A Comparison of Arthropod Communities in Transgenic *Bt* and Conventional Cotton in Australia

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ABSTRACT Transgenic *Bacillus thuringiensis* (*Bt*) cotton has had a major impact on the Australian cotton industry by largely controlling lepidopteran pests. However, it also may have other impacts on the invertebrate community that need to be identified. We compared the canopy invertebrate community in sprayed conventional, unsprayed conventional, and unsprayed *Bt* cotton over three seasons using suction sampling methods. We found that the diversity or species richness of the beneficial communities was reduced in the sprayed crops at two sites. Although spraying had the strongest effect on the community, there was a slight difference between the total community in unsprayed conventional and *Bt* crops, with crop type accounting for 4.5% of the variance between these communities. Out of over 100 species groups examined, the most consistent differences between unsprayed *Bt* and conventional communities were higher numbers of *Helicoverpa* in conventional crops (as would be expected) and slightly higher numbers of Chloropidae and Drosopillidae (Diptera), damsel bugs (Hemiptera, Nabidae), and jassids (Hemiptera, Cicadellidae) in conventional crops. With the advent of Bollgard II and the possibility that 80% of the cotton crop in Australia could be transgenic, the effects of these small differences in the transgenic and conventional communities should be monitored over the long-term to assess if any modifications to cotton management practices need to be made.

KEY WORDS *Bacillus thuringiensis*, *Helicoverpa*, Cry1Ac, beneficals, biodiversity

LEPIDOPTERAN SPECIES, particularly *Heliothis* and *Helicoverpa* species, are key pests of cotton worldwide, capable of dramatically reducing cotton yield through damage to flower buds (squares) or maturing fruit (bolls) (Luttrell et al. 1994). Control of these pests has often relied on the use of broad-spectrum insecticides, which disrupt beneficial populations, often leading to pest resurgence and outbreaks of secondary pests, as well as risks of off-farm movement of pesticides and environmental contamination. Therefore, these pests have been a major challenge to the development of integrated pest management (IPM) systems in cotton.

IPM practitioners have long sought alternatives that are efficacious against lepidopteran pests, selective against beneficials and with low mammalian toxicity or environmental risk. One such option has been the bacterium *Bacillus thuringiensis* variety *kurstaki* Berliner (*Bt*), which has been cultured commercially and formulated as a biopesticide spray against *Heliothis*/*Helicoverpa* for over 30 yr (Van Rie 2000). The sprayed formulations contain a number of Cry toxins as well as the infective spore. Unfortunately, although the spray is selective against most beneficial groups and has low mammalian toxicity and environmental risk, its efficacy is variable and generally poor compared with conventional insecticides, and this has limited its use and value for IPM in cotton.

One *Bt* protein, the Cry IAc δ endotoxin, also has been available commercially in genetically modified cotton (*Bt* cotton) since 1996 (Perlak et al. 2001). Here we use *Bt* cotton to designate plants expressing only the Cry1Ac protein (known as Ingard in Australia and Bollgard® elsewhere in the world). In contrast to *Bt* sprays, *Bt* cottons have provided much more consistent control of *Heliothis*/*Helicoverpa* spp. and have had a major impact on cotton production wherever they have been commercially adopted by significantly reducing pesticide inputs (Benedict and Altman 2001, Fitt and Wilson 2000, Perlak et al. 2001, Qaim 2003). As a result, *Bt* cotton has provided a valuable tool for developing IPM strategies in cotton (Wilson et al. 1998, Fitt and Wilson 2000, Wu 2001).

The sprayable form of *Bt* differs from the transgenic form. The *Bt* spray contains Cry proteins, present in a nonactivated form that must be activated in the insect's gut to be toxic, whereas *Bt* cotton has a truncated form of one insecticidal protein (Cry IAc) that does not require additional activation (Van Rie 2000). *Bt*

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sprays are UV susceptible, breaking down quickly, and because coverage is variable, the toxic components in the spray are not delivered to the target in a consistent dose. In contrast, in *Bt* cotton, the *Bt* is present throughout the growing season, although parts of the plant express different amounts of *Bt*, and the overall concentration of *Bt* declines as the season progresses (Fitt et al. 1994, 1998). Because of these differences, it is possible that the Cry1Ac protein produced in the plant interacts differently with the arthropod community than do *Bt* sprays.

Bt cotton will alter the arthropod community directly by reducing the abundance of *Helicoverpa* spp. (Hoffmann et al. 1992, Jenkins 1994) and some other lepidopteran species (Wilson et al. 1992, Flint et al. 1995). *Bt* cotton may also have indirect, although expected, effects on the abundance of predators and parasitoids that specialize on larvae of *Helicoverpa* spp. or other lepidopteran species controlled by Cry1Ac (Luttrell et al. 1994, Fitt and Wilson 2000). Whether such indirect effects extend to other, nontarget organisms is less clear and was the main rationale for this work. Hilbeck reported a tritrophic effect in laboratory studies of *Bt* corn, where lacewings [*Chrysoperla carnea* (Stephens)] that were fed on the caterpillar *Spodoptera littoralis* (Boisduval) that had fed on Cry1Ab had reduced survival compared with controls *Bt* corn containing the toxin Cry1Ab (Hilbeck et al. 1998, 1999). Lacewings were not affected when they fed on mites that had fed on *Bt* corn even though the mites contained more *Bt* toxin than the larvae, suggesting that there was an interaction between the toxin and the caterpillar (Dutton et al. 2002, 2003) that reduced the suitability of this already low-quality prey. Romeis et al. (2004) showed no direct effect of *Bt* protein on lacewings and showed clearly that the effects reported by Hillbeck were caused by reduced prey quality. Furthermore, because lacewings prefer aphids (which retain little or no *Bt* in their bodies) rather than caterpillars as prey (Meier and Hilbeck 2001), it is unclear whether the tritrophic effect observed in the laboratory would translate into lower numbers of this predator in *Bt* maize fields or in *Bt* cotton fields.

With the introduction of Bollgard II® cotton, which has two *Bt* genes (Cry1Ac and Cry2Ab), the majority (≈70% in 2004) of the cotton crop in Australia is now transgenic (A. Hurst, personal communication). Under these conditions, even a small difference in the invertebrate community could have a compounding effect. For instance, subtle effects on a component of the beneficial fauna, not easily detected in small plot research, may become more significant in the survival of a particular organism as the scale of *Bt* cotton production increases. In addition, changes in abundances of other animals that are neither pests nor beneficials could influence pest and beneficial abundances through the food web. Thus, it is important to look for changes in the whole community as well as changes in the beneficials. If there is a change in species composition in cotton, this could influence how cotton is managed using an IPM approach.

The aim of this study was to establish if the insect community in transgenic *Bt* cotton differs from that in unsprayed conventional cotton and, further, to compare these with the community found in the conventionally sprayed cotton system. Preliminary analysis of these data (Fitt and Wilson 2002) reported little numerical difference in the abundance of key beneficial and pest groups between unsprayed *Bt* cotton (Ingard; Cry 1Ac), stacked *Bt* cotton (Cry1Ac + Cry2Aa; a forerunner of later Cry1Ac/Cry2Ab combinations now commercialized as Bollgard II®), or conventional cotton. Sprayed cotton, in contrast, had significantly reduced beneficial populations. In this analysis, the aim was to more thoroughly explore the data set using ordination techniques to examine "whole of community" patterns and to ask the following: (1) are there functional groups within invertebrate families that are more affiliated with *Bt* or conventional cotton; (2) are there specific species more affiliated with *Bt* or conventional cotton; and (3) if there is no significant change in individual species, does the overall community structure of *Bt* and conventional cotton differ?

Materials and Methods

Study Sites and Agronomic Management. Experiments were carried out on three commercial cotton farms: Doreen (30°00′, 149°17′) in the Namoi Valley, Auscott Ewenmar "Ewenmar" (31°42′, 147°56′) in the Macquarie Valley, and Auscott Narrabri "Auscott" (30°12′, 149°33′) in the Namoi Valley (see Table 1 for details). Fields were selected because they were relatively isolated from other sprayed cotton, therefore reducing the chance of insecticide drift across the unsprayed areas. All the experiments involved fertilized, irrigated cotton grown on beds 1 m apart with agronomic practices that followed commercial "best practice."

Experiments were conducted over three seasons (1995/96, 1997/98, 1998/99). Doreen and Ewenmar were sampled in 1995/96, Doreen in 1997/98, and Auscott in 1998/99 (Table 1).

