Composition of Arthropod Species Assemblages in *Bt*-expressing and Near Isogenic Eggplants in Experimental Fields

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ABSTRACT The environmental impact of genetically modified (GM) plants in experimental fields has been examined in several ways, in particular with respect to the dynamics of specific nontarget organisms. The approach of sampling for biodiversity in agroecosystems to compare complex patterns could also be useful in studying potential disruptions caused by GM crops. In this study, we set up replicated field plots of *Bt*-expressing eggplants and near isogenic untransformed eggplants as a control. We monitored the presence and abundance of herbivore and predator arthropods in weekly visual samplings of the plant canopy for three growing seasons (2001–2003). Insect species were pooled in organismal taxonomic units (OTUs); three multivariate methods were used to compare species assemblage as an estimate of insect biodiversity. This multistep statistical approach proved to be efficient in recognizing association patterns, as evidenced by the data for the target species *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) clearly showing a significant association with the control plots. All the analyses indicate a comparable species assemblage between transgenic and near isogenic eggplant areas. Our results suggest that some taxa may warrant more specific study. For example, Alticinae beetles (Coleoptera: Chrysomelidae) were alternatively more abundant in either of the two treatments, and their overall abundance was significantly higher on transgenic eggplants. In light of these results and because of their taxonomic proximity to the target species, these herbivores may represent an important nontarget group to be further studied. Moreover, some sap feeders (e.g., Homoptera: Cicadellidae) were more abundant on *Bt*-expressing plants in some samples in all 3 yr.

KEY WORDS biodiversity, biosafety, transgenic plants, multivariate analysis, Cry3Bb

The agricultural area that has been planted with genetically modified (GM) crops has continuously increased since they became commercially available about a decade ago (James 2005). A significant portion of this area is covered by transgenic plants expressing toxins of the soil bacterium *Bacillus thuringiensis* Berliner (*Bt* crops) for insect pest control. These plants are frequently assumed to be specific to a limited number of target pests, mainly Lepidoptera or Coleoptera. However, concerns have been raised that extensive and long-term use of *Bt* crops especially could directly or indirectly affect the nontarget arthropod fauna (Agrawal 2000).

In the field of applied ecology, the debate on "broad view of the ecosystem" versus "selection of a few key organisms or indicator species to test" is still open and ongoing. The "key species" approach attempts to put together a working system by a detailed analysis of single components. An alternative philosophy starts with the "big picture" and subsequently zooms in to focus on some aspects or components of the system, but only if this is necessary.

Early studies on GM plants were devoted mainly to highlighting possible hazards and pathways of transgenic toxin exposure to higher trophic levels under controlled laboratory conditions and only for a limited period of time (Hilbeck et al. 1998, 1999, Birch et al. 1999, Losey et al. 1999). Subsequently, the analysis of potential risks by examining several components of the arthropod fauna along the food web under natural conditions has attracted more interest (Oberhauser and Rivers 2003, Cowgill et al. 2004, French et al. 2004).

Agroecosystems are simplified but nevertheless complex ecosystems where, albeit temporarily, multitrophic interactions involving numerous species are established in communities and food webs. It is therefore clear that an ecological analysis based on one or a few preconceived key species, while economically and technically easier to conduct, may provide incomplete information about the complex interactions between GM crops and higher trophic levels. Agriculture depends on several ecological functions that are essential to soil fertility and crop productivity (e.g., microbial decomposition and nutrient cycling, crop pollination by animals, food turnover). All zoological groups that mediate these functions,

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Table 1. Criteria for pooling taxonomic groups found in experimental eggplant fields (2001–2003) in OTUs

Taxon/stage	OTU	Trophic function in the studied agro-ecosystem	Potential exposure	Criteria for visual discrimination
Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae), adults	"CPB adults"	Target herbivores	Leaf feeders, suffer sublethal effects on transgenic eggplants, frequently moving between plants	Identified based on size and distinctive colors
L. decemlineata, third and fourth instars	"CPB large"	Target herbivores	Voracious eaters of eggplant leaves, less sensitive to the toxin compared with younger larvae	Identified based on size and color
L. decemlineata, first and second instars	"CPB small"	Target herbivores, possible prey for large predators	Young larvae are the most sensitive to the Cry toxins, although their food intake rate is lower	Identified based on size and color
L. decemlineata, eggmasses	"CPB eggs"	Food source for generalist predators	Eggs are likely not to be exposed to the Cry toxin, females might have been exposed and egg laying behavior might be driven by food quality	Identified, based on their shape and color
Flea beetles (Chrysomelidae: Alticinae), adults of all species	"Flea beetles[a]"	Nontarget herbivores	Adults are exposed to the toxin while feeding on the leaves	Identified based on size and shape
Potato tuber moth, *Phthorimaea operculella*, (Lepidoptera: Gelechiidae), larvae (only intact mines)	"PTM mines"	Nontarget herbivores	Exposed to the Cry toxin while feeding on leaf tissues	Mines of *P. operculella* were distinguished from those of Agromyzidae larvae based on size and shape
Leafminers (Diptera: Agromyzidae)	"Leafminers"	Nontarget herbivores	Exposed to the Cry toxin while feeding on leaf tissues	Mines distinguished from *P. operculella* based on size and shape
Stinkbugs (Heteroptera: Pentatomidae) all stages except eggs	"Pentatomidae[b]"	Nontarget herbivores	Cry toxins may be ingested while feeding on plants	Identification at family level, based on body shape and size
Lygus spp. (Hemiptera: Miridae)	"Lygus"	Nontarget herbivores	Cry toxins may be ingested while feeding on plants	Identification at family level, based on body color and size
Green peach aphid, *Myzus persicae* (Homoptera: aphidoidea), all stages except eggs	"Green peach aphid"	Sap feeders, both adults and larvae are prey for generalist predators	Exposure of aphids to Cry toxins is still unclear (Raps et al., 2001; Zhang et al, 2004)	Identified based on color
Cotton aphid, *Aphys gossypi* (Homoptera: aphidoidea), all stages except eggs	"Cotton aphid"	Sap feeders, both adults and larvae are prey for generalist predators	Exposure of aphids to Cry toxins is still unclear	Identified based on color
Cicadella viridis (Homoptera: Cicadellidae)	"Cicadella"	Sap feeders	Their exposure to the toxin is unknown, would be possible if Cry proteins enter phloem	Identified based on size and color
All individuals belonging to this family, except the former species	"Cicadellidae"	Sap feeders	Their exposure to the toxin is unknown, would be possible if Cry proteins enter phloem	Identification at family level based on appearance
All individuals belonging to this family	"Thripidae"	Sap feeders, some predatory species might also have been present	Their exposure to the toxin is unknown, it may theoretically be either direct (herbivores) or indirect (predatory species)	Identification at family level based on appearance and size
Green lacewings, *Chrysoperla* spp. (Neuroptera: Chrysopidae), adults	"Lacewing adults"	Generalist predators	Possibly exposed while feeding on plant pollen or exudates	Identified based on their appearance, identification at species level is not possible in the field
Chrysoperla spp., larvae	"Lacewing larvae"	Generalist predators	Likely to be exposed to the toxin via their prey	Identified based on their appearance, identification at species level is not possible in the field

Table 1. Continued

Taxon/stage	OTU	Trophic function in the studied agro-ecosystem	Potential exposure	Criteria for visual discrimination
Chrysoperla spp., eggs	"Lacewing eggs"	Possible hosts of specialized parasitoids (pers. observ.)	Eggs are likely not to be exposed to the Cry toxin (egg laying females might have been exposed)	Identified based on their appearance, identification at species level in field conditions is not possible
Macrolophus caliginosus (Hemiptera: Miridae), adults	"Macrolophus"	Generalist predators, herbivory occasionally is reported for this species	Individuals can be exposed to the toxins either directly, if feeding on plants, or via their prey	Identification at species level is possible for adults, based on the color pattern of the antennae
Cyrtopeltis tenuis (Hemiptera: Miridae), adults	"Cyrtopeltis"	Generalist predators, herbivory occasionally is reported for this species	Individuals can be exposed to the toxins either directly, if feeding on plants, or via their prey	Identification at species level based on color
Dicyphus pallidus (Hemiptera: Miridae), adults	"Dicyphus"	Generalist predators, herbivory occasionally is reported for this species	Individuals can be exposed to the toxins either directly, if feeding on plants, or via their prey	Identification at species level based on color
Leaf bugs (Heteroptera: Miridae), adults not belonging to the previous species, and all larval stages pooled	"Miridae"	Various feeding regimes, including predators	Individuals can be exposed to the toxins either directly, if feeding on plants, or via their prey	Identification at family level based on appearance and dimensions
Pirate bugs (Heteroptera: Anthocoridae), all individuals belonging to this family	"Anthocoridae"	Generalist predators	Likely to be exposed to the toxin via their preys	Identified at family level based on body form and size
Damselbugs (Heteroptera: Nabidae), all individuals belonging to this family	"Nabidae"	Generalist predators	Likely to be exposed to the toxin via their preys	Identification at family level based on appearance and size
Rove beetles (Coleoptera: Staphylinidae), all individuals belonging to this family	"Staphylinidae"	Usually predatory species	Their exposure to the toxin is unknown, most likely is indirect via their prey	Identification at family level based on appearance
Coccinella septempunctata (Coleoptera: Coccinellidae), adults	"Coccinella"	Predators of aphids	Indirect via prey or direct while feeding on plant pollen or exudates	Identified based on color
Hippodamia variegata (Coleoptera: Coccinellidae), adults	"Hippodamia"	Predators	Indirect via prey or direct while feeding on plant pollen or exudates	Identified based on color
Ladybirds (Coleoptera: Coccinellidae), larvae	"Ladybird larvae"	Predators	Possibly exposed to the toxin via their prey or directly while feeding on plant pollen or exudates	Identification at family level based on appearance
Ladybirds (Coleoptera: Coccinellidae), pupae	"Ladybird pupae"	Quiescent stage	Exposed during the larval stage	Identification at family level based on appearance
Stethorus punctillum (Coleoptera: Coccinellidae), all stages except eggs	"Stethorus"	Specialized predators of spider mites	Possibly exposed to the toxin via their prey	Identified based on size and appearance
Spiders (Araneae), all individuals	"Araneae"	Generalist predators	Indirect exposure through the food web	Identified based on appearance
Psyllobora vigintiduopunctata (Coleoptera: Coccinellidae), adults	"22-spots"	Fungal feeders	Unknown	Identified based on color pattern

should therefore be considered to avoid a common intellectual shortcut that equates population density with function (Arpaia 2004).

