Landscape effects of transgenic cotton on non-target ants and beetles

Yves Carrière\textsuperscript{a},*, Christa Ellers-Kirk\textsuperscript{a}, Manda G. Cattaneo\textsuperscript{a}, Christine M. Yafuso\textsuperscript{a}, Larry Antilla\textsuperscript{b}, Cho-Ying Huang\textsuperscript{d}, Magfurar Rahman\textsuperscript{c}, Barron J. Orr\textsuperscript{c}, Stuart E. Marsh\textsuperscript{c}

\textsuperscript{a}Department of Entomology, University of Arizona, 410 Forbes Building, P.O. Box 210036, Tucson, AZ 85721-0036, USA
\textsuperscript{b}Arizona Cotton Research and Protection Council, 3721 East Weir Avenue, Phoenix, AZ 85040-2933, USA
\textsuperscript{c}Arizona Remote Sensing Center, Office of Arid Lands Studies, University of Arizona, 1955 East Sixth Street, Tucson, AZ 85719, USA
\textsuperscript{d}Department of Geomatics, National Cheng Kung University, 1 University Road, Tainan 70101, Taiwan