At each site there were three or four treatments: unsprayed conventional cotton, unsprayed *Bt* cotton (Ingard, Cry1Ac only), unsprayed stacked *Bt* cotton (Cry1Ac + Cry2Aa), and sprayed conventional cotton (Fig. 1). In the 1995/96 and 1997/98 seasons, the unsprayed conventional, unsprayed *Bt*, and unsprayed *Bt* stacked plots (included in 1997/98) were replicated twice, whereas during the 1998/99 season, all three unsprayed treatments were replicated three times. The sprayed conventional cotton treatment was not replicated in any year, although there was replication of the sampling effort. Replication of the insecticide sprayed treatment among the unsprayed treatments would have greatly increased the risk of disruption of the unsprayed treat-

Table 1. Cotton varieties and constructs used at the different sites

Season	Farm	Plot size (ha)	Planting date	Treatment	Cotton variety	*Bt* gene	*Bt* construct
1995/96	Doreen	5.17 and 4.8	12th Oct	Unsprayed *Bt*	DPL5415	Cry1Ac	MON531
		1.0 and 1.2	12th Oct	Unsprayed Conventional	DPL5415	—	—
		40.0	12th Oct	Sprayed Conventional	DPL5415	—	—
1995/96	Ewenmar	5.4 and 5.3	27th Oct	Unsprayed *Bt*	Sicala V2i	Cry1Ac	MON757
		2.4 and 1.6	27th Oct	Unsprayed Conventional	Sicala V2	—	—
		80.0	6th Oct	Sprayed Conventional	Sicala V2	—	—
1997/98	Doreen	1.9	15th Oct	Unsprayed *Bt*	Siokra V15i	Cry1Ac	MON757
		1.9	15th Oct	Unsprayed *Bt* stacked	Siokra V15 Stacked	Cry1Ac Cry2Aa	MON757 MON1849
		1.9	15th Oct	Unsprayed Conventional	Siokra V15	—	—
		4.56	15th Oct	Sprayed Conventional	Siokra V15	—	—
1998/99	Auscott	1.2	30th Sept	Unsprayed *Bt*	Siokra V15i	Cry1Ac	MON757
		1.2	30th Sept	Unsprayed *Bt* stacked	Siokra V15 Stacked	Cry1Ac Cry2Aa	MON757 MON1849
		1.2	30th Sept	Unsprayed Conventional	Siokra V15	—	—
		3.2	10th Oct	Sprayed Conventional	Siokra V15	—	—

ments with insecticide drift. This risk is exacerbated late in the season when the frequency of irrigation and the dense crop canopy preclude the use of a ground sprayer and necessitates aerial application of insecticides.

Pests in the sprayed portion of the field were managed by a professional cotton consultant who checked the field every 3–4 d and advised the grower when the crop needed spraying and the most appropriate insecticide to apply. In the 1995/96 season, the unsprayed plots were sprayed with a selective aphicide, Pirimicarb (Pirimor at 500 g/ha) to control aphids (*Aphis gossypii* Glover) that would have caused economic damage to the cotton line through honey dew contamination if left unchecked. These applications occurred on 22 February at Doreen (before the fourth to last sample) and 13 February at Ewenmar (before the fourth to last sample).

Sampling. To assess insect abundance in the crop canopy, we used a suction sampler (mini Blower Vac, Homelite B180v; Ryobi Technologies, Milperra, Australia). Samples were taken weekly (1995/96) or fortnightly (1997/98, 1998/99) from the central rows of the replicated plots. In the sprayed plots, samples were taken from two (1995/96, 1997/98) or three sites (1998/99) within the sprayed plot. Sampling began at seedling emergence and continued until ≈20% of the bolls had opened. At each replicated plot or sampling site we took five (1995/96, 1997/98) or three (1998/99) replicate suction samples, each of 10 m along a row.

To sample the cotton using the suction sampler, a single pass was made over the cotton while it was young, but for larger plants, the suction sampler was swept back and forth three times from the bottom of the plants to the top in a zigzag pattern. This was done to ensure that all strata of the plant were sampled. Collected samples were taken back to the laboratory where they were killed and counted under a dissecting microscope.

Taxonomy. The 1995/96 samples were identified at least to family and often to species for most insects and to order for most other invertebrates. Identifications were conducted using a reference collection of cotton insects at ACRI, Narrabri, or were sent for identification to the Australian National Insect Collection, Commonwealth Scientific and Industrial Research Organization Entomology, Canberra (where voucher specimens are located). Because of the large number of "unknown" insect species and the chance that many of these could have been the same species, we analyzed these samples at the level of family for most insects and order for most other invertebrates (Table 2). For the 1997/98 and 1998/99 samples, we applied a standard suction sampling classification scheme used in Australia where only key pests, predators, and parasites were identified to species level, sometimes even to developmental stage, and other insect species were identified only to order (Room and Wardhaugh 1977, Pyke and Brown 1996, Deutscher et al. 2005; Table 2). The animals were classified as predators, pests, or others.

Statistical Analysis. Because diversity indices differ in their strengths and weaknesses, it is unwise to rely on one index (Tothmeresz 1995). Consequently, we

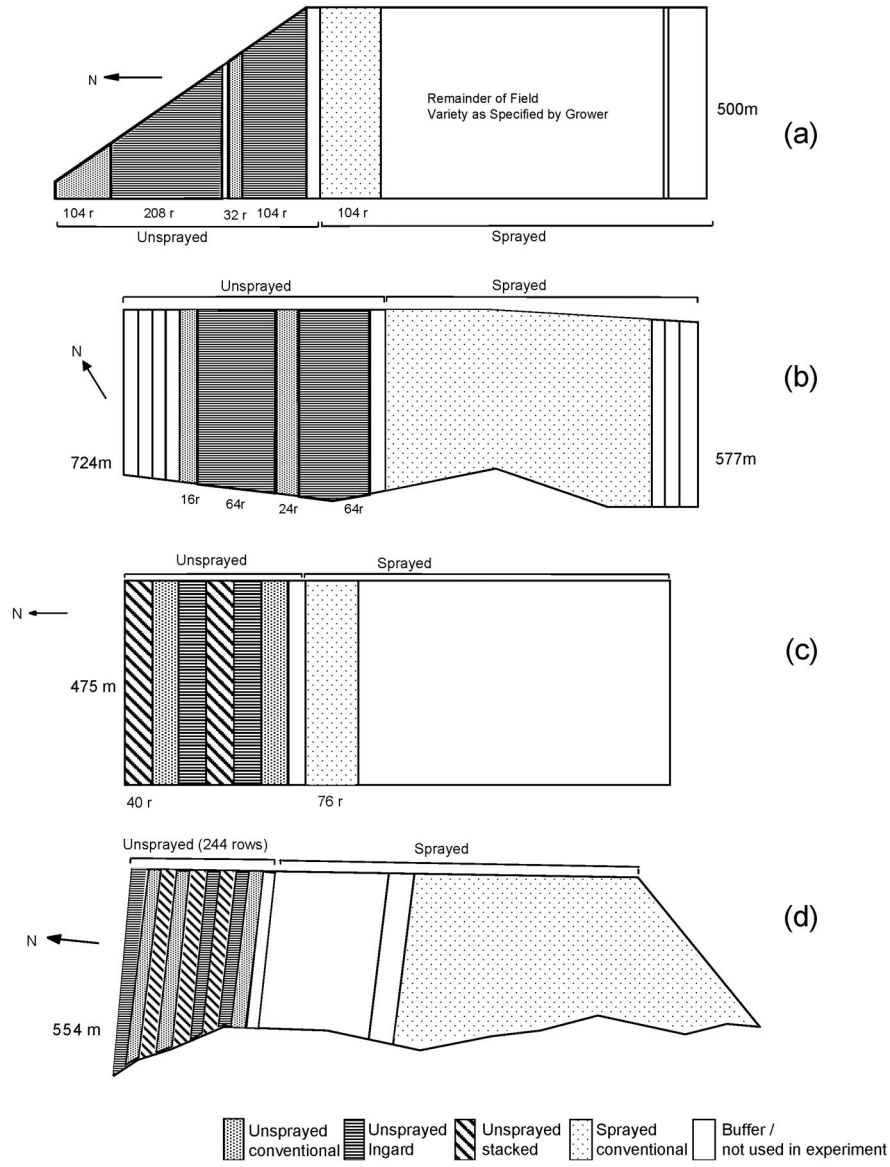

Fig. 1. Layout of the fields used in this analysis. The shaded sections are those used in the experiments. (a) Doreen, 1995/96 season. (b) Ewenmar, 1995/96 season. (c) Doreen, 1997/98 season. (d) Auscott, 1998/99 season.

used the Simpson index (SI; Simpson 1949) and Shannon Weaver index (SW; Shannon and Weaver 1949), both of which are members of Renyi's diversity index family, to measure diversity, and rarefaction curves (Sanders 1968) to measure species richness of beneficial populations. Diversity indices of the beneficial communities in each plot for each date were compared for each site using repeated-measure analyses of variance (ANOVAs), calculated using the program GENSTAT (Payne 2000). When a significant difference was detected, we compared indices using the LSD. To calculate the rarefaction curves, we used the program developed by S. M. Holland, which is available at www.uga.edu/≈strata/software/AnRare Readme.html. The Simpson index was corrected for sample-size bias $\{SI = \Sigma[(n^2 - n)/(N^2 - N)]\}$ and modified ($-\ln SI$) following Rosenzweig (1995) so that the units increase with an increase in diversity. The Simpson index is more sensitive to dominant species, whereas the Shannon Weaver index ($H = -\Sigma p_i \ln p_i$) is more sensitive to rare species.