The use of more realistic field studies has been advocated as a fundamental requirement for the study of potential ecological impacts of GM crops (Firbank et al. 2005). The recently published series of field studies in this section of *Environmental Entomology* has provided an avenue for improving our knowledge of transgenic corn and cotton agroecosystems. A significant reduction in the populations of some taxa was detected in multi-year field experiments (Daly and Buntin 2005, Dively 2005, Naranjo 2005a, Pilcher et al. 2005, Whitehouse et al. 2005). Nevertheless, these effects occurred only in a minority of sampled species and were of lesser magnitude than the effects of insecticidal sprays (Bhatti et al. 2005a, b, Dively 2005, Naranjo 2005a, Whitehouse et al. 2005). Even in the

Table 2. Mean no. of individuals per plant (±SE) recorded in eggplant field sampling in Southern Italy during the growing season 2001

OTU	26 July		2 Aug.		9 Aug.		13 Aug.		21 Aug.		24 Aug.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0	0	0	0	0	0	0	0	0	0	0	0
Flea beetles	0.33 ± 0.07	0.45 ± 0.13	0.13 ± 0.05	0.35 ± 0.08	0.62 ± 0.12	0.48 ± 0.10	1.33 ± 0.24	0.97 ± 0.20	2.75 ± 0.40	3.77 ± 0.47	5.78 ± 0.60	4.40 ± 0.53
PTM mines	0.17 ± 0.07	0.25 ± 0.07	0.37 ± 0.10	0.18 ± 0.06	0.40 ± 0.12	0.33 ± 0.08	0.28 ± 0.09	0.18 ± 0.05	0.52 ± 0.11	0.32 ± 0.09	0.55 ± 0.12	0.37 ± 0.11
Green peach aphid	0	0	0	0	0	0	0.45 ± 0.26	1.05 ± 0.55	0	0.02 ± 0.02	0	0
Cotton aphid	0	0.02 ± 0.02	0.02 ± 0.02	0	0	0	0	0.02 ± 0.02	0	0.05 ± 0.03	0	1.95 ± 1.95
Cicadellidae	0.42 ± 0.10	0.67 ± 0.12	0.70 ± 0.12	0.67 ± 0.11	0.40 ± 0.08	0.30 ± 0.06	3.90 ± 0.64	3.49 ± 0.50	3.87 ± 0.61	2.83 ± 0.42	3.37 ± 0.43	3.55 ± 0.53
Thripidae	0.03 ± 0.02	0.07 ± 0.03	0.07 ± 0.03	0.02 ± 0.02	0.05 ± 0.03	0.05 ± 0.03	0.20 ± 0.06	0.25 ± 0.09	0.13 ± 0.08	0.18 ± 0.09	2.40 ± 0.30	1.55 ± 0.24
Lacewing larvae	0	0	0	0	0	0	0.05 ± 0.03	0.02 ± 0.02	0	0.05 ± 0.04	0.18 ± 0.09	0
Lacewing eggs	0.07 ± 0.03	0.03 ± 0.02	0.15 ± 0.05	0.17 ± 0.05	0.47 ± 0.08	0.43 ± 0.06	1.35 ± 0.25	1.55 ± 0.24	2.25 ± 0.21	2.45 ± 0.21	0.88 ± 0.20	1.25 ± 0.27
Miridae	0	0	0	0	0	0	0.13 ± 0.05	0.18 ± 0.13	0.40 ± 0.13	0.53 ± 0.14	0.97 ± 0.11	0.78 ± 0.14
Anthocoridae	0	0	0.05 ± 0.03	0.02 ± 0.02	0.18 ± 0.05	0.10 ± 0.04	0.30 ± 0.08	0.25 ± 0.09	0.28 ± 0.11	0.35 ± 0.09	0.57 ± 0.12	0.65 ± 0.12
Nabidae	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.08 ± 0.04	0.17 ± 0.05	0.30 ± 0.06	0.15 ± 0.05	0.03 ± 0.02	0.27 ± 0.06	0.18 ± 0.06	0.18 ± 0.05	0.12 ± 0.04
Staphylinidae	0	0	0	0	0	0	0.05 ± 0.04	0.08 ± 0.04	1.07 ± 0.23	0.28 ± 0.12	0.13 ± 0.05	0.07 ± 0.03
Coccinella	0	0	0.02 ± 0.02	0	0	0	0.02 ± 0.02	0	0	0	0	0
Ladybird larvae	0	0	0	0	0	0	0.20 ± 0.13	0.38 ± 0.30	0	0	0	0
Araneae	0.07 ± 0.03	0.10 ± 0.05	0.02 ± 0.02	0	0.02 ± 0.02	0.02 ± 0.02	0.10 ± 0.04	0.02 ± 0.02	0	0.18 ± 0.06	0.03 ± 0.02	0
22-spots	0	0	0	0	0	0	0	0	0.02 ± 0.02	0.03 ± 0.02	0	0

OTU	28 Aug.		31 Aug.		4 Sept.		7 Sept.		11 Sept.		14 Sept.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0	0.02 ± 0.02	0	0	0.05 ± 0.03	0.02 ± 0.02	0.03 ± 0.02	0.05 ± 0.03	0	0	0	0.02 ± 0.02
Flea beetles	11.25 ± 1.28	10.12 ± 0.87	15.85 ± 0.95	13.63 ± 1.26	25.03 ± 2.67	18.10 ± 1.63	20.22 ± 1.34	17.23 ± 1.22	15.05 ± 0.88	14.32 ± 1.16	20.95 ± 1.05	17.68 ± 1.11
PTM mines	0.32 ± 0.11	0.27 ± 0.09	0.28 ± 0.09	0.50 ± 0.12	0.20 ± 0.07	0.38 ± 0.12	0.10 ± 0.05	0.13 ± 0.06	0.13 ± 0.06	0.12 ± 0.07	0.07 ± 0.03	0.05 ± 0.03
Green peach aphid	0.07 ± 0.04	0	0	0.02 ± 0.02	0.02 ± 0.02	0.10 ± 0.05	0.02 ± 0.02	0.02 ± 0.02	0.07 ± 0.05	0	0	0
Cotton aphid	0	0.03 ± 0.02	0.32 ± 0.10	0.17 ± 0.07	0.07 ± 0.03	0.07 ± 0.05	0.12 ± 0.05	0.03 ± 0.02	0	0.02 ± 0.02	0.03 ± 0.02	0.05 ± 0.03
Cicadellidae	4.28 ± 0.43	3.18 ± 0.46	6.42 ± 0.53	5.83 ± 0.61	6.17 ± 0.59	3.97 ± 0.51	6.55 ± 0.66	4.65 ± 0.43	1.33 ± 0.26	1.62 ± 0.27	3.67 ± 0.43	3.30 ± 0.47
Thripidae	0.20 ± 0.11	0.15 ± 0.06	0.18 ± 0.07	0.05 ± 0.03	0.03 ± 0.02	0.12 ± 0.05	0.25 ± 0.10	0.08 ± 0.06	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.07 ± 0.04
Lacewing larvae	0.07 ± 0.03	0.08 ± 0.07	0.05 ± 0.03	0.02 ± 0.02	0.07 ± 0.04	0.02 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0	0	0.02 ± 0.02	0
Lacewing eggs	1.60 ± 0.17	1.10 ± 0.16	1.25 ± 0.24	2.25 ± 0.22	2.58 ± 0.24	2.02 ± 0.25	2.28 ± 0.23	2.92 ± 0.25	2.30 ± 0.28	2.45 ± 0.25	5.08 ± 0.32	4.93 ± 0.29
Miridae	0.68 ± 0.14	1.15 ± 0.28	1.40 ± 0.18	1.00 ± 0.19	0.87 ± 0.14	0.97 ± 0.21	0.77 ± 0.13	0.50 ± 0.11	0.15 ± 0.05	0.08 ± 0.04	0.10 ± 0.05	0.08 ± 0.07
Anthocoridae	0.23 ± 0.07	0.32 ± 0.08	0.52 ± 0.13	0.25 ± 0.07	0.32 ± 0.11	0.28 ± 0.08	0	0.02 ± 0.02	0.07 ± 0.03	0.12 ± 0.05	0	0
Nabidae	0.30 ± 0.07	0.42 ± 0.10	0.52 ± 0.09	0.47 ± 0.10	0.37 ± 0.08	0.35 ± 0.09	0.30 ± 0.08	0.15 ± 0.05	0.42 ± 0.07	0.33 ± 0.07	0.15 ± 0.06	0.05 ± 0.03
Staphylinidae	1.53 ± 0.28	0.67 ± 0.16	0.40 ± 0.15	0.47 ± 0.13	0.40 ± 0.10	0.13 ± 0.06	0.40 ± 0.10	0.15 ± 0.05	0.02 ± 0.02	0	0	0
Coccinella	0.03 ± 0.02	0.05 ± 0.03	0	0	0	0	0	0.05 ± 0.03	0	0	0	0
Ladybird larvae	0	0	0	0	0	0	0	0	0	0	0	0
Araneae	0.15 ± 0.05	0.05 ± 0.04	0.13 ± 0.05	0.25 ± 0.08	0.17 ± 0.05	0.12 ± 0.04	0.17 ± 0.05	0.17 ± 0.05	0.08 ± 0.04	0.05 ± 0.03	0.10 ± 0.05	0.03 ± 0.02
22-spots	0	0.017 ± 0.017	0.083 ± 0.036	0.017 ± 0.017	0.017 ± 0.017	0.05 ± 0.028	0	0	0	0	0.017 ± 0.017	0

