (see Glantz 1992). The species abundances were considered significantly different between treatments when  $P \le 0.05$ . All data analyses were performed with Sigma Stat<sup>®</sup> software (1994).

### Results

The abundance of the insidious flower bug, *O. insidiosus*, differed significantly between treatments in the 1994 season ( $T = 3.2 \times 10^3$ , P < 0.0001); more were captured in the transgenic fields than in the nontransgenic fields (Fig. 1). But, in 1995 no significant difference in abundance was detected ( $T = 6.0 \times 10^3$ , P = 0.78). The abundance of *G. punctipes* did not differ significantly between treatments in 1994 ( $T = 4.0 \times 10^3$ , P = 0.99), or in 1995 ( $T = 5.9 \times 10^3$ , P = 0.40). Similarly, the abundance

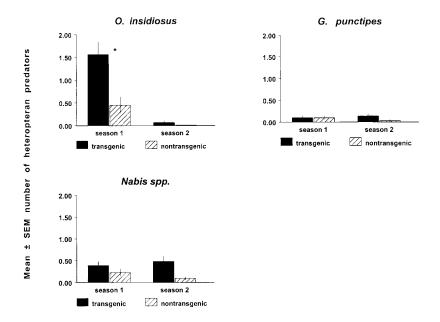


Fig. 1. Mean  $\pm$  SEM number of heteropteran predators captured per sweep per day in treatment fields for consecutive seasons. These included *O. insidiosus, G. punctipes,* and *Nabis* spp. \*, denotes significant difference ( $P \le 0.05$ ) in abundance between treatments. Sample size (n) was 63 sweep samples per treatment field in 1994 (season 1); 78 sweep samples in 1995 (season 2).

of *Nabis* spp. was not measurably affected by the treatments in 1994 ( $T = 3.8 \times 10^3$ , P = 0.29) nor in 1995 ( $T = 5.6 \times 10^3$ , P = 0.08).

We found no significant difference in the abundance of lady beetles (in sweep net samples) between the treatment fields in either season (Fig. 2), as indicated for the adults of each of the 3 lady beetle species, *C. septempunctata* (1994,  $T = 3.8 \times 10^3$ , P = 0.29; in 1995,  $T = 6.3 \times 10^3$ , P = 0.51), *H. axyridis* (1994,  $T = 3.9 \times 10^3$ , P = 0.75; in 1995,  $T = 6.1 \times 10^3$ , P = 0.91), and *H. convergens* (1994,  $T = 4.2 \times 10^3$ , P = 0.28; in 1995,  $T = 6.2 \times 10^3$ , P = 0.89).

The abundance of *C. p. punctulata* foraging on the ground was not affected by the treatments in 1994 ( $T = 4.1 \times 10^3$ , P = 0.82) or in 1995 ( $T = 1.9 \times 10^3$ , P = 0.17), although there seemed to be an undetectable trend toward more beetles in the transgenic treatment fields (Fig. 3). Similarly, the abundance of *Poecilus* spp. was not affected by the treatments in 1994 ( $T = 4.1 \times 10^3$ , P = 0.95) or in 1995 ( $T = 1.94 \times 10^3$ , P = 0.38). Also, the abundance of *Scarites* spp. was not affected significantly by the treatments in 1994 ( $T = 4.37 \times 10^3$ , P = 0.19) or in 1995 ( $T = 1.9 \times 10^3$ , P = 0.36).

The abundance of unidentified ants was not affected by the treatments in 1995 ( $T = 2.0 \times 10^3$ , P = 0.75; see Fig. 4). But, spiders were significantly more abundant in the transgenic treatment fields ( $T = 1.6 \times 10^3$ , P < 0.0001). Unfortunately, we did not collect data on ant or spider abundance in 1994.

The abundance of L. decemlineata larvae (young instars that had fallen to the

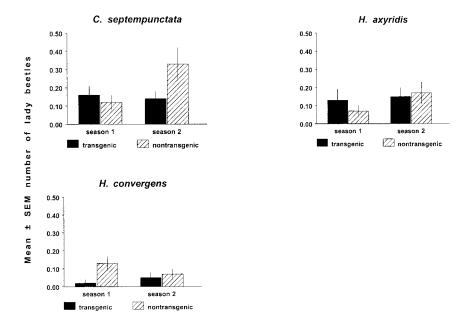


Fig. 2. Mean ± SEM number of lady beetles captured per sweep sample per day in treatment fields for consecutive seasons. These included *C. septempunctata*, *H. axyridis*, and *H. convergens*. Sample size (n) was 63 sweep samples per treatment fields in 1994; 78 sweep samples in 1995.

ground and late instars that were crawling on the ground prior to digging into the soil and constructing pupation chambers) was influenced by the treatments in 1994 ( $T = 3.6 \times 10^3$ , P = 0.01) and in 1995 ( $T = 2.5 \times 10^3$ , P < 0.0001). As anticipated, significantly more larvae were present on the ground in the nontransgenic fields (Fig. 5).

Aphids, 1° *M. persicae*, on the plants and on the ground were not affected by the treatments in 1995 ( $T = 6.6 \times 10^4$ , P = 0.095;  $T = 2.0 \times 10^3$ , P = 0.73; respectively; see Fig. 6).