The data were examined using principle response curve (PRC) analysis, which is a multivariate method for the analysis of repeated measures and is designed

Table 2. All the animal groups identified in the surveys, including their common name (when present) and the number of individuals found at each site

Role	Order	Family	Genus	Common name	Other characteristics	Ewenmar 1995/96 Totals	Doreen 1995/96 Totals	Doreen 1997/98 Totals	Auscott 1998/99 Totals
P	Acarina	Tetranychidae		Mites	Adult	1	3,964	39	2,247
P	Acarina	Tetranychidae		Mites	Juvenile			17	671
P	Acarina			Brown mites					7
B	Araneae	Clubionidae		Sac spider		180			
B	Araneae	Oxyopidae		Lynx spider		167			
B	Araneae	Salticidae		Jumping spider		63			
B	Araneae	Theridiidae		Tangle-web spider		45		16	209
B	Araneae	Thomisidae		Crab spider		21			
B	Araneae		"Unknown"	Spiders		1,701	1,317	1,251	1,911
P	Blattodea			Cockroaches			3		
O	Coleoptera	Anthicidae				271	93	15	
B	Coleoptera	Cantharidae				1			
B	Coleoptera	Carabidae		Ground beetles/tiger beetles		19	13		
P	Coleoptera	Cerambycidae		Long horn beetles		9			
P	Coleoptera	Chrysomelidae		Flea Beetle		226	1,265	85	244
B	Coleoptera	Coccinellidae	"Unknown"	Lady beetles		283	307	99	170
B	Coleoptera	Coccinellidae	*Diomus noescens* (Blackburn)	"Minute two-spotted ladybird"				88	86
P	Coleoptera	Curculionidae		Weevils		38	70		34
P	Coleoptera	Elateridae		Wire worms			5		
O	Coleoptera	Languriidae				4			
O	Coleoptera	Lathridiidae				598	217		
B	Coleoptera	Melyridae	*Dicranolaius belulus* (Guérin-Méneville)			692	123	110	73
O	Coleoptera	Mycetophagidae		Hairy fungus beetles		13	20		
O	Coleoptera	Nitidulidae	*Carpophilus*	Dried fruit beetles		191	159		3
O	Coleoptera	Phalacridae				23	264		
O	Coleoptera	Staphylinidae		Rove beetles		6	4		
P	Coleoptera	Tenebrionidae		Flour beetles		1	2		
O	Coleoptera		"Unknown"			2	2	256	146
B	Coleoptera		"Unknown"		Predatory			25	581
P	Coleoptera		"Unknown"		Pests				19
B	Dermaptera		"Unknown"	Earwigs			3	1	
B	Dermaptera	Labiduridae		Earwigs		1			
P	Diptera	Agromyzidae			Leaf-mining flies		3		
O	Diptera	Anthomyiidae				20			
O	Diptera	Cecidomyiidae				4	4		
O	Diptera	Ceratopogonidae				102	23		
O	Diptera	Chironomidae				1,862	794		
O	Diptera	Chloropidae				10,802	11,196		
P	Diptera	Dolichopodidae				5	2		
P	Diptera	Drosophilidae		Fruit flies		460	188		
O	Diptera	Empididae					10		
O	Diptera	Ephydridae				29	21		
O	Diptera	Fergusoninidae				5	1		
O	Diptera	Heleomyzidae					21		
O	Diptera	Lauxaneidae					30		
O	Diptera	Muscidae		House flies		18	35		
O	Diptera	Phoridae				11	20		

O	Diptera	Pipunculidae				3			
O	Diptera	Platystomatidae				31	1		
O	Diptera	Psychodidae				3	8		
O	Diptera	Pyrogtidae					3		
O	Diptera	Scatopsidae				2			
O	Diptera	Sciaridae				150	478		
B	Diptera	Syrphidae		Hoverfly		3	3	4	2
O	Diptera	Tephritidae				1	5		
O	Diptera	Teratomysidae					1		
O	Diptera	Tipulidae				1	2		
B	Diptera	Tachinidae			Parasitoid flies				4
O	Diptera		"Unknown"			453	65	1,476	2,781
P	Hemiptera	Aleyrodidae		Whitefly		14	18		2,759
O	Hemiptera	Alydidae					1		
B	Hemiptera	Anthocoridae	*Orius* spp	Pirate Bug			1	15	53
P	Hemiptera	Aphididae		Aphids		25,171	72,404	9,128	158,044
P	Hemiptera	Cicadellidae		Jassids	Adults	9,450	9,252	3,827	8,826
P	Hemiptera	Cicadellidae		Jassids	Juveniles			6,601	18,008
P	Hemiptera	Scutelleridae	*Tectocoris*			19	9		
P	Hemiptera	Pyrrhocoridae	*Dysdercus sidae* Montrouzier	"Cotton stainer"		4			1
O	Hemiptera	Lygaeidae	"Unknown"			351	257		
B	Hemiptera	Lygaeidae	*Geocoris lubra* Kirkaldy	"Bigeyed bug"				124	17
P	Hemiptera	Lygaeidae	*Nysius vinitor* Bergroth					39	361
P	Hemiptera	Lygaeidae	*Oxycarenus luctuosus* (Montrouzier)					24	23
P	Hemiptera	Miridae	"Unknown"			887	675		
P	Hemiptera	Miridae	*Campylomma liebknechti* (Girault)		Adult			131	2,717
P	Hemiptera	Miridae	*Campylomma liebknechti* (Girault)		Juvenile			8	669
P	Hemiptera	Miridae	*Creotiades dilutus* (Stål)		Adults			9	111
P	Hemiptera	Miridae	*Creotiades dilutus* (Stål)		Juvenile				232
B	Hemiptera	Miridae	*Deraeocoris signatus* (Distant)					4	34
B	Hemiptera	Miridae	*Taylorilygus pallidulus* (Blanchard)	Brokenback bug				22	
B	Hemiptera	Nabidae	*Nabus kinbergii* Reuter	"Damsel bug"		181	90	9	349
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)	Southern green stink bug		21	6		
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)		Adults				6
P	Hemiptera	Pentatomidae	*Nezara viridula* (Linnaeus)		Juvenile				88
B	Hemiptera	Pentatomidae	*Cermatulus nasalis* (Westwood)			2		4	
B	Hemiptera	Pentatomidae	*Oechalia schellengergii* (Guérin-Méneville)						15
O	Hemiptera		"Unknown"			29		53	
B	Hemiptera		"Unknown"		Predatory			2	6
P	Hemiptera		"Unknown"		Pests				15
O	Hymenoptera	Agaonidae					3		
O	Hymenoptera	Aphelinidae				3	6		
B	Hymenoptera	Apidae		Bees		15	1		
O	Hymenoptera	Bethylidae				10	13		
B	Hymenoptera	Braconidae			Parasitoid wasps	350	93		
O	Hymenoptera	Ceraphronidae				4	2		
B	Hymenoptera	Chalcididae			Parasitiod wasps	16	5		
O	Hymenoptera	Cynipidae	*Eucoilidea*		Aphid hyperparasite	7			
O	Hymenoptera	Diapriidae				28	40		
O	Hymenoptera	Elasmidae				1	2		

Table 2. Continued.

Role	Order	Family	Genus	Common name	Other characteristics	Ewenmar 1995/96 Totals	Doreen 1995/96 Totals	Doreen 1997/98 Totals	Auscott 1998/99 Totals
O	Hymenoptera	Eucoilidae					8		
O	Hymenoptera	Encyrtidae				102	56		
O	Hymenoptera	Eulophidae	"Unknown"			346	360		
O	Hymenoptera	Eulophidae	*Hemiptarsenus*		Hyperparasitoid of Leafmining fly	7	3		
O	Hymenoptera	Eupelmidae				7	6		
O	Hymenoptera	Figitidae				4			
O	Hymenoptera	Formicidae	"Unknown"	Ants		62	469	342	13
B	Hymenoptera	Formicidae	*Iridomyrmex*						84
B	Hymenoptera	Formicidae	*Paratrechina*						1
B	Hymenoptera	Formicidae	*Rhytidoponera*						2
B	Hymenoptera	Halictidae		Bees		11	1		
B	Hymenoptera	Ichneumonidae	"Unknown"		Parasitoid wasps	9	4		
B	Hymenoptera	Ichneumonidae	*Lissopimpla excelsa* (Costa)	Orchard dupe	Parasitoid wasps	1			
B	Hymenoptera	Mymaridae			Egg parasitoids	349	120		
O	Hymenoptera	Mymarommatidae					1		
O	Hymenoptera	Perilampidae				2			
B	Hymenoptera	Platygastridae	"Unknown"		Egg parasitoids	21	26		
B	Hymenoptera	Platygastridae	*Telenomus*		Egg parasitoids				22
O	Hymenoptera	Pteromalidae				132	84		
O	Hymenoptera	Scelionidae				186	83		
O	Hymenoptera	Torymidae				22	2		
B	Hymenoptera	Trichogrammatidae			Egg parasitoids	198	150	4	1,448
O	Hymenoptera		"Unknown"	Wasps		43		350	3,196
P	Isopoda			Slater				2	
P	Lepidoptera	Noctuidae	*Helicoverpa*		Larvae	741		135	370
P	Lepidoptera	Noctuidae	*Helicoverpa*		Eggs			48	
P	Lepidoptera	Tortricidae	*Crocidosema plebejana* Zeller						24
P	Lepidoptera		"Unknown"			82	407	7	41
P	Lepidoptera		"Unknown"		Moth				12
B	Neuroptera		"Unknown"	Lacewings	Juveniles		27	19	104
B	Neuroptera		"Unknown"	Lacewings	Eggs				10
B	Neuroptera	Chrysopidae	*Mallada*	"Green lacewing"	Adult	18		12	167
B	Neuroptera	Hemerobiidae	*Micromus tasmaniae* Walker	"Brown lacewing"	Adult	40			70
O	Odonata			Dragon fly		11	4		
P	Orthoptera	Acrididae		Locust				2	
P	Orthoptera		"Unknown"	Grasshoppers		12	33		14
P	Thysanoptera	Thripidae		Thrips		1,066	1,371		
P	Thysanoptera	Thripidae		Thrips	Adults			4,267	4,219
P	Thysanoptera	Thripidae		Thrips	Juveniles			7,350	1,303
	TOTALS					58,556	106,838	36,020	212,622

P, pest; B, beneficial; O, other (i.e., not a pest or beneficial).