OTU	18 Sept.		21 Sept.		25 Sept.		28 Sept.		2 Oct.		5 Oct.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0	0	0	0	0.02 ± 0.02	0	0	0	0	0	0	0
Flea beetles	32.90 ± 3.43	17.90 ± 2.25	36.78 ± 2.85	25.25 ± 1.94	35.05 ± 2.73	30.68 ± 3.16	24.75 ± 1.63	26.15 ± 1.65	32.25 ± 2.03	30.13 ± 1.61	35.58 ± 1.95	39.10 ± 1.71
PTM mines	0.10 ± 0.06	0.30 ± 0.13	0	0.13 ± 0.06	0.08 ± 0.04	0.10 ± 0.06	0.15 ± 0.06	0.07 ± 0.04	0.35 ± 0.08	0.10 ± 0.04	0.07 ± 0.05	0.20 ± 0.07
Green peach aphid	0.03 ± 0.03	0.03 ± 0.02	0.07 ± 0.05	0.05 ± 0.03	0	0.05 ± 0.04	0	0	0	0.08 ± 0.04	0	0
Cotton aphid	0.02 ± 0.02	0.15 ± 0.05	0.15 ± 0.08	0.42 ± 0.24	0.25 ± 0.10	0.07 ± 0.05	0	0	0.35 ± 0.09	0	0.17 ± 0.06	0.15 ± 0.06
Cicadellidae	4.02 ± 0.43	2.67 ± 0.36	4.28 ± 0.41	3.93 ± 0.38	4.22 ± 0.47	3.77 ± 0.42	1.85 ± 0.19	1.88 ± 0.15	4.37 ± 0.28	2.73 ± 0.29	3.23 ± 0.31	3.02 ± 0.29
Thripidae	0	0	0.12 ± 0.05	0.12 ± 0.05	0.35 ± 0.12	0.10 ± 0.05	0	0	0.67 ± 0.17	0.37 ± 0.15	0.48 ± 0.16	0.62 ± 0.16
Lacewing larvae	0.02 ± 0.02	0.08 ± 0.04	0.08 ± 0.04	0	0.10 ± 0.70	0.02 ± 0.02	0	0	0.02 ± 0.02	0	0.03 ± 0.02	0

Lacewing eggs	4.92 ± 0.44	4.08 ± 0.45	4.95 ± 0.32	4.63 ± 0.44	3.95 ± 0.41	3.52 ± 0.32	1.52 ± 0.15	1.73 ± 0.17	3.10 ± 0.37	2.37 ± 0.21	2.17 ± 0.24	3.37 ± 0.30
Miridae	0.57 ± 0.11	0.38 ± 0.11	0.38 ± 0.09	0.38 ± 0.10	1.17 ± 0.23	0.43 ± 0.13	0.45 ± 0.08	0.52 ± 0.09	0.20 ± 0.07	0.17 ± 0.06	0.20 ± 0.06	0.25 ± 0.07
Anthocoridae	0.28 ± 0.13	0.22 ± 0.07	0.08 ± 0.04	0.25 ± 0.08	0.15 ± 0.05	0.12 ± 0.06	0.12 ± 0.04	0.10 ± 0.04	0.22 ± 0.06	0.22 ± 0.06	0.18 ± 0.06	0.13 ± 0.05
Nabidae	0.20 ± 0.08	0.17 ± 0.05	0.37 ± 0.08	0.15 ± 0.05	0.50 ± 0.09	0.58 ± 0.12	0.08 ± 0.04	0.20 ± 0.05	0.25 ± 0.07	0.42 ± 0.08	0.15 ± 0.05	0.10 ± 0.04
Staphylinidae	0.02 ± 0.02	0.07 ± 0.03	1.03 ± 0.24	0.57 ± 0.16	0.47 ± 0.14	0.13 ± 0.07	0.17 ± 0.05	0.20 ± 0.06	0.53 ± 0.10	0.20 ± 0.05	0.15 ± 0.06	0.15 ± 0.06
Coccinella	0.02 + 0.02	0.03 + 0.02	0	0	0	0	0	0	0.02 + 0.02	0	0.03 + 0.02	0.02 + 0.02
Ladybird larvae	0.02 ± 0.02	0.03 ± 0.02	0	0	0	0	0	0	0.02 ± 0.02	0	0.03 ± 0.02	0.02 ± 0.02
Araneae	0.22 ± 0.06	0.12 ± 0.05	0.23 ± 0.06	0.28 ± 0.08	0.18 ± 0.06	0.10 ± 0.04	0.07 ± 0.03	0.02 ± 0.02	0.18 ± 0.06	0	0.12 ± 0.05	0.12 ± 0.05
22-spots	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.02	0.03 ± 0.03	0	0	0	0	0	0	0.02 ± 0.02	0

case of reduced abundance of some generalist predators, Naranjo (2005b) found no difference in the overall intensity of natural predation. Potential hazards for some nontarget species interacting with GM plants were found in laboratory conditions (Lovei and Arpaia 2005), but no ecological impacts have been verified specifically in the field.

This study has two objectives: first, we aimed at detecting the potential impact of growing transgenic eggplants (*Solanum melongena* L.) expressing Cry3Bb toxin on nontarget herbivores and on generalist predators. Transgenic eggplants were tested in the field for their resistance to Coleoptera (Acciarri et al. 2000), but only limited information about nontarget insects was collected. The second goal is the use of community ecology methods for evaluating detectable changes in the structure of arthropod assemblages as a proxy for the overall change in biotic communities associated with these plants. We therefore propose the use of a multistep approach, based on multivariate tests.

Materials and Methods

Plants. The transgenic eggplant line 9–8 expressing the *Bt* toxin Cry3Bb for the control of Colorado potato beetle was obtained by genetic transformation of the eggplant line DR2 (Arpaia et al. 1997). These transformed and control plants were used for the first field trial in 2001. In the two following cropping seasons (2002 and 2003), F1 hybrid progeny were used for field experiments to use more productive plants. The hybrids were derived from the transgenic line 9–8 used as a female parent, whereas near isogenic controls were obtained from the DR2 line as a female parent. To test for the presence of the transgene, all seedlings were selected in vivo by spraying them with a kanamicin solution according to the protocol of Sunseri et al. (1993) before transplanting them in the field. In addition, a polymerase chain reaction (PCR) analysis was performed with 20 randomly chosen transgenic eggplants. Genomic DNA was extracted from young leaves and amplified using the specific Cry3Bb primers: seven forward (5′-GTGCCACAGGATTCTATCGAC-3′) and four reverse (5′-GATATCGTTGCAACAAGGCA-3′).

Transgenic plants were tested for toxin expression in previous field studies (Acciarri et al. 2000) and showed expression in all above-ground plant tissues (young and old leaves, flowers, fruits) as expected when using a 35S promoter. The same plants were previously assessed for several years in field trials and evidenced higher yield compared with their isolines under heavy herbivore pressure caused by *L. decemlineata* (Acciarri et al. 2000, Mennella et al. 2005).

Experimental Design. Three field trials were carried out in Metaponto (Southern Italy) from 2001 to 2003. Restrictions imposed by the local government obliged us to change the site of the deliberate field release in every cropping season; therefore, eggplant fields were alternately prepared in two different experimental stations in the same area (Pantanello and Campo 7). The chosen fields are usually cultivated

Table 3. Mean no. of individuals per plant (±SE) recorded in eggplant field sampling in Southern Italy during the growing season 2002