## Discussion

Three factors are likely responsible for the greater abundance of the insidious flower bug, *O. insidiosus*, in the transgenic fields in 1994. First, esfenvalerate (a pyrethroid) was used in the nontransgenic fields. The spraying of this chemical may have decimated the populations of *O. insidiosus* in these fields on each farm in 1994. Another possible factor could be the greater abundance of prey, such as potato leafhopper immatures, in the transgenic fields (see Riddick et al. 1998). Also, unidentified thrips were quite abundant in the transgenic fields in the 1994 season, but not in the 1995 season (EWR, pers. obs.). Another factor could be the density of plant foliage in the transgenic fields, particularly late in the season. Although not measured directly, transgenic plants did not suffer significant damage by *L. decemlineata*, but the nontransgenic plants were heavily defoliated near the end of the season, despite

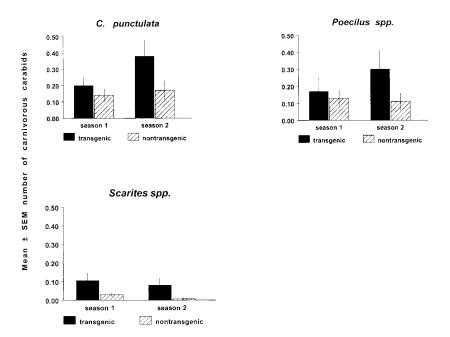


Fig. 3. Mean ± SEM number of generalist carnivorous carabids captured per plant row per trap per day in treatment fields for consecutive seasons. These included *C. p. punctulata, Poecilus* spp., and *Scarites* spp. Sample size (n) was 63 plant rows per transgenic fields and 66 per nontransgenic fields in 1994. In 1995, sample size was 45 plant rows for both treatment fields.

the use of the additional insecticides. In addition, because of the plant-imbibing tendencies of *O. insidiosus*, foliage of transgenic plants would be more suitable as an alternate source of nutrients. Unfortunately, we did not determine which of these factors had the most impact on *O. insidiosus* populations in this study. However, we postulate that the high numbers of thrips in 1994 (but not in 1995) in transgenic fields was the primary factor causing the higher abundance of *O. insidiosus*. Thrips are considered as important prey for *Orius* spp. (McCaffrey and Horsburgh 1986, Hirose 1998).

We observed that aphids, 1° *M. persicae*, on plants were marginally less abundant (P = 0.09) in the transgenic fields in this study. The Cry3A-endotoxins contained within the foliage of transgenic potato does not alter the foliage-probing behavior of *M. persicae* (Shieh et al. 1994). Thus, aphids were not deterred from feeding and reproducing on transgenic plants. A reason for their lower abundance could be due to the action of natural enemies in the transgenic fields. Hoy et al. (1998) indicated that aphidophagous predators may have suppressed aphid populations in *Bt*-potato (same as Cry3A-transgenic potato) plots, in comparison to standard (nontransgenic) potato plots, which had been treated with pyrethroid insecticides. They demonstrated that aphid predators, foraging on transgenic potato plants, were very important in the control of aphids during their early phase of population increase. In nontransgenic

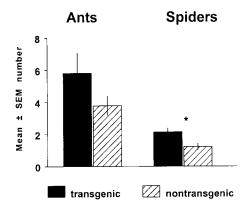
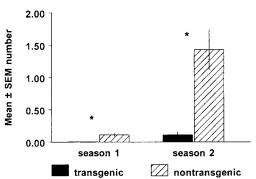


Fig. 4. Mean  $\pm$  SEM number of ants and spiders per row per trap per day in treatment fields in 1995. \*, denotes significant difference ( $P \le 0.05$ ) in abundance between treatments. Sample size (n) was 63 plant rows per transgenic fields and 66 per nontransgenic fields in 1994. In 1995, sample size was 45 plant rows for both treatment fields.



L. decemlineata

Fig. 5. Mean ± SEM number of fallen *L. decemlineata* (Colorado potato beetle) larvae per row per trap per day in treatment fields for consecutive seasons. \*, denotes significant difference ( $P \le 0.05$ ) in abundance between treatments. Sample size (n) was 63 plant rows per transgenic fields, and 66 per nontransgenic fields in 1994. In 1995, sample size was 45 plant rows for both treatment fields.

fields, the use of pyrethroid insecticides reduced the abundance of aphid predators without controlling the aphids; resulting in an aphid outbreak. In transgenic fields, in which no insecticides were used, aphidophagous predators remained abundant and apparently prevented an aphid outbreak (Hoy et al. 1998).

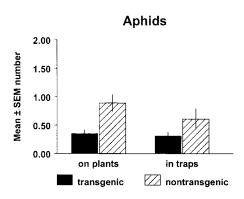


Fig. 6. Mean ± SEM number of aphids per branch stem per plant per day and the number per row per trap per day in treatment fields in 1995. Sample size (n) was 252 direct counts for both treatment fields. Sample size was 45 plant rows for both treatment fields.

We were not able to demonstrate any such greater abundance of *G. punctipes* or the 3 species of lady beetles (*C. septempunctata, H. axyridis, H. convergens*) in the transgenic fields in our study. The observation that *Nabis* spp. were marginally more abundant (P = 0.08) in the transgenic fields in 1995 was probably due to the reduction in pesticides and prevalence of plant foliage in the transgenic fields.

We observed that the carnivorous carabids showed a slight tendency toward being more abundant in the transgenic fields, although no significant effects were detected. We observed that the abundance of spiders, potential competitors of the carnivorous carabids, was greater in fields containing transgenic potatoes. Perhaps the spiders benefitted more from the ground cover and plant foliage associated with the transgenic plants. A number of spider species may regularly move from the ground into the plant canopy to search for prey. Spider abundance may have been strongly associated with herbivores (like leafhoppers) that were common on transgenic potato foliage. In a companion study, leafhoppers were more abundant in the seed-mixed and pure transgenic potato fields, than in the nontransgenic fields (Riddick et al. 1998).

In conclusion, we have shown that deployments of transgenic potatoes had no deleterious effect on the abundance of some plant and ground-dwelling predators. There remains an urgent need for augmenting and conserving predator populations in potato ecosystems. Only then will the full predation potential of these natural enemies be realized.

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