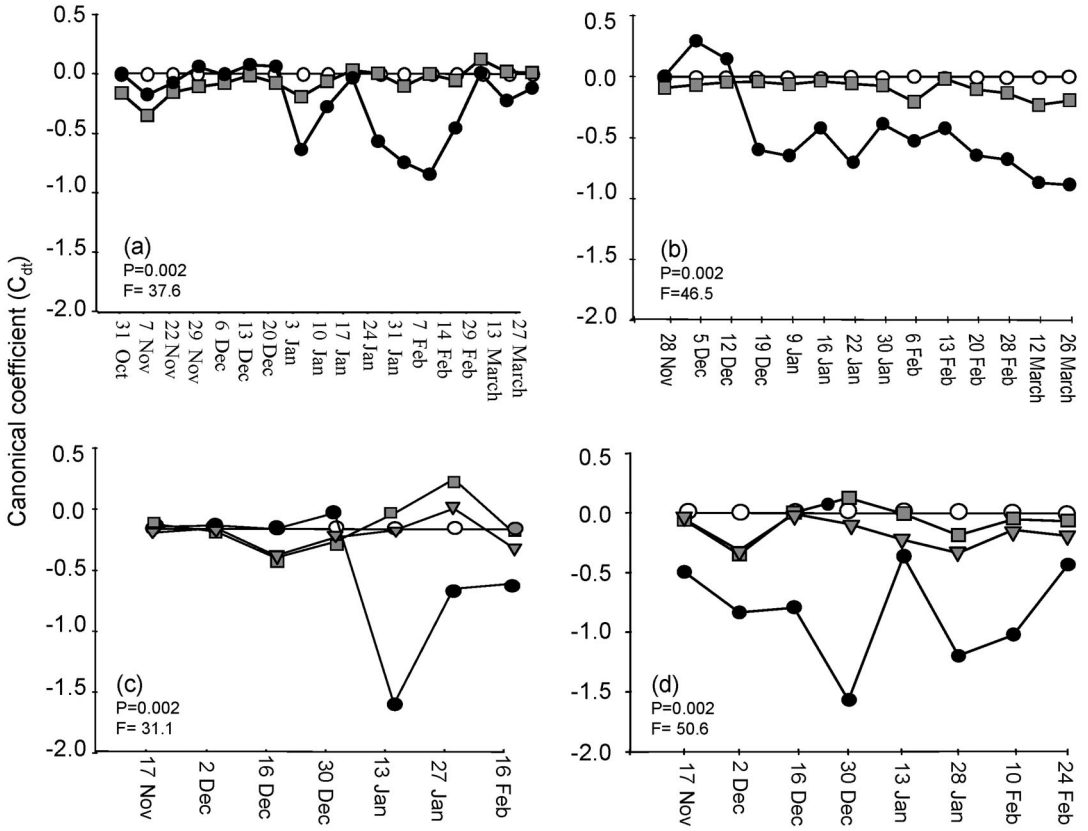

Fig. 2. PRCs of arthropod communities found in sprayed conventional, unsprayed conventional, and unsprayed *Bt*. At all sites, there was a significant difference between the communities (Monte Carlo simulation, 499 permutations). (a) Doreen, 1995/96 season: 80 taxa, 510 samples. (b) Ewenmar, 1995/96 season: 88 taxa, 420 samples. (c) Doreen, 1997/98 season: 43 taxa, 280 samples. (d) Auscott, 1998/99 season: 55 taxa, 288 samples. White circles, conventional unsprayed (control); black circles, conventional sprayed; gray squares, Ingard *Bt* cotton; gray triangles, stacked *Bt* cotton.

to test and display treatment effects that change across time. Treatment "curves" are presented relative to a standard, in this case unsprayed conventional cotton. The PRC is based on a partial redundancy analysis (the y-axis in a PRC is the first ordination axis "axis 1" of a redundancy analysis [RDA], whereas the x-axis is time) and was generated using the program CANOCO (ter Braak and Smilauer 2002). To test that the PRC explains significant treatment variance, we conducted permutation tests using the Monte Carlo method (available within the program CANOCO) on the first canonical axis of the RDA. To ensure all samples taken at each plot "traveled" together during each permutation, we did random permutations of the whole plots only.

Species groups with species weights that contributed to the overall community response (from PRC) were further analyzed by comparing their distribution on *Bt* (Cry1Ac only) and conventional cotton throughout the season at all four sites. Because of sparse data, insect counts from all samples per crop per date were combined ($n = 10$ samples for sites Doreen 95/96, Ewenmar 95/96, and Doreen 97/98 and $n = 9$ samples for site Auscott 98/99). To ensure the data from Auscott were comparable with the other sites, it was multiplied by $10/9_{\text{ths}}$. To meet the assumptions of normally distributed residuals, the counts of insect numbers were log-transformed. Despite combining the sample counts, there were still a number of zero counts, so one was added to the counts before transformation. Plots of the log-transformed insect counts versus time of sampling showed that no particular function could be adequately fitted to all the data. Therefore, the logged insect counts over time were modeled using smoothing splines (Verbyla et al. 1999), which uses the data to determine the shape of the response. This was done within a linear mixed model using ASREML (Gilmour et al. 2000). Each spline curve consists of a linear component (slope and intercept terms) and a nonlinear component (spline term). The fixed terms in the model are crop and day and their interaction. If the crop term is significant, the spline curves for each crop type have differing intercepts. Because so many species were tested, signifi-

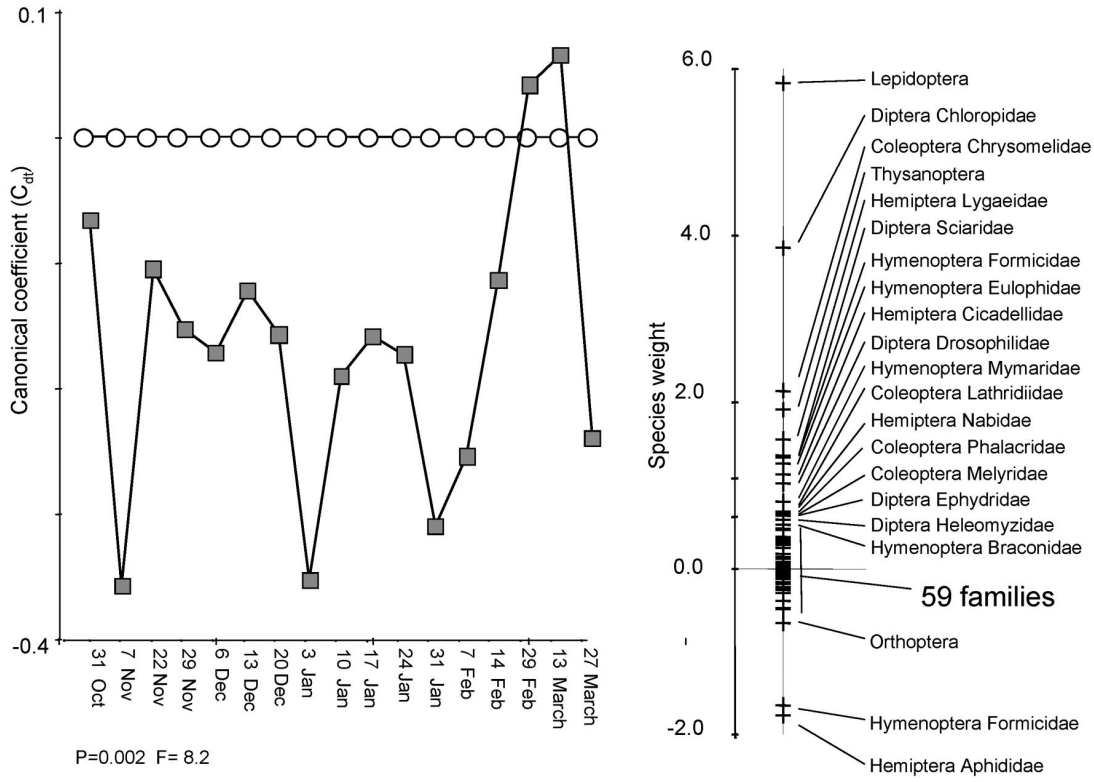

Fig. 3. PRCs and species weights of the unsprayed conventional and *Bt* cotton at site Doreen 1995/96. Taxa with species weights between −0.5 and 0.5 are not listed because these have little influence on the curves. The symbols are the same as those used in Fig. 2.