OTU	7 Aug.		13 Aug.		21 Aug.		5 Sept.		19 Sept.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0.23 ± 0.07	0.22 ± 0.06	0.32 ± 0.09	0.15 ± 0.05	0.10 ± 0.05	0.08 ± 0.05	0.40 ± 0.09	1.70 ± 0.32	0.20 ± 0.06	1.52 ± 0.28
CPB large	0	0	0.07 ± 0.05	1.55 ± 0.81	0.23 ± 0.11	1.05 ± 0.51	0	0.57 ± 0.20	0.03 ± 0.03	0.30 ± 0.10
CPB small	0.28 ± 0.28	1.02 ± 0.91	0.48 ± 0.25	3.97 ± 1.52	0.30 ± 0.30	1.85 ± 0.82	0.25 ± 0.25	0.12 ± 0.12	0	0.02 ± 0.02
CPB eggs	0.10 ± 0.05	0.25 ± 0.08	0.32 ± 0.28	0.30 ± 0.09	0	0.08 ± 0.85	0	0.02 ± 0.02	0	0
Flea beetles	0.65 ± 0.11	0.98 ± 0.19	3.45 ± 0.40	0.95 ± 0.18	7.27 ± 0.96	4.33 ± 0.57	6.57 ± 0.51	5.98 ± 0.56	11.58 ± 0.65	11.18 ± 0.72
PTM mines	0.22 ± 0.07	0.48 ± 0.12	0.72 ± 0.13	0.73 ± 0.15	1.55 + 0.27	2.13 + 0.32	0.37 ± 0.09	0.37 ± 0.109	0.97 ± 0.13	0.77 ± 0.20
Green peach aphid	0.70 ± 0.23	0.27 ± 0.18	7.85 ± 4.69	1.27 ± 0.44	2.77 ± 0.70	1.82 ± 0.50	0.07 ± 0.04	0.07 ± 0.07	0.03 ± 0.02	0.03 ± 0.03
Cotton aphid	0.25 ± 0.08	0.75 ± 0.44	0.47 ± 0.10	3.68 ± 2.02	7.02 ± 1.41	15.25 ± 6.37	0.65 ± 0.17	0.05 ± 0.03	0.18 ± 0.07	0.20 ± 0.06
Cicadellidae	2.75 ± 0.44	1.78 ± 0.33	5.85 ± 0.55	4.33 ± 0.63	3.05 ± 0.36	1.62 ± 0.22	4.95 ± 0.47	1.75 ± 0.34	6.55 ± 0.45	7.92 ± 0.81
Thripidae	0.02 ± 0.02	0	0.20 ± 0.07	0	0.32 ± 0.15	0.03 ± 0.02	0.43 ± 0.15	0.17 ± 0.06	0.35 ± 0.16	0
Lacewing larvae	0	0	0.02 ± 0.02	0	1.55 ± 0.27	2.13 ± 0.32	0.03 ± 0.02	0.02 ± 0.02	0	0.95 ± 0.29
Lacewing eggs	0.07 ± 0.03	0	0.27 ± 0.09	0.05 ± 0.03	0.03 ± 0.02	0.13 ± 0.08	1.52 ± 0.16	0.87 ± 0.22	2.73 ± 0.28	1.22 ± 0.18
Macrolophus	0.02 ± 0.02	0	0.02 ± 0.02	0.08 ± 0.04	0.08 ± 0.04	0	0.20 ± 0.05	0.03 ± 0.02	0.50 ± 0.09	0.32 ± 0.06
Cyrtopeltis	0	0	0.07 ± 0.04	0	0.27 ± 0.08	0	0.32 ± 0.08	0.02 ± 0.02	0.13 ± 0.04	0.03 ± 0.02
Dicyphus	0	0	0.02 ± 0.02	0	0.12 ± 0.04	0.18 ± 0.05	0.10 ± 0.06	0.22 ± 0.08	0.38 ± 0.10	0.52 ± 0.09
Miridae	0	0	0	0	0.10 ± 0.06	0.13 ± 0.07	0.25 ± 0.07	0.22 ± 0.09	0	0
Anthocoridae	0.02 ± 0.02	0	0.10 ± 0.07	0	0	0.08 ± 0.04	0.03 ± 0.02	0.17 ± 0.05	0.22 ± 0.11	0.05 ± 0.03
Nabidae	0.02 ± 0.02	0	0.05 ± 0.04	0.08 ± 0.03	0.05 ± 0.03	0.08 ± 0.06	0.03 ± 0.02	0.10 ± 0.05	0.12 ± 0.05	0.10 ± 0.04
Staphylinidae	0	0	0	0.03 ± 0.02	0.05 ± 0.03	0	0.22 ± 0.11	0.20 ± 0.07	0.20 ± 0.05	0.27 ± 0.08
Coccinella	0	0	0	0.03 ± 0.02	0.12 ± 0.05	0.22 ± 0.10	0.75 ± 0.14	0.12 ± 0.05	0.18 ± 0.06	0.05 ± 0.03
Ladybird larvae	0.02 ± 0.02	0	0	0.07 ± 0.03	0	0	0.55 ± 0.12	0.13 ± 0.06	0.03 ± 0.02	0.03 ± 0.02
Ladybird pupae	0	0.02 ± 0.02	0.07 ± 0.04	0.02 ± 0.02	0	0	0.05 ± 0.03	0	0.02 ± 0.02	0.07 ± 0.05
Stethorus	0.05 ± 0.03	0.05 ± 0.03	0.15 ± 0.07	0.10 ± 0.04	4.30 ± 1.35	3.97 ± 1.22	0.03 ± 0.02	0.12 ± 0.05	0.02 ± 0.02	0.02 ± 0.02
Araneae	0.03 ± 0.02	0.02 ± 0.02	0.08 ± 0.04	0.10 ± 0.04	0.02 ± 0.02	0.02 ± 0.02	0	0	0.17 ± 0.05	0.07 ± 0.04
22-spots	0	0	0	0.02 ± 0.02	0	0.02 ± 0.02	0.03 ± 0.02	0.05 ± 0.03	0.03 ± 0.02	0.08 ± 0.04

OTU	26 Sept.		4 Oct.	
	Bt	Control	*Bt*	Control
CPB adults	0.39 ± 0.22	0.68 ± 0.15	0.70 ± 0.54	0.53 ± 0.12
CPB large	0.03 ± 0.02	0.08 ± 0.04	0.25 ± 0.25	0.25 ± 0.11
CPB small	0.03 ± 0.02	0.07 ± 0.04	0.12 ± 0.12	0.12 ± 0.08
CPB eggs	0	0	0	0
Flea beetles	14.03 ± 1.30	10.95 ± 0.75	14.67 ± 6.24	6.35 ± 0.54
PTM mines	0.90 + 0.15	0.52 + 0.11	0.90 ± 0.36	0.35 ± 0.08
Green peach aphid	0.05 ± 0.03	0.07 ± 0.03	0.12 ± 0.12	0.12 ± 0.06
Cotton aphid	0.15 ± 0.06	0.15 ± 0.05	0.33 ± 0.13	0.05 ± 0.03
Cicadellidae	4.50 ± 0.54	3.17 ± 0.032	3.72 ± 2.17	2.17 ± 0.31
Thripidae	0.17 ± 0.07	0.05 ± 0.03	0.03 ± 0.03	0.03 ± 0.03
Lacewing larvae	0.90 ± 0.15	0.52 ± 0.11	0.02 ± 0.02	0.02 ± 0.02
Lacewing eggs	0.42 ± 0.13	0	1.55 ± 0.67	0.63 ± 0.12
Macrolophus	1.71 ± 0.51	0.17 ± 0.05	0.32 ± 0.16	0.15 ± 0.05
Cyrtopeltis	0.25 ± 0.07	0.18 ± 0.05	0.18 ± 0.11	0.10 ± 0.04
Dicyphus	0.58 ± 0.15	0.33 ± 0.11	0.30 ± 0.23	0.23 ± 0.06
Miridae	0	0	0.10 ± 0.04	0
Anthocoridae	0.63 ± 0.12	0.43 ± 0.12	0.20 ± 0.15	0.15 ± 0.07
Nabidae	0.58 ± 0.24	0.20 ± 0.05	0.25 ± 0.19	0.18 ± 0.06
Staphylinidae	0.07 ± 0.03	0.03 ± 0.02	0.07 ± 0.04	0
Coccinella	0.08 ± 0.04	0.03 ± 0.02	0.10 ± 0.04	0.08 ± 0.04
Ladybird larvae	0.02 ± 0.02	0	0.03 ± 0.02	0.02 ± 0.02
Ladybird pupae	0	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
Stethorus	0.07 ± 0.03	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.02
Araneae	0.07 ± 0.04	0	0.03 ± 0.02	0.65 ± 0.04
22-spots	0.03 ± 0.02	0.02 ± 0.02	0	0

Table 4. Mean no. of individuals per plant (±SE) recorded in eggplant field sampling in Southern Italy during the growing season 2003