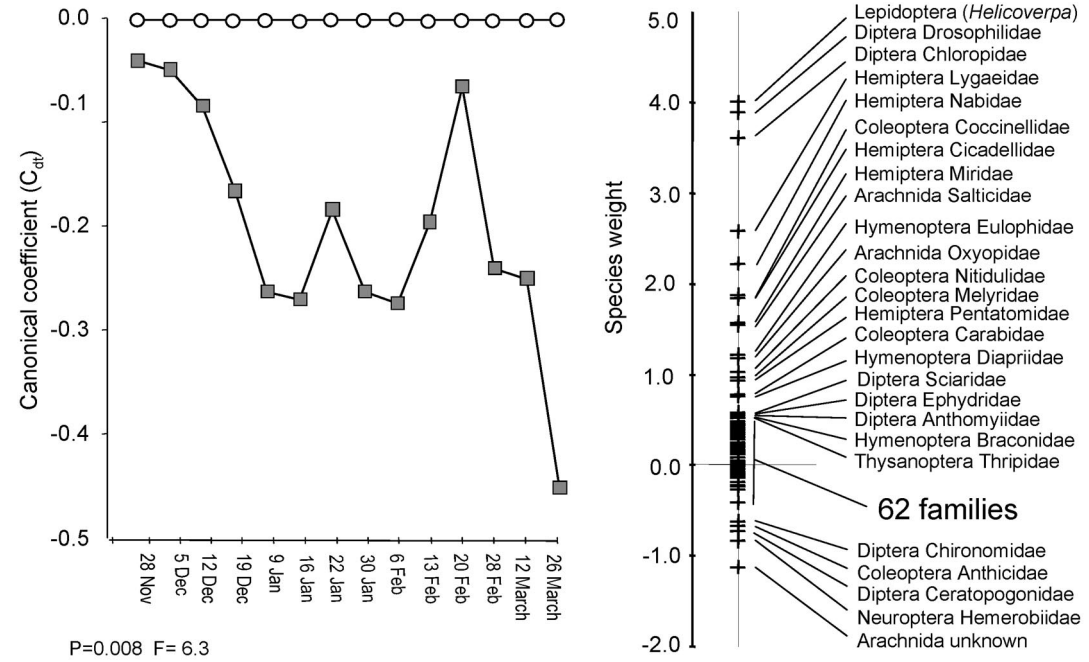

Fig. 4. PRCs of the unsprayed conventional and *Bt* cotton at site Ewenmar 1995/96. The symbols are the same as those used in Fig. 2.

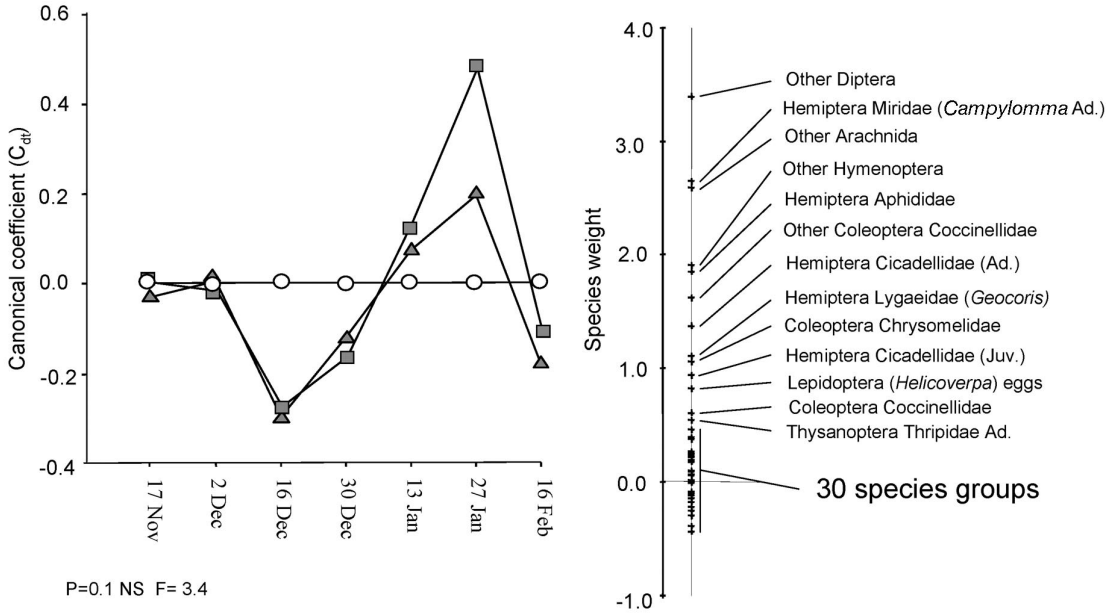

Fig. 5. PRCs of the unsprayed conventional and *Bt* cotton at site Doreen 1997/98. The symbols are the same as those used in Fig. 2.

cance for species data were accepted at 0.01, with 0.05–0.01 indicating a strong trend.

When comparing communities at the four sites, we were effectively asking the same question four times, thus possibly increasing the chance of committing a type 1 error. To correct for this, we used a modified Bonferroni procedure where *P* values were sorted from the highest to the lowest and compared with the corresponding adjusted α value (α, $\alpha/2$, $\alpha/3$, etc.; Haccou and Meelis 1992). If any *P* value was less than its adjusted α value, the null hypothesis was rejected.

Results

Community Differences in *Bt* and Conventional Cotton. PRCs (Fig. 2) indicated that at all four sites there was a significant difference between the communities of different crop types ($P = 0.002$; Fig. 2), with the community in the sprayed conventional cotton showing the most divergence. Crop type accounted for 8.9, 16.3, 9.5, and 16.6% of the variance for Doreen 1995/96, Ewenmar 1995/96, Doreen 1997/98, and Auscott 1998/99, respectively, of which 40.7, 44.4. 54.2 and 49.6%, respectively, of this variance was captured by axis 1. A large proportion of the variance in the communities was explained by changes during the season, because sampling dates accounted for 52.1, 33.7, 53, and 50.1% of the variance in the communities for Doreen 1995/96, Ewenmar 1995/96, Doreen 1997/98, and Auscott 1998/99, respectively.

To test if there was any influence of the Cry proteins on the communities, the sprayed treatment was removed from the analysis. This revealed a significant difference between the communities of unsprayed *Bt* and conventional treatments in three of the four data sets (Figs. 3–6; Table 3). The three communities with significant differences all had *P* values smaller than their adjusted α values (Table 3). This indicates that unsprayed *Bt* communities are significantly different from unsprayed conventional communities. In the communities with a significant effect of crop type, sampling dates explained 43–60% of the variance in the communities, whereas crop type accounted for 4.3–5.5% of the variance.

Species weights >0.5 (Figs. 3–6) are most likely to follow the abundance changes shown in the PRCs, whereas those less than −0.5 show a trend in the opposite direction (values between −0.5 and 0.5 do not contribute strongly to the community response; Van den Brink and Ter Braak 1999). Of the species groups that contributed to the changes depicted in the PRCs, *Helicoverpa* and Lepidoptera had high species weights, as expected (Figs. 3, 4, and 6). In addition the fly family Chloropidae also had high species weights (Figs. 4 and 5). As the *Bt* curves in the PRCs were negative and most of the species with high species weights were positive, these species were less abundant in unsprayed *Bt* crops compared with unsprayed conventional crops (Figs. 3–6).