OTU	17 July		23 July		7 Aug.		13 Aug.		20 Aug.		28 Aug.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0.03 ± 0.02	0	0.25 ± 0.07	0.50 ± 0.09	0.95 ± 0.13	0.58 ± 0.15	0.45 ± 0.10	0.30 ± 0.06	0.55 ± 0.12	0.97 ± 0.17	0.63 ± 0.12	0.68 ± 0.10
CPB large	0	0	0	0	0	0	0	0.07 ± 0.07	1.72 ± 0.52	0.81 ± 0.29	5.42 ± 1.01	2.52 ± 0.60
CPB small	0	0	0	0	2.55 ± 1.02	2.88 ± 0.68	12.42 ± 2.66	7.82 ± 1.91	10.00 ± 2.42	6.07 ± 1.08	7.59 ± 1.07	5.23 ± 1.04
CPB eggs	0	0	0.42 ± 0.15	0.30 ± 0.09	1.02 ± 0.15	0.87 ± 0.12	2.05 ± 0.22	0.98 ± 0.18	1.28 ± 0.17	0.95 ± 0.29	1.05 ± 0.43	0.32 ± 0.07
Flea beetles	0.35 ± 0.13	0.57 ± 0.16	2.02 ± 0.19	2.33 ± 0.23	0.85 ± 0.15	1.55 ± 0.21	3.48 ± 0.62	1.12 ± 0.16	0.57 ± 0.22	3.00 ± 0.37	3.59 ± 0.47	4.77 ± 0.57
PTM mines	0.23 ± 0.08	0.18 ± 0.06	0.10 ± 0.04	0.73 ± 0.18	1.38 ± 0.17	0.40 ± 0.10	1.18 ± 0.24	0.084 ± 0.04	0.82 ± 0.15	0.68 ± 0.10	0.27 ± 0.07	0.23 ± 0.06
Green peach aphid	0.12 ± 0.07	0.02 ± 0.02	0.18 ± 0.06	0.22 ± 0.06	0.92 ± 0.20	0.28 ± 0.11	0.07 ± 0.04	0	0.02 ± 0.02	0	0.36 ± 0.14	0.67 ± 0.16
Cotton aphid	0.07 ± 0.04	0.05 ± 0.03	0.13 ± 0.07	0.17 ± 0.07	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.02	0	0.05 ± 0.04	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02
Cicadella viridis	0	0.18 ± 0.07	0.18 ± 0.06	0.23 ± 0.06	0.07 ± 0.04	0.08 ± 0.04	0.20 ± 0.07	0.17 ± 0.07	0	0.07 ± 0.03	0	0
Cicadellidae	0.03 ± 0.02	0.18 ± 0.08	0.37 ± 0.08	0.55 ± 0.09	0.53 ± 0.11	0.82 ± 0.31	0.67 ± 0.16	1.37 ± 0.23	0.83 ± 0.18	3.14 ± 0.31	2.34 ± 0.32	1.60 ± 0.20
Thripidae	0	0.03 ± 0.02	0.02 ± 0.02	1.38 ± 0.38	0.80 ± 0.23	0.03 ± 0.02	0.17 ± 0.05	0.03 ± 0.02	0	0.14 ± 0.06	0.12 ± 0.06	0
Lygus	0	0	0.07 ± 0.03	0.12 ± 0.05	0	0.10 ± 0.05	0	0.03 ± 0.02	0	0	0	0.02 ± 0.02
Pentatomidae	0	0	0	0	0.17 ± 0.05	0.05 ± 0.03	0.10 ± 0.05	0.08 ± 0.04	0.13 ± 0.06	0.19 ± 0.07	0.14 ± 0.05	0.15 ± 0.05
Agromyzidae	0	0.03 ± 0.03	0.02 ± 0.02	0	0.10 ± 0.04	0.33 ± 0.13	0.47 ± 0.09	0.10 ± 0.04	0.23 ± 0.06	0.35 ± 0.13	0	0.15 ± 0.05
Lacewing adults	0	0	0	0	0.10 ± 0.04	0.17 ± 0.05	0.42 ± 0.10	0.03 ± 0.08	0.18 ± 0.05	0.31 ± 0.09	0.09 ± 0.04	0.03 ± 0.02
Lacewing larvae	0	0	0.10 ± 0.04	0.22 ± 0.06	0.15 ± 0.05	0	0.07 ± 0.03	0.03 ± 0.02	0.20 ± 0.06	0.14 ± 0.05	0.07 ± 0.03	0.10 ± 0.04
Lacewing eggs	0.43 ± 0.14	0.72 ± 0.16	1.25 ± 0.19	1.73 ± 0.28	7.22 ± 0.88	3.65 ± 0.41	5.00 ± 0.67	4.57 ± 0.46	5.23 ± 0.53	6.64 ± 0.63	1.88 ± 0.21	2.48 ± 0.35
Macrolophus	0.10 ± 0.039	0	0.20 ± 0.06	0.32 ± 0.07	0.05 ± 0.03	0	0	0.03 ± 0.02	0	0.45 ± 0.11	0.25 ± 0.06	0.30 ± 0.06
Cyrtopeltis	0	0	0.52 ± 0.14	0	0	0.07 ± 0.03	0	0.13 ± 0.06	0.13 ± 0.05	0.32 ± 0.11	0.27 ± 0.09	0.07 ± 0.04
Dicyphus	0	0	0.03 ± 0.02	0.05 ± 0.04	0	0	0.13 ± 0.05	0.07 ± 0.03	0.03 ± 0.02	0	0	0
Miridae	0.10 ± 0.04	0	0.82 ± 0.17	0.48 ± 0.10	0.05 ± 0.03	0.43 ± 0.10	0.13 ± 0.05	0.27 ± 0.08	0.17 ± 0.05	1.34 ± 0.25	0.51 ± 0.10	1.02 ± 0.21
Anthocoridae	0.25 ± 0.16	0.27 ± 0.16	0	0	0.55 ± 0.13	0.98 ± 0.22	0.53 ± 0.09	0.17 ± 0.05	0.18 ± 0.06	0.70 ± 0.18	1.66 ± 0.21	2.10 ± 0.25
Nabidae	0.02 ± 0.02	0.03 ± 0.03	0.02 ± 0.02	0.18 ± 0.07	0.27 ± 0.07	0.08 ± 0.036	1.02 ± 0.21	0.07 ± 0.03	0.32 ± 0.09	0.31 ± 0.22	0.02 ± 0.02	0.03 ± 0.02
Staphylinidae	0.02 ± 0.02	0	0.08 ± 0.04	0.33 ± 0.09	0.03 ± 0.02	0.07 ± 0.03	0.12 ± 0.05	0	0	0.03 ± 0.02	0	0.03 ± 0.02
Coccinella	0	0	0.03 ± 0.02	0.03 ± 0.02	0.25 ± 0.06	0.12 ± 0.04	0.02 ± 0.02	0.02 ± 0.02	0.03 ± 0.02	0.12 ± 0.04	0.22 ± 0.05	0.20 ± 0.05
Hippodamia	0	0	0.22 ± 0.05	0.20 ± 0.052	0.18 ± 0.05	0.33 ± 0.06	0.18 ± 0.05	0.25 ± 0.06	0.23 ± 0.06	0.15 ± 0.05	0.02 ± 0.02	0.03 ± 0.02
Ladybird larvae	0	0	0	0	0	0	0	0	0	0.02 ± 0.02	0.03 ± 0.03	0.12 ± 0.12
Stethorus	0.05 ± 0.03	0.02 ± 0.02	0	0	0.05 ± 0.04	0.13 ± 0.09	0.08 ± 0.04	0.05 ± 0.03	0.03 ± 0.02	0.09 ± 0.04	0	0.07 ± 0.032
Araneae	0.017 ± 0.017	0.07 ± 0.03	0.18 ± 0.06	0.47 ± 0.10	0.53 ± 0.09	0.25 ± 0.07	1.00 ± 0.16	0.30 ± 0.06	1.02 ± 0.13	0.70 ± 0.13	0.63 ± 0.12	0.47 ± 0.09
22-spots	0	0	0.13 ± 0.04	0.03 ± 0.02	0.15 ± 0.05	0.42 ± 0.06	0.28 ± 0.06	0.37 ± 0.06	0.22 ± 0.05	0.22 ± 0.05	0.12 ± 0.04	0.15 ± 0.05

OTU	5 Sept.		17 Sept.		25 Sept.		2 Oct.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
CPB adults	0.23 + 0.08	0.22 + 0.10	1.50 + 0.22	2.10 + 0.37	1.88 + 0.23	2.30 + 0.29	5.18 + 0.60	3.00 + 0.34
CPB large	5.40 + 0.68	2.83 + 0.60	2.20 + 0.30	4.73 + 0.67	2.30 + 0.44	3.18 + 0.59	0.65 + 0.19	2.58 + 0.49
CPB small	6.03 + 1.09	4.72 + 1.43	1.23 + 0.25	4.47 + 0.73	0.22 + 0.14	0.98 + 0.33	0.32 + 0.27	1.37 + 0.41
CPB eggs	0.13 + 0.05	0.13 + 0.04	0.07 + 0.03	0.07 + 0.03	0.02 + 0.02	0.03 + 0.02	0.02 + 0.02	0.03 + 0.02
Flea beetles	5.57 + 0.76	5.52 + 0.63	5.97 + 0.68	6.25 + 0.76	8.47 + 1.02	4.25 + 0.43	9.42 + 0.73	4.30 + 0.61
PTM mines	0.27 ± 0.13	0.18 ± 0.07	0.30 ± 0.09	0.38 ± 0.09	0.10 ± 0.05	0.20 ± 0.07	0.03 ± 0.02	0.28 ± 0.07
Green peach aphid	0	0	0	0	0	0	0	0
Cotton aphid	0	0	0	0	0.02 ± 0.02	0	0	0
Cicadella viridis	0	0.02 ± 0.02	0	0.02 ± 0.02	0	0	0	0
Cicadellidae	1.65 ± 0.21	0.47 ± 0.10	0.57 ± 0.14	0.67 ± 0.11	0.40 ± 0.09	0.85 ± 0.12	0.57 ± 0.12	0.45 ± 0.09
Thripidae	0	0.05 ± 0.03	0	0	0	0.58 ± 0.15	0	0
Lygus	0	0	0.02 ± 0.02	0	0.02 ± 0.02	0	0	0
Pentatomidae	0.10 ± 0.04	0	0.10 ± 0.05	0.03 ± 0.02	0.15 ± 0.05	0.10 ± 0.05	0.07 ± 0.03	0.13 ± 0.06

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Table 4. Continued

OTU	5 Sept.		17 Sept.		25 Sept.		2 Oct.	
	Bt	Control	*Bt*	Control	*Bt*	Control	*Bt*	Control
Agromyzidae	0.45 ± 0.09	0	0	0.17 ± 0.08	0.02 ± 0.02	0	0.02 ± 0.02	0
Lacewing adults	0.23 ± 0.07	0.07 ± 0.03	0.17 ± 0.05	0.12 ± 0.05	0	0.02 ± 0.02	0.13 ± 0.04	0.02 ± 0.02
Lacewing larvae	0.07 ± 0.03	0.20 ± 0.06	0.05 ± 0.03	0.02 ± 0.02	0.02 ± 0.02	0	0.08 ± 0.04	0.07 ± 0.03
Lacewing eggs	2.33 ± 0.27	1.98 ± 0.26	0.77 ± 0.12	1.30 ± 0.18	0.40 ± 0.08	0.83 ± 0.12	0.32 ± 0.08	0.55 ± 0.09
Macrolophus	0	0	0.12 ± 0.04	0.70 ± 0.21	1.17 ± 0.06	0.15 ± 0.05	0.17 ± 0.05	0.13 ± 0.04
Cyrtopeltis	1.24 ± 0.19	0.48 ± 0.13	0.05 ± 0.05	0.02 ± 0.02	0.13 ± 0.07	0	0.12 ± 0.04	0.13 ± 0.04
Dicyphus	0	0.18 ± 0.08	0.10 ± 0.05	0	0	0.05 ± 0.03	0	0
Miridae	2.48 ± 0.78	0.70 ± 0.17	0.48 ± 0.11	1.00 ± 0.27	0.40 ± 0.10	0.27 ± 0.07	0.32 ± 0.07	0.28 ± 0.06
Anthocoridae	1.93 ± 0.34	0.55 ± 0.19	0.13 ± 0.04	0.55 ± 0.14	0.80 ± 0.16	0.48 ± 0.09	0.43 ± 0.10	0.08 ± 0.04
Nabidae	0.02 ± 0.02	0.18 ± 0.06	0.07 ± 0.03	0.03 ± 0.02	0	0.05 ± 0.03	0.03 ± 0.02	0.12 ± 0.04
Staphylinidae	0	0	0	0.02 ± 0.02	0	0	0	0
Coccinella	0	0	0	0.02 ± 0.02	0	0.10 ± 0.04	0	0.12 ± 0.04
Hippodamia	0.07 ± 0.034	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.02	0	0	0
Ladybird larvae	0	0	0	0.02 ± 0.02	0	0.10 ± 0.04	0	0.12 ± 0.04
Stethorus	0	0	0	0.02 ± 0.02	0	0.10 ± 0.04	0	0.12 ± 0.04
Araneae	0.87 ± 0.13	0.58 ± 0.11	1.13 ± 0.28	0.52 ± 0.20	0.87 ± 0.15	0.72 ± 0.12	0.58 ± 0.10	0.65 ± 0.11
22-spots	0.03 ± 0.02	0.05 ± 0.03	0	0	0.02 ± 0.02	0	0	0

with wheat and various vegetables. Six 200-m^2 plots were prepared in each year, three of which were planted with transgenic eggplants and three with their near isogenic control, according to a completely randomized design. Eggplants were mulched and placed in paired rows spaced 2 m apart. The distance between rows in each pair and between plants along rows was 50 cm. Plant density was 2 plants/m^2; therefore, a total number of 1,200 plants were placed per treatment in each field experiment. Eggplants were cultivated following traditional cultural practices (La Malfa 1990). No pesticides were sprayed. Biosafety measures were adopted according to EU legislation for the deliberate environmental release of genetically modified organisms.