Are Any Species Groups More Affiliated with *Bt* or Conventional Cotton? We tested eight general groups to see if there were consistent differences between the number of individuals on Ingard® and conventional cotton over the four sites by fitting a model of their distribution using smoothing splines. The *F* statistic and denominator degrees of freedom for the crop term (numerator df is 1) are shown in Table 4, together with the retransformed value of the spline curves for both crop types at the time midway through the experiment. Of these, we identified five general groups

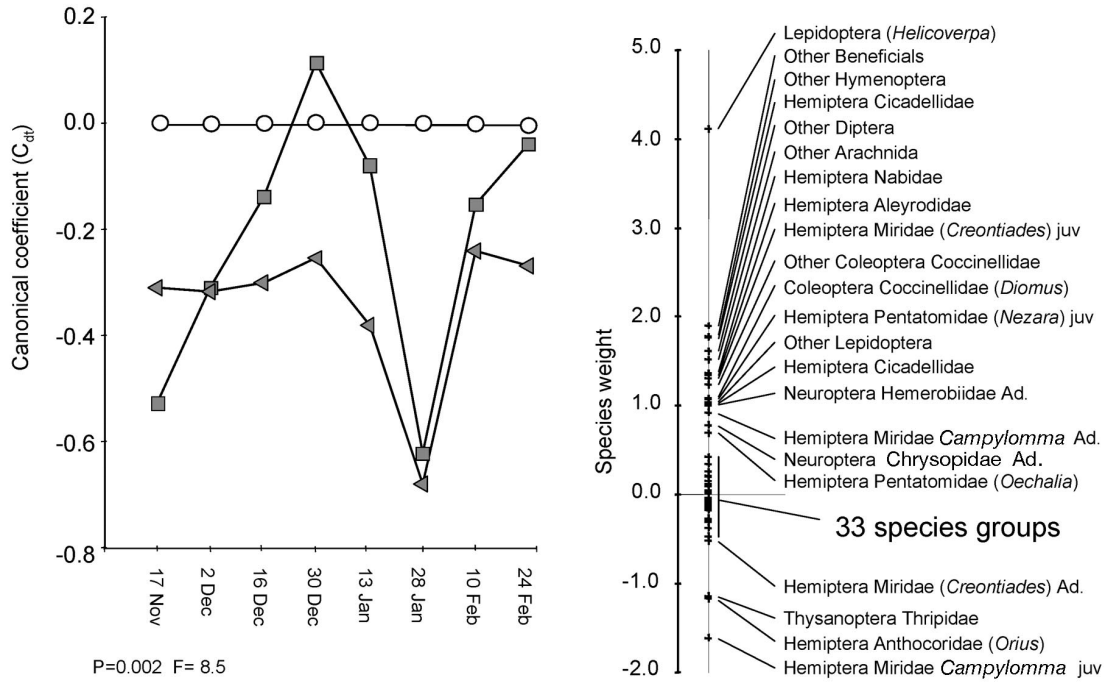

Fig. 6. PRCs of the unsprayed conventional and *Bt* cotton at site Auscott 1998/99. The symbols are the same as those used in Fig. 2.

in which $P < 0.01$, indicating that crop type significantly improved the fit of the model (Table 4; Fig. 7). For spiders and pest Hemiptera in particular, the SE of the predicted values for unsprayed conventional and *Bt* strongly overlapped. This indicates that, although there were only slight differences in the abundances of these animals in *Bt* and conventional cotton, the differences were consistent over time and between sites. For Lepidoptera and Beneficial and other Hemiptera, the SEs were more distinctive (Fig. 7).

Table 3. Characteristics of the PRC of unsprayed communities

Site	Doreen 1997/98	Ewenmar 1995/96	Doreen 1995/96	Auscott 1998/99
F value	3.4	6.3	8.2	8.5
P value (calculated using monte carlo simulation, 499 permutations)	0.1	0.008	0.002	0.002
α (calculated using improved Bonferroni procedure)	0.05	0.025	0.0167	0.0125
Variance explained by crop type	3.7%	5.2%	4.3%	5.5%
Proportion of this variance captured by axis 1	20.5%	26.7%	27.9%	30.9%
Variance explained by sampling date	56.6%	43%	55.9%	59.9%

There were 39 species groups (identified in Figs. 3–6) with high species weights. Of these, seven were discarded from further analysis because of sparse data. We modeled the distribution of individuals in Ingard® and conventional cotton over time for the remaining 32 taxa using smoothing splines. The *F* statistic and denominator degrees of freedom for the crop term (numerator df is 1) are shown in Table 5, together with the retransformed value of the spline curves for both crop types at the time midway through the experiment. Of these, we identified five taxa (two Diptera: Chloropidae and Drosopillidae; two Hemiptera: Cicadellidae and Nabidae; and one Lepidoptera: *Helicoverpa*) in which crop type improved the fit of the model (Table 5; Fig. 8). Again there was substantial overlap of the SEs of the predicted values for unsprayed conventional and *Bt* for most taxa, indicating a slight but consistent difference in the abundances of the taxa in the two crop types. *Helicoverpa* showed the least amount of overlap of the SEs.

Abundance, Diversity, and Species Richness of Beneficial Arthropods. Diversity indices of the beneficial communities (species identified in Table 2) were not affected by crop type at Doreen 1995/96 (SI: $F = 0.61$, $P = 0.55$, df = 2,27; SW: $F = 1.35$, $P = 0.27$, df = 2,30; Fig. 9a). At Doreen 1997/98, the SW index was also unaffected ($F = 0.65$, $P = 0.59$, df = 3,19), although the SI indicated that sprayed cotton was significantly more diverse than either Ingard® or stacked (SI: $F = 3.55$, $P = 0.035$, df = 3,18; LSD = 0.2165; Fig. 9c). The SI was

Table 4. Results of a spline analysis examining the effect of "crop type" (Ingard or conventional cotton) on general groups

Role	General groups	Conventional	Ingard®	df	*F*	*P*
P	Lepidoptera	7.7	3.7	42	19.5	<0.001[b]
P	Pest Hemiptera	465.0	374.6	38	10.9	0.002[b]
P	Pest Coleoptera	3.5	2.8	40	2.67	0.11
B	Hymenoptera	22.0	21.7	30	0.03	0.874
B	Spiders	31.3	24.5	44	11.5	0.001[b]
B/O	Beneficial and other Coleoptera	21.8	17.6	21	5.79	0.025[a]
B/O	Beneficial and other Hemiptera	6.8	4.0	39	25.4	<0.001[b]
O	Diptera	112.6	62.1	39	44.9	<0.001[b]

The *F* and *P* values indicate whether adding "crop type" significantly improved the model. The conventional and Ingard values are the predicted number of individuals in 10 samples in the middle of the season.

[a] *P* value between 0.05 and 0.01 (indicates a trend).

[b] *P* value <0.01 (indicates a significant difference).

P, pest; B, beneficial; O, other.

unaffected by crop type for both Ewenmar 1995/96 (SI: $F = 3.21$, $P = 0.057$, df = 2,25) and Auscott 1998/99 (SI: $F = 2.15$, $P = 0.107$, df = 3,45), although in both cases there was a significant difference in the SW index (Ewenmar 1995/96: $F = 22.86$, $P < 0.001$, df = 2,26, LSD = 0.1166; Auscott 1998/99: $F = 21.15$, $P < 0.001$, df = 3,47, LSD = 0.1562), with diversity in sprayed cotton either significantly lower than the other crops (Auscott; Fig. 9d) or significantly lower than Ingard®, which was significantly lower than unsprayed conventional (Ewenmar; Fig. 9b). There was no consistent pattern in the rarefaction curves at the four sites.

Discussion

To date, most field studies have indicated little or no change in the beneficial community on *Bt* crops in comparison to conventional crops (Sims 1995, Orr and Landis 1997). We also found little difference in the diversity or species richness of beneficial arthropods in the unsprayed *Bt* and conventional crop types. We did find that the beneficial community in sprayed crops was significantly less diverse than that in unsprayed crops at two sites according to the SW index. This pattern, however, was not supported by the SI, which, at one site, indicated that the sprayed crop was more diverse than the *Bt* crops. Because the SW index is more sensitive to rare species, the differences in the effect of crop type on the indices suggest that spraying had a stronger affect on rarer species.

Although most differences in the communities were attributable to the effect of spraying, we did identify slight differences in the invertebrate communities found in unsprayed conventional compared with unsprayed *Bt* cotton or stacked Ingard®. These differences accounted for ≈4.5% of the variability between unsprayed conventional and unsprayed transgenic cotton.

Some difference between the invertebrate communities found in unsprayed conventional and *Bt* cotton is to be expected, given that the abundance of many lepidopteran larvae has been greatly reduced in the *Bt* cotton community. Indeed, the species weights of Lepidoptera and *Helicoverpa* had the strongest influence on the PRCs. The potential for lower numbers of parasitoids or predators, which specialize on larvae of *Helicoverpa* spp. or other lepidopterans, could also contribute to the difference. The drop in larval density may account for the slight drop in spider numbers in *Bt* crops (Table 4; Fig. 7). Some workers report no effect of *Bt* crops on either the numbers of lepidopteran parasitoids present (Johnson et al. 1997, Wu and Guo 2003) or their activity (Johnson and Gould 1992, Orr and Landis 1997), whereas others report lower numbers of lepidopteran parasitoids in *Bt* crops (Pilcher et al. 2005). Overall, we found no consistent differences between the number of egg and larval parasitoids of Lepidoptera throughout the season, although the Eulophidae (Hymenoptera) showed a trend to be lower in *Bt* cotton (Table 5).

We found slightly lower numbers of Hemiptera in Ingard® and the stacked *Bt* cotton in comparison with unsprayed conventional cotton. Hemiptera includes damsel bugs (Nabidae, *Nabis kinbergii*) and jassids (Cicadellidae), both of which were in lower numbers in *Bt* cotton and may have influenced results (Fig. 8). Although there are reports of no change in damsel bug numbers in some *Bt* crops such as corn (Wold et al. 2001), our findings are in agreement with Naranjo (2005), who reported a reduction in the number of damsel bugs in a 5-yr study in *Bt* cotton, as did Daly and Buntin (2005) in their multi-year study on *Bt* corn. Observations in commercially grown *Bt* cotton crops in Australia have also shown lower numbers of damsel bugs compared with conventional crops (M. Dillon, unpublished data). Why there should be lower numbers of damsel bugs is unclear. Laboratory experiments have found no effect on the development, fecundity, or survival of damsel bugs when fed on Lepidoptera prey, *Spodoptera exigua* (Ponsard et al. 2003) that had been fed on *Bt* or conventional cotton. Damsel bugs are generalist predators (Snyder and Ives 2003) that may attack Lepidoptera larvae and eggs (Ehler 2004), but are also predators of aphids (Hesler et al. 2000, Elliott et al. 2002, Östman and Ives 2003)

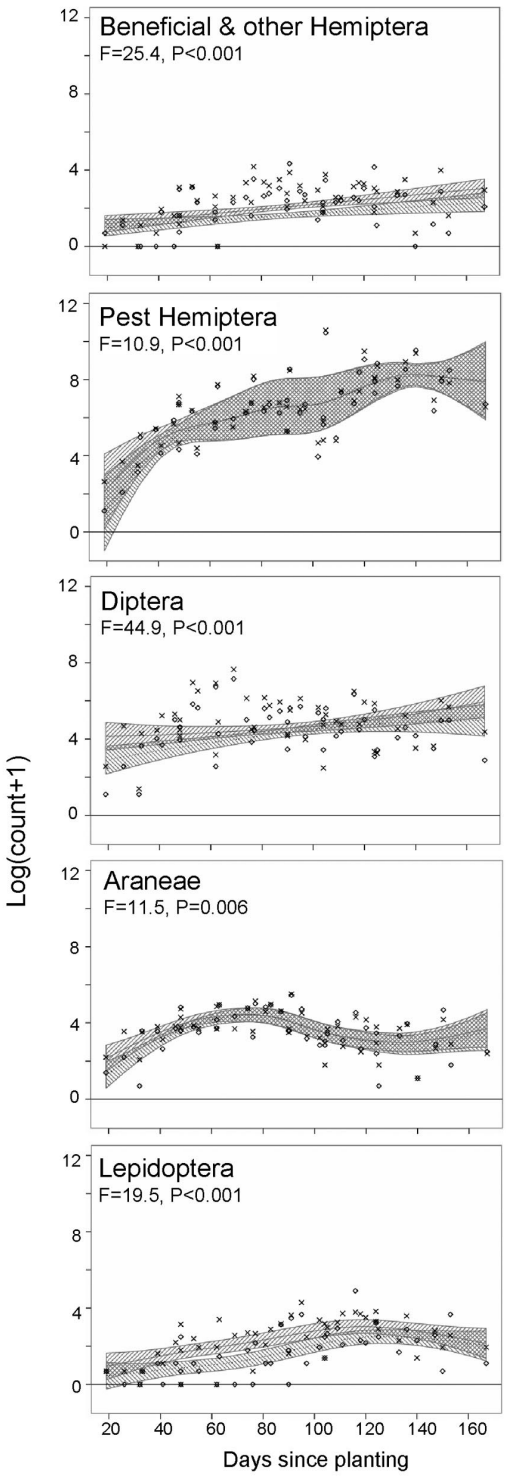

Fig. 7. Graphs of general groups at all sites modeled using smoothing splines. The plots show predicted values for both crop types versus time, with the shaded area within 1 SE of the predicted value. Forward diagonal shading, conventional; backward diagonal shading, *Bt*. Crosses are conventional data points, and circles are *Bt* data points.

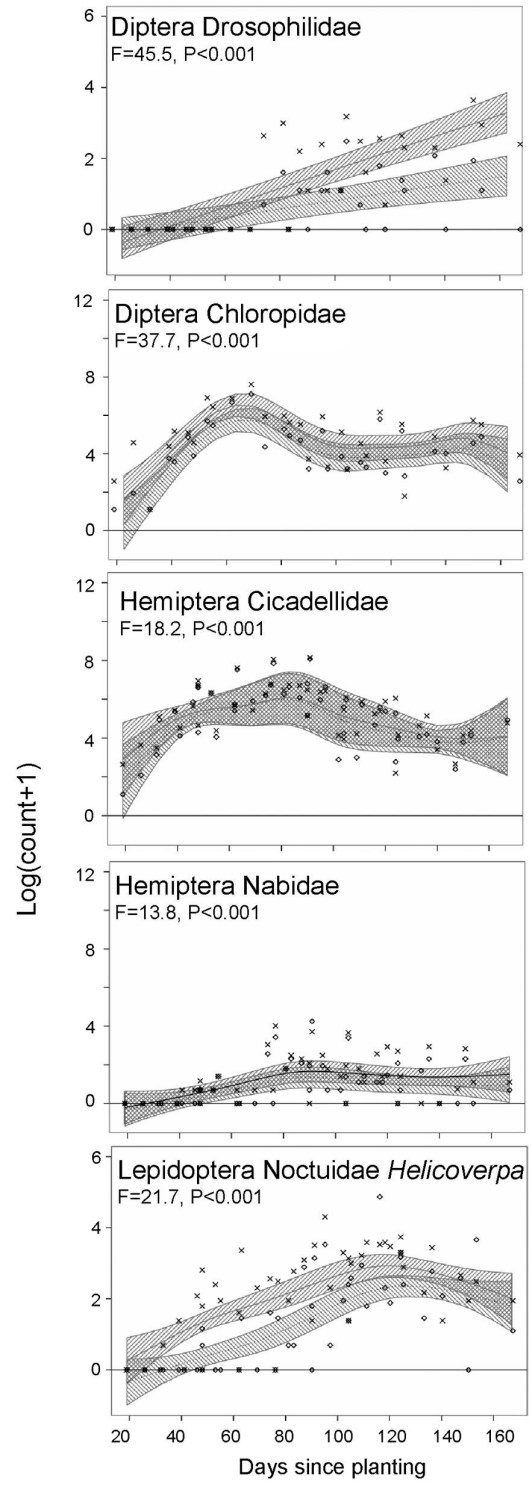

Fig. 8. Graphs of selected taxa counts at all sites modeled using smoothing splines. The plots show predicted values for both crop types versus time, with the shaded area within 1 SE of the predicted value. Forward diagonal shading, conventional; backward diagonal shading, *Bt*. Crosses are conventional data points, and circles are *Bt* data points.

Table 5. Results of a spline analysis examining the effect of "crop type" (Ingard or conventional cotton) on species groups identified from the PRC

Role	Site				Species groups	Conventional	Ingard®	df	*F*	*P*
	1	2	3	4						
B		+			Arachnida Oxyopidae	4.8	2.7	12	3.5	0.087
B		+			Arachnida Salticidae	1.9	0.7	12.5	5.7	0.035[a]
O		−			Coleoptera Anthicidae	1.2	1.2	37.3	0	0.974
P	+		+		Coleoptera Chrysomelidae	3.5	2.8	40.3	2.7	0.107
B				+	Coeloptera Coccinellidae (*Diomus*)	1.3	1.0	59.3	2	0.167
B		+	+	+	Coleoptera Coccinellidae (others)	1.3	1.0	60.7	2	0.164
O	+				Coleoptera Lathridiidae	5.0	4.4	27.4	1	0.324
B	+	+			Coleoptera Melyridae	2.5	2.1	36.1	1.5	0.233
O		+			Coleoptera Nitidulidae	1.0	0.9	53.7	0.1	0.768
O	+				Coleoptera Phalacridae	0.7	0.6	18.4	1	0.34
O		−			Diptera Ceratopogonidae	0.3	0.4	29	0.4	0.534
O		−			Diptera Chironomidae	8.2	9.3	28.6	1.9	0.177
O	+	+			Diptera Chloropidae	89.3	41.8	29	38	<0.001[b]
P	+	+			Diptera Drosophilidae	2.9	0.9	29	46	<0.001[b]
O	+	+			Diptera Sciaridae	2.6	1.8	29.1	2.5	0.126
P				+	Hemiptera Aleyrodidae	1.0	0.8	37.5	2.1	0.156
P	−				Hemiptera Aphididae	25.6	29.7	34.2	1.8	0.185
P	+	+	+	+	Hemiptera Cicadellidae	109.4	79.8	40.2	18	<0.001[b]
P/B	+	+	+		Hemiptera Lygaeidae	3.7	2.3	38	5.8	0.021[a]
B			+		Hemiptera Lygaeidae (*Geocoris*)	1.0	0.5	46.3	4.3	0.045[a]
P			+	−	Hemiptera Miridae (*Campylomma*)	8.3	7.1	46.2	2.2	0.142
P		+		+	Hemiptera Miridae (*Creontiades*)	3.0	2.7	11.2	0.7	0.41
B	+	+		+	Hemiptera Nabidae	1.8	1.1	43.9	14	<0.001[b]
P/B		+		+	Hemiptera Pentatomidae	0.7	0.4	26.1	3	0.097
B	+	+			Hymenoptera Braconidae	2.4	2.1	27.8	0.8	0.385
O	+	+			Hymenoptera Eulophidae	5.1	3.8	29.2	4.7	0.038[a]
B	−				Hymenoptera Formicidae	2.2	2.9	47.4	2.2	0.144
B	+				Hymenoptera Mymaridae	2.3	1.8	26.8	1.8	0.186
P	+	+		+	Lepidoptera Noctuidae (*Helicoverpa*)	5.6	2.3	44.1	22	<0.001[b]
B				+	Neuroptera Chrysopidae	0.4	0.5	19	1.1	0.317
B		−		+	Neuroptera Hemerobiidae	0.6	0.4	28.8	0.2	0.689
P	+	+		−	Thysanoptera Thripidae	24.9	26.7	43.1	0.5	0.485

A plus indicates sites where the species had positive species weights; a minus indicates sites where the species had negative species weights. The F and P values indicate whether adding "crop type" significantly improved the model. The conventional and Ingard values are the predicted number of individuals in 10 samples in the middle of the season.