Species Sampling Procedures. Information was collected on the arthropod assemblages by making weekly visual observations of the plant canopy. All aerial parts of 20 randomly selected plants per plot were carefully checked for arthropods. Leaves were checked on both sides, but insects were not removed from leaves. Observations started at 0800 hours, and all sampling was completed in ~3 h. All specimens found on the plants were recorded, and data were pooled in organismal taxonomic units (OTUs) based on (1) their ecological role in the food web, (2) their potential exposure to the Cry3Bb toxin expressed in plants, and (3) feasibility of visual identification on plants. The complete list of OTUs is given in Table 1.

Community Analysis. Species assemblages were compared between treatments by means of correspondence analysis (CA; Benzécri 1973), whereas differences between treatments were tested using the multi-response permutation procedure (MRPP; Zimmerman et al. 1985). Associations between taxa and treatments were defined on the basis of using an indicator species analysis (ISA; Dufrene and Legendre 1997).

Ordination techniques are widely used for summarizing species responses to environmental factors, both along gradients (thus analyzing coenoclines) and through time (thus analyzing ecological successions). They can be divided into two broad categories relative to the way environmental information is considered. In cases where environmental data are explicitly included in the analysis, usually constraining the ordination of species, a "direct gradient analysis" is performed. An "indirect gradient analysis" is performed in cases where only species composition is considered, and relationships with environmental variables are inferred based on patterns in species distribution. Nonmetric multidimensional scaling (Kruskal 1964) and correspondence analysis (Benzécri 1973) are the most widely used indirect gradient analysis methods. Each method has its own strengths and weaknesses, but when species count data are considered, and unimodal species responses are assumed, CA is the most suited ordination technique, and this is the reason why it was selected for this study. Unlike many other ordination techniques, CA is aimed at maximizing a weighted correlation between species scores and sample scores, the weight being the abundance of the species. Therefore, the eigenvalue of the first CA axis

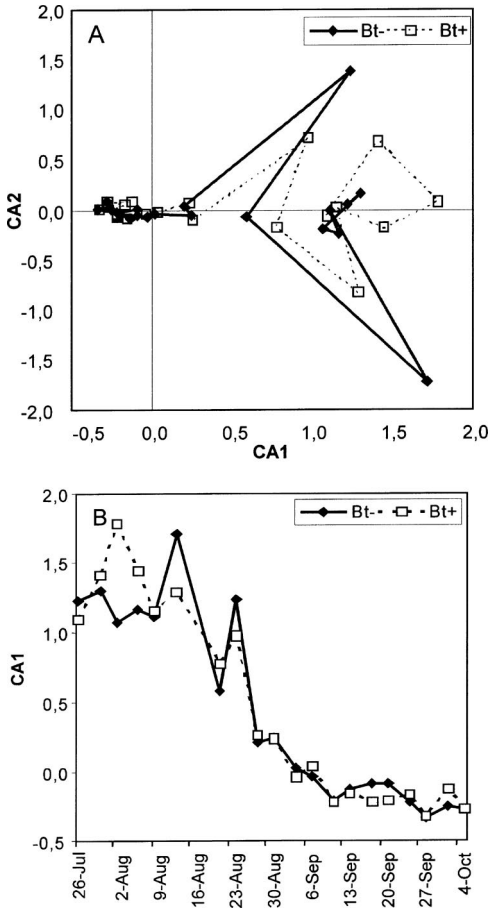

Fig. 1. Correspondence analysis ordination of 2001 field observations. (A) Ordination of arthropod samples from transgenic (*Bt*+) and control (*Bt*−) eggplant plots in the space defined by the first two axes (CA1 and CA2). (B) Successional dynamics of species assemblage in transgenic (*Bt*+) and control (*Bt*−) plots as summarized by CA1 scores.

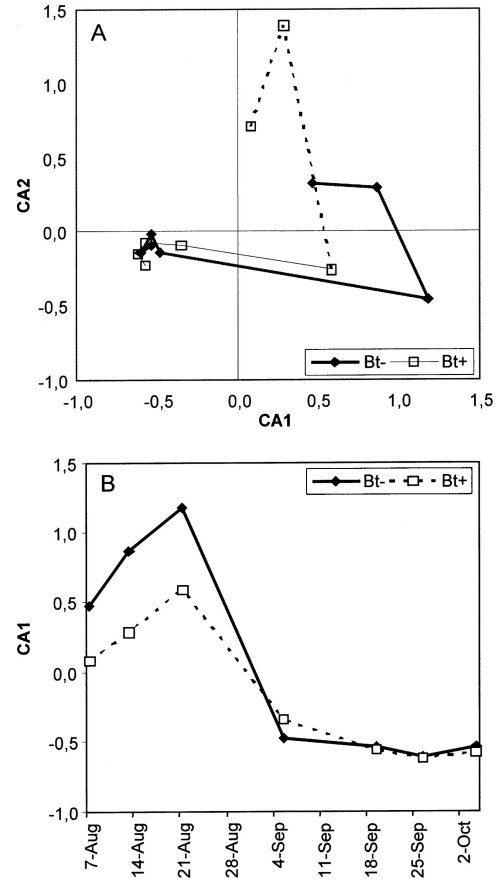

Fig. 2. Correspondence analysis ordination of 2002 field observations. (A) Ordination of arthropod samples from transgenic (*Bt*+) and control (*Bt*−) eggplant plots in the space defined by the first two axes (CA1 and CA2). (B) Successional dynamics of species assemblage in transgenic (*Bt*+) and control (*Bt*−) plots as summarized by CA1 scores.

is equivalent to the correlation coefficient between species scores and sample scores (Gauch 1982, Pielou 1984). The second and higher axes also maximize the correlation between species scores and sample scores, but they are constrained to be uncorrelated with (orthogonal to) the previous axes. In CA ordinations, each species is represented by a point, which can be regarded as an estimate of the species optimum relative to the environmental features of samples.

The MRPP was first introduced by Mielke et al. (1976) as a technique for detecting the difference between a priori classified groups. It turned out to be an extremely versatile data-analytic framework from which a number of applications are spin-offs, such as the measurement of agreement, multivariate correlation and association coefficients, and the detection of autocorrelation (see Mielke and Berry 2001 for a complete coverage of applications of the MRPP framework). MRPP is often analogous to parametric tests such as the *t*-test or analysis of variance (ANOVA). Indeed, it has been shown that many "classical" tests are special cases of MRPP. For instance, Mielke and Berry (1994) showed the equivalence between members of the MRPP family of statistics and the ANOVA/MANOVA test statistics. What makes MRPP more attractive than the parametric counterparts is its robustness under violations of the parametric assumptions (Mielke and Berry 1994), which are the rule in community ecology data sets. The MRPP statistic is a weighted average of within-group distances, where the weights are determined by the group sizes. The MRPP statistics can be tested either by means of an exact procedure based on permutations of the data set or by means of an approximated procedure, which can be applied when dealing with very large data sets.

To detect and describe the association between species and treatments, ISA (Dufrene and Legendre 1997) was applied. This is a very common goal in community analysis when groups of samples are defined either a priori or after a classification procedure. ISA provides a straightforward solution for defining species properties

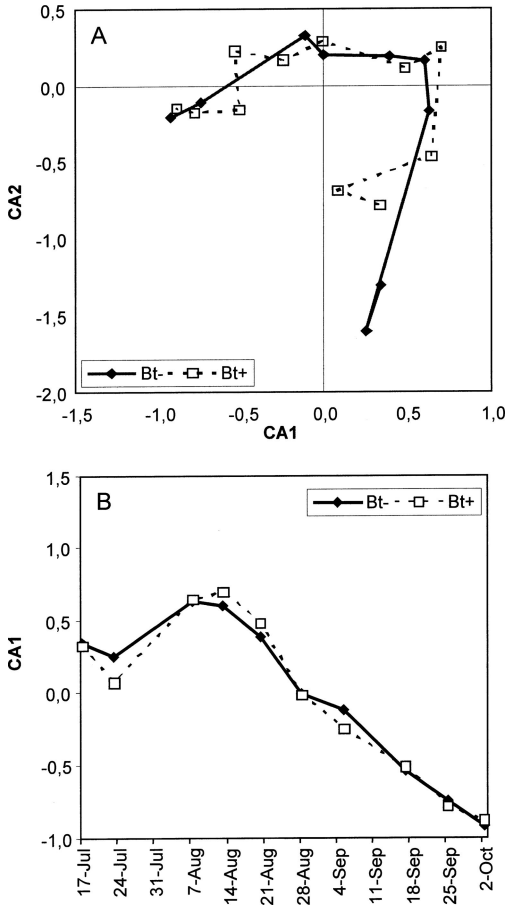

Fig. 3. Correspondence analysis ordination of 2003 field observations. (A) Ordination of arthropod samples from transgenic (*Bt+*) and control (*Bt−*) eggplant plots in the space defined by the first two axes (CA1 and CA2). (B) Successional dynamics of species assemblage in transgenic (*Bt+*) and control (*Bt−*) plots as summarized by CA1 scores.

by combining information on the abundance and frequency of occurrence of species in different groups. On this basis, an indicator value can be obtained for each species, and these values can be tested for statistical significance using a Monte Carlo technique.

Although each one of the above-mentioned methods has its own strengths, when combined into a single data analysis procedure, they are even more effective in summarizing the overall pattern of species distributions relative to treatments and time, in testing differences between groups of samples, and in identifying species that are significantly associated with groups of samples (e.g., treatments).