[a] *P* value between 0.05 and 0.01 indicates a trend.

[b] *P* value <0.01 indicates a significant difference.

Site 1, Doreen 1995/96; 2, Ewenmar 1995/96; 3, Doreen 1997/98; 4, Auscott 1998/99; P, pest; B, beneficial; O, other.

and spider mites (Wilson et al. 1998). It may be that damsel bugs are more dependant on lepidopteran larvae than currently realized, which could partially explain their reduced abundance.

Jassid densities were slightly but significantly lower in *Bt* compared with conventional cotton. Because jassids are sometimes considered a pest (Deutscher et al. 2005), this could be a bonus for the grower. Nevertheless, the slight differences in jassid numbers between conventional and *Bt* cotton would be masked in commercially grown cotton by any differences in spray regimen between the two crop types.

We also found that the number of Chloropidae and Drosophillidae (Diptera) was lower in *Bt* cotton compared with conventional cotton. Why this occurred is unclear. Purified insecticidal proteins known to be effective against some Diptera include Cry4Aa1, Cry4Ba1, Cry10Aa1, and Cry11Aa1 (Benedict and Altman 2001). Cry2Aa1 (old name, CryIIA; Benedict and Altman 2001) is effective against Lepidoptera and some Diptera, including the mosquito *Anopheles quadrimaculatus* Say, and to a lesser extent, the mosquito *Culex pipiens* L., but it has no effect on other Diptera, including *Musca* and *Drosophila* (Sims 1997). CryBI is effective against the lepidopterans *Heliothis virescens* (Fabricius) and *Lymantria dispar* L., and to a lesser extent the mosquito *Aedes aegypti* L. (Donovan et al. 1988). The *Bt* cotton used in this study expresses Cry1Ac, which is specific to Lepidoptera (Sims 1995, Peferoen 1997). Even the two-gene cotton, Bollgard II, which has been recently licensed for general release in Australia, expresses Cry1Ac and Cry2Ab, both of which are specific to Lepidoptera. Thus, it is unlikely that the *Bt* gene in cotton had a direct effect on Drosophillidae or Chloropidae.

The role of Chloropidae in cotton is also unclear. The larvae of this family are reported to feed on a range biota, including bacteria, vegetative matter (both living and rotting), the eggs of other insects and spiders, beneath the skins of living frogs, and as parasites of Hymenoptera (Spencer 1986). Because Chloropidae do not seem to be pests or beneficials in cotton, its role from an IPM perspective is probably limited to providing an alternative source of food for some predators.

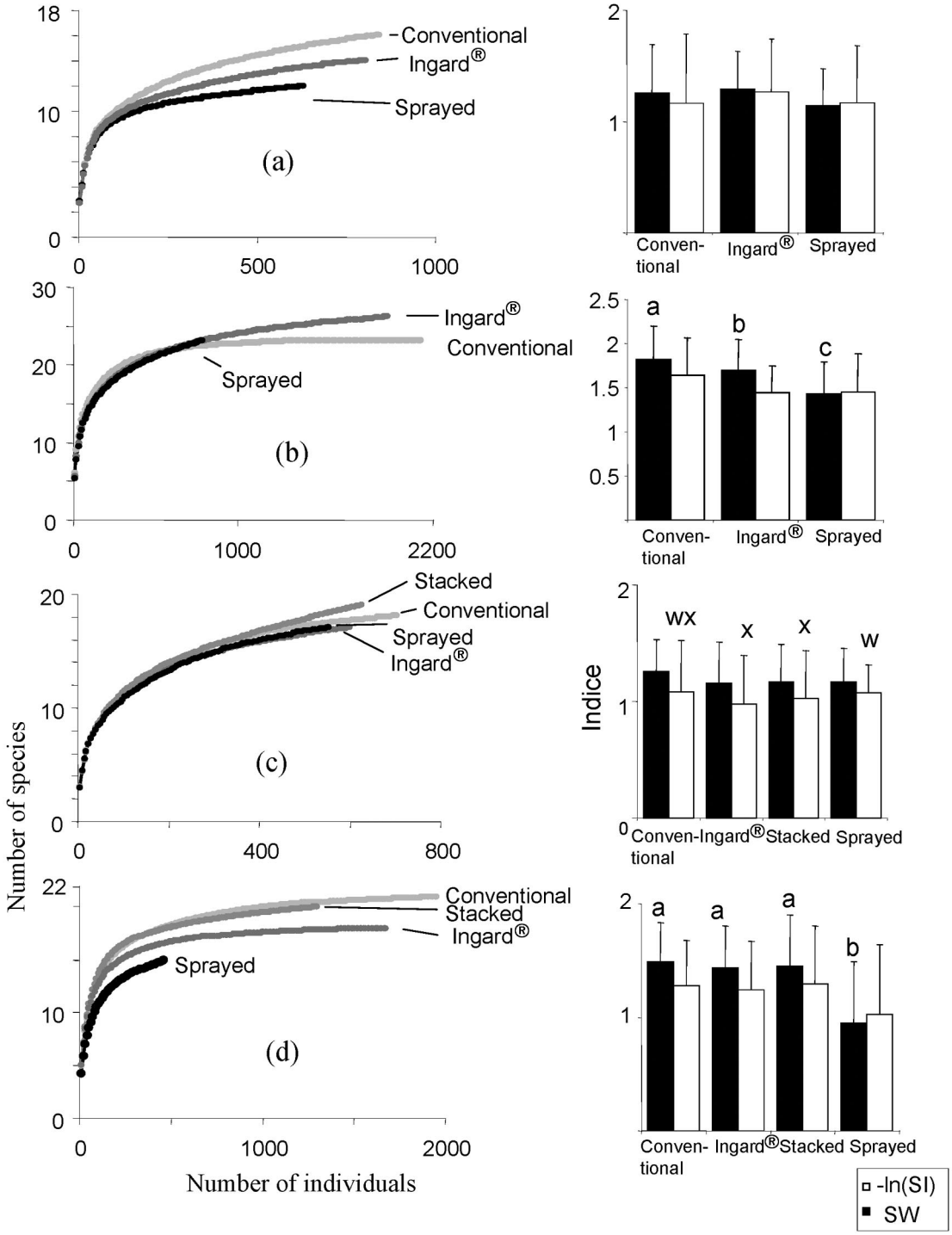

Fig. 9. Rarefaction curves and diversity indices of the beneficials found at the four sites. Rarefaction curves were calculated in increments of five specimens for sites (a) and (c), and increments of 10 specimens for sites (b) and (d). SDs are shown for the diversity indices. There was no difference in the indices calculated for sites (a) and (c). At sites (b) and (d), different letters above the histograms indicate statistical differences among the indices (a–c, SW; w,x, SI; as calculated using the LSD). (a) Doreen 1995/96. (b) Ewenmar 1995/96. (c) Doreen 1997/98. (d) Auscott 1998/99.

We found no difference in the number of green lacewings in *Bt* crops over the course of a season. Other studies that have focused specifically on lacewings have also found no effect (Orr and Landis 1997, Pilcher et al. 1997). Lepidopteran prey that have fed on *Bt* appear to be a poor-quality food source for lacewings, probably because of a change in the amino acid composition of the lepidopteran's hemolymph (Dutton et al. 2003). Lacewings readily consume other prey such as mites, which have been shown to accumulate higher levels of *Bt* toxin than lepidopteran larvae, without harm (Dutton et al. 2002, 2003).

The greatest influences on invertebrate communities in cotton are insecticide sprays, and the advent of *Bt* cotton has fostered a large drop in insecticide applications, with a 56% reduction in pesticide applications for *Helicoverpa* (Fitt 2004) and a 50% reduction in active ingredient overall. Nevertheless, when managing *Bt* cotton, it is important to understand how the dynamics of pest and beneficial species may be affected so that management practices can be adjusted if necessary. Our results indicated only a subtle shift in the arthropod community between *Bt* and conventional cotton, some of which was probably driven by the reduction in *Helicoverpa* and other lepidopterans. Our analyses did not indicate significant and consistent intrinsic effects of *Bt* cotton on key species that would warrant a different pest management approach.

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