Results

Community Analysis. The mean number of individuals sampled is given in Tables 2–4. A taxon was retained for analysis if an individual was found on at least three different sampling dates.

Table 5. Results of ISA and MRPP on the data from eggplant field season 2001

OTU	26 July	2 Aug.	9 Aug.	13 Aug.	21 Aug.	24 Aug.	28 Aug.	31 Aug.	4 Sept.	7 Sept.	11 Sept.	14 Sept.	18 Sept.	21 Sept.	25 Sept.	28 Sept.	2 Oct.	5 Oct.
CPB adults							n.s.		n.s.	n.s.		n.s.			n.s.			
Flea beetles	n.s.	Control	n.s.	n.s.	Control	n.s.	n.s.	n.s.	*Bt+*	n.s.	n.s.	*Bt+*	*Bt+*	*Bt+*	n.s.	n.s.	n.s.	n.s.
PTM mines	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	Control	n.s.	n.s.	*Bt+*	n.s.
Green peach aphid				n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.		n.s.	
Cotton aphid	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	Control	n.s.	n.s.		*Bt+*	n.s.
Cicadellidae	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*Bt+*	n.s.	*Bt+*	n.s.	n.s.	n.s.	*Bt+*	n.s.	n.s.	n.s.	*Bt+*	n.s.
Thripidae	n.s.	n.s.		n.s.	n.s.	*Bt+*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	*Bt+*		n.s.	n.s.
Lacewing larvae				n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.		n.s.	n.s.		n.s.	n.s.
Lacewing eggs	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*Bt+*	Control	n.s.	Control	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	Control
Miridae				n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*Bt+*	n.s.	n.s.	n.s.
Anthocoridae		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Nabidae	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*Bt+*	n.s.	n.s.	n.s.	n.s.
Staphylinidae				n.s.	*Bt+*	n.s.	*Bt+*	n.s.	*Bt+*	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.	*Bt+*	n.s.
Coccinella		n.s.		n.s.			n.s.			n.s.			n.s.				n.s.	
Ladybird larvae				n.s.									n.s.				n.s	n.s
Araneae	n.s.	n.s.	n.s.	n.s.	Control	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*Bt+*	n.s.
22-spot					n.s.		n.s.	n.s.	n.s.			n.s.	n.s.	n.s.				n.s.
MRPP *P* value	n.s.	n.s.	n.s.	n.s.	0.041	n.s.	n.s.	0.007	0.035	0.019	n.s.	n.s.	0.000	0.001	n.s.	n.s.	n.s.	n.s.

OTUs that were present on a given sampling date have n.s., or *Bt+*, or Control in the corresponding column. While n.s., which stands for not significant, indicates the lack of association between a taxon and a group of samples (*Bt+* or Control), a cell with "*Bt+*" or "Control" indicates a significant association between a taxon and the displayed treatment. In the last row, the results of MRPP are indicated. Probability levels are only shown for significant values.

Table 6. Results of ISA and MRPP on data from eggplant field season 2002

OTU	7 Aug.	13 Aug.	21 Aug.	5 Sept.	19 Sept.	26 Sept.	4 Oct.
CPB adults	n.s.	n.s.	n.s.	Control	Control	Control	Control
CPB large		Control	n.s.	Control	Control	n.s.	Control
CPB small	n.s.	Control	n.s.	n.s.	n.s.	n.s.	n.s.
CPB eggs	n.s.	Control	n.s.	n.s.			
Flea beetles	n.s.	Control	*Bt*+	n.s.	n.s.	*Bt*+	Control
PTM mines	n.s.	Control	n.s.	n.s.	n.s.	*Bt*+	n.s.
Green peach aphid	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Cotton aphid	n.s.	Control	n.s.	*Bt*+	n.s.	n.s.	n.s.
Cicadellidae	n.s.	Control	*Bt*+	*Bt*+	n.s.	*Bt*+	n.s.
Tripidae	n.s.	n.s.	n.s.	n.s.	*Bt*+	n.s.	n.s.
Lacewing larvae		n.s.	n.s.	n.s.	Control	*Bt*+	n.s.
Lacewing eggs	n.s.	n.s.	*Bt*+	*Bt*+	*Bt*+	n.s.	n.s.
Macrolophus	n.s.	n.s.	n.s.	*Bt*+	n.s.	*Bt*+	n.s.
Cyrtopeltis		*Bt*+	*Bt*+	*Bt*+	n.s.	n.s.	n.s.
Dyciphus		n.s.	n.s.	n.s.	n.s.	n.s.	Control
Miridae			n.s.	n.s.			*Bt*+
Anthocoridae	n.s.	n.s.	n.s.	Control	n.s.	n.s.	n.s.
Nabidae	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Staphylinidae		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Coccinella		n.s.	n.s.	*Bt*+	n.s.	n.s.	n.s.
Ladybird larvae	n.s.			*Bt*+	n.s.	n.s.	n.s.
Ladybird pupae	n.s.	n.s.		n.s.	n.s.	n.s.	n.s.
Stethorus	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Araneae	n.s.	n.s.	n.s.		n.s.	n.s.	n.s.
22-spot		n.s.	n.s.	n.s.	n.s.	n.s.	
MRPP *P* value	n.s.	0.000	n.s.	0.000	0.001	0.003	0.002

OTUs that were present on a given sampling date have n.s., *Bt*+, or Control in the corresponding column. While n.s., which stands for not significant, indicates the lack of association between a taxon and a group of samples (*Bt*+ or Control), a cell with *Bt*+ or Control indicates a significant association between a taxon and the displayed treatment. In the last row, the results of MRPP are indicated. Probability levels are only shown for significant values.

The degree of infestation by the target insect pest, *L. decemlineata*, increased each year, and during 2003, the action threshold for their control was reached in the nontransgenic plots. Among the nontarget herbivores, the Chrysomelidae, Alticinae, and Cicadellidae were always very abundant. With regard to generalist predators, the eggs laid by Chrysopidae were always abundant, whereas the density of Coccinellidae was variable between years, being the most abundant and diverse in terms of populations during the 2003 field season. A fungivore species, *Psyllobora vigintiduopunctata* L. (Coleoptera:Coccinellidae), was commonly found in the experimental fields.

Correspondence Analysis. The results are separately presented for each field season in Figs. 1–3. The ordination in the space defined by the first two axes is displayed. Sample scores relative to the first axis, which condense the most relevant features in community structure changes, also are shown against time in a separate plot, aimed at summarizing successional patterns.

In Fig. 1A, samples are plotted in the space defined by the first two axes, which explain 39.85 and 19.11% of the total variance, respectively. For data collected in 2001, the observations in the left part of the ordination are very close to the first axis. They are much more scattered at the opposite end of this axis, where the first samples in the time series are located. However, there is no clear separation between the treatments. The successional pattern in species assemblages, obtained by plotting the first axis score of samples against time (Fig. 1B), clearly shows similar trends for transgenic and control samples, especially in the last section of the time series, where the curves tend to overlap.

Analysis of the 2002 field data (Fig. 2) shows a similar situation, although somewhat simplified in terms of successional dynamics. As in the previous case, the first axis is related to temporal changes in community structure, whereas no major differences exist between treatments.

The ordination of field data collected in the 2003 growing season is shown in Fig. 3. Again, there is no clear-cut separation between the two treatments, and positive coordinates along the first axis are observed for early samples.

MRPP and ISA. For each sampling date throughout the 3 yr of field studies, species distribution relative to treatments and differences between treatments were analyzed by means of ISA and MRPP, respectively. The results of these statistical tests are shown in Tables 5–7, in which each column corresponds to a sampling date, i.e., to a set of samples collected in both treatment and control plots. For each sampling date and OTU, significant association with either of the treatments are indicated. In the last row, *P* values are given for comparisons in which the within-group variability was significantly lower than expected, thus suggesting that differences between arthropod assemblage structure in *Bt*+ and control plots were not observed by chance.

Years 2002 and 2003, in which the target insect was abundant, have a higher number of significant values in the MRPP test during late season, when CPB is almost constantly associated with control plots. Apart from the target species, some groups are significantly

Table 7. Results of ISA and MRPP on the data from eggplant field season 2003

OTU	17 July	23 July	7 Aug.	13 Aug.	20 Aug.	28 Aug.	05 Sept.	17 Sept.	25 Sept.	02 Oct.
CPB adults	n.s	n.s	n.s	n.s	Control	*Bt+*	n.s.	Control	Control	n.s
CPB large				n.s	n.s	n.s	Control	Control	Control	n.s
CPB small			n.s	n.s	n.s	n.s	n.s.	Control	n.s.	n.s
CPB eggs		n.s	n.s	n.s	n.s	n.s	n.s.	n.s.	n.s.	n.s
Flea beetles	n.s	n.s	n.s	Control	n.s	n.s	n.s.	n.s.	*Bt+*	*Bt+*
PTM mines	n.s	Control	*Bt+*	n.s	n.s	*Bt+*	Control	n.s.	n.s.	*Bt+*
Green peach aphid	n.s	Control	n.s	n.s	n.s	n.s				
Cotton aphid	n.s	Control	*Bt+*	n.s	n.s	n.s			n.s.	
Cicadella viridis	Control	n.s	n.s	*Bt+*	n.s		n.s	n.s		
Cicadellidae	n.s	n.s	*Bt+*	n.s	Control	n.s	n.s.	n.s.	n.s	n.s
Thripidae	n.s	Control	*Bt+*	Control	n.s	n.s	n.s.		Control	
Lygus		n.s.	n.s	n.s		n.s		n.s.	n.s	
Pentatomidae			n.s	n.s	n.s	n.s	n.s.	n.s.	n.s	n.s
Agromyzidae	n.s	n.s	n.s	n.s	n.s	n.s	n.s.	n.s.	n.s	n.s
Lacewing adults			n.s	n.s	n.s	Control	n.s.	*Bt+*	n.s.	n.s
Lacewing larvae		n.s	n.s	n.s	n.s	n.s	n.s.	n.s.	n.s.	n.s
Lacewing eggs	n.s	Control	*Bt+*	n.s	n.s	Control	Control	n.s.	n.s.	n.s
Macrolophus	n.s	n.s.	n.s	n.s	*Bt+*	n.s		n.s.	*Bt+*	Control
Cyrtopeltis		Control	n.s	n.s	*Bt+*	Control	n.s	n.s.	n.s	*Bt+*
Dicyphus		n.s.		n.s	n.s		n.s	n.s.	n.s	
Miridae	n.s.	n.s.	*Bt+*	n.s.	Control	Control	Control	*Bt+*	n.s	n.s
Anthocoridae	n.s		*Bt+*	n.s	Control	n.s	n.s.	n.s.	n.s.	n.s
Nabidae	n.s	Control	n.s	n.s	n.s	n.s	n.s.	n.s.	n.s.	n.s
Staphylinidae	n.s	Control	n.s	Control	n.s	n.s		n.s.		
Coccinella		n.s	n.s	n.s	Control	n.s		n.s.	n.s.	
Hippodamia		n.s	n.s	n.s	n.s	n.s	*Bt+*	n.s	n.s	n.s.
Ladybirds larvae					n.s	n.s		n.s.	n.s	n.s
Stethorus	n.s	n.s	n.s	n.s	n.s	n.s		n.s.	n.s.	n.s.
Araneae	n.s	Control	n.s	n.s	n.s	Control	*Bt+*	*Bt+*	*Bt+*	n.s
22-spot		n.s	n.s	n.s	n.s	n.s	n.s.		n.s.	
MRPP *P* value	n.s	0.0000	0.000	n.s	n.s	n.s	0.000	0.000	0.000	0.000

OTUs that were present in a given sampling date have n.s., *Bt+*, or Control in the corresponding column. While n.s., which stands for not significant, indicates the lack of association between a taxon and a group of samples (*Bt+* or Control), a cell with *Bt+* or Control indicates a significant association between a taxon and the displayed treatment. In the last row, the results of MRPP are indicated. Probability levels are only shown for significant values.

associated with either of the treatments at specific dates. Groups that are consistently associated with the same treatment in more than a single instance suggest group-specific biotic responses. However, these results are not independent of the abundance of each group throughout the sampling season, and therefore, they have to be regarded as clues rather than as evidence for treatment effects.

The results of the ISA reinforce the above reported findings about the role of Alticinae in explaining the difference between the treatments. The seasonal variation of flea beetle populations in the two plot types is shown in Fig. 4. None of the other taxa showed such a significant difference in terms of abundance in any field season (data not shown).

Some other groups (Staphylinidae, Cicadellidae, *Aphis gossypii* Glover) also showed an interesting pattern of association (Tables 5–7). These groups were subjected to exploratory data analyses to seek possible indications of their spatial structure under the two experimental conditions. As a general trend, when a significant association was found, three criteria were always met (data not shown): first, the species were distributed in the field according to a contagious model, their variances being much larger than their means; second, when means were significantly larger in one of the two treatments, variances and median values were proportionally higher, so that not much difference in aggregation patterns is to be expected; finally, the differences appeared more often during peaks of populations.

Discussion

The main goal of sampling for biodiversity in agroecosystems is recognizing, characterizing, and comparing patterns in specific habitats. Species-based biodiversity has been extensively studied, categorized, evaluated, and reviewed (Magurran 1988); a large array of biodiversity indices exists, along with several attempts to compare them. In the last decades, there have been significant developments in community ecology, and effective protocols based on this faunistic approach were established, for instance, in the recent Water Framework Directive (EU 2000). In this study, we aimed at detecting possible effects of Cry3Bb-expressing eggplants on selected groups of nontarget insects using a community approach.

In the first step, we analyzed the spatial and temporal structure of the arthropod fauna by means of CA, which proved to be a very useful tool for summarizing successional patterns in the species assemblage. Further details about species composition and differences between treatments were obtained from multivariate methods based on distance measures and permutation statistics (ISA and MRPP). These methods are com-

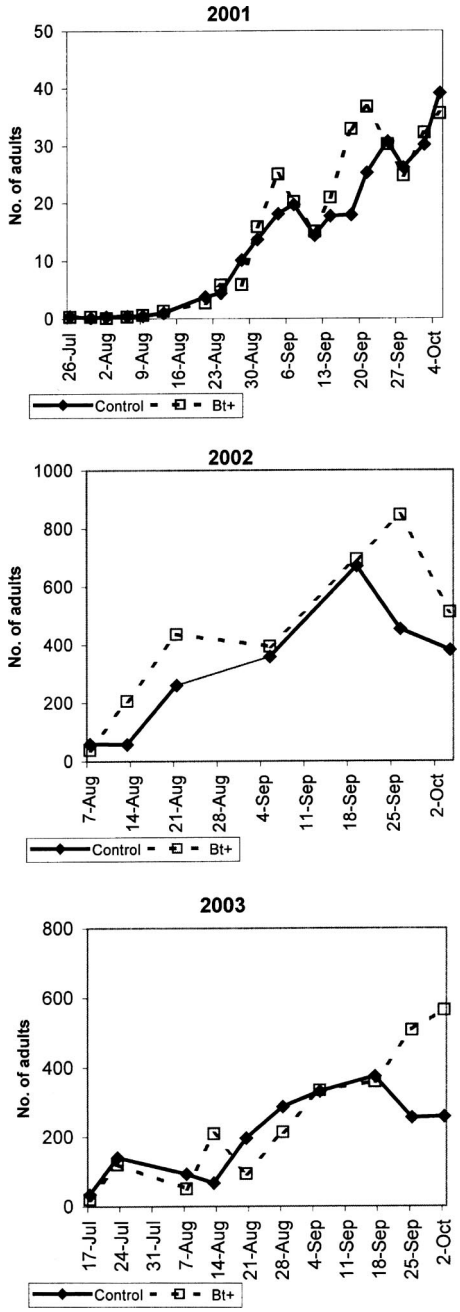

Fig. 4. Population dynamics of flea beetles in transgenic (*Bt*+) and control plots during three growing seasons.

pletely independent of those assumptions that limit the application of parametric statistics in ecological research (e.g., normal distribution of species abundances). In particular, ISA provides an effective nonparametric way for identifying taxa that are significantly associated with one among several previously defined groups (treatments). This method has been applied recently in different ecological fields (Morgan et al. 2003) to study the change in community composition in relation to common environmental variables. The combined use of MRPP and ISA provided dependable results about species assemblage composition and biotic response to different treatments while allowing the identification of species that can be used as effective indicators for further biological monitoring.

One major concern in planning surveys is that taxonomic knowledge is often partial and imperfect. Therefore, the use of organismal taxonomic units is acceptable and is preferred to lumping species into larger units (orders, families). The use of families of higher taxonomic units is not appropriate because there are few families and even fewer orders where the constituent species have the same ecological role, belong to the same guild, or have the same feeding habits. Moreover, it is likely that technicians or parataxonomists will sometimes conduct monitoring; in such situations, recognizing distinct taxonomic units will usually be reliable, but the allocation to higher taxonomic units will not.

As a prerequisite for indicating the validity of our multistep numerical approach, *Leptinotarsa decemlineata*, the target insect for the crop used, is clearly recognizable as being associated to the control plots. The comparative study of arthropod biodiversity generally indicated a similar species assemblage between the two treatments (*Bt*-expressing and near isogenic eggplants) in our experimental fields for each of the 3 yr. Pooling species together may potentially obscure any existing effects of a *Bt* crop on single species (cf. Naranjo, 2005b). However, the results obtained with this simplified sampling technique also agree with the outcome of a parallel study (Schmidt 2006), where a faunal list was obtained by identifying, in the laboratory, specimens collected with plant eclector traps.

The group of Coleoptera Alticinae (mainly *Chaetocnema tibialis* Illiger and *Epitrix hirtipennis* Melsheimer) was associated on different dates with either of the two treatments. Their overall abundance, however, was significantly higher on transgenic eggplants compared with control plots. Our results correspond with the study of Daly and Buntin (2005), who found a higher abundance of the flea beetle *Chaetocnema pulicaria* Melsheimer on *Bt*-expressing corn compared with the control. One possible explanation for this finding is that transgenic eggplants were much healthier later in the season than control plants because of their resistance to *L. decemlineata* attack. This might allow other herbivores to feed on plants where there is less competition for the same resources. The Alticinae are coleopterans in the family Chrysomelidae; therefore, they have a taxonomic proximity to the target species (the Colorado potato beetle) of Cry3Bb-expressing eggplants. This suggests these herbivores are important nontarget species that should be further studied.

This paper is the first report of a specific study on the biosafety of a GM horticultural crop, whose field management is very different in terms of area planted, agricultural practices, and resistance management from that of the more commonly studied commodity crops (corn, cotton, canola). In agreement with field studies on *Bt*-expressing cotton and corn (Daly and

Buntin 2005, Naranjo 2005b), we found no major effects on selected nontarget species caused by the presence of Cry toxins in crop plants.

The analysis of field results over a 3-yr period seems adequate to guarantee a generally acceptable sensitivity to detect the effects expected from the use of GM crops (Naranjo 2005a). Our field size, while not very different from what small farmers may devote to single horticultural crops, may have been a limitation for detecting effects on very mobile organisms (e.g., adult lacewings). Nevertheless, the size of our plots was larger than the critical minimal size indicated for field studies in corn (9 m width; Prasifka et al. 2005).

The use of a faunistic approach has been applied only recently in the fast growing literature on the biosafety of GM crops (Naranjo et al. 2005). We believe that, with a reasonably limited effort, this approach might furnish valuable ecological data about these particular agroecosystems, where the most common or abundant species might not always be the ones potentially affected by the new cropping system (Jasinski et al. 2003). Moreover, only explicit consideration of the matricial nature of food webs can avoid gross underestimates of type I errors committed while isolating one organism's dynamics from that of other co-occurring and competing taxa. This approach may also prove helpful in postrelease monitoring designs, where no case-specific monitoring is planned, but rather a general surveillance of long-term effects is requested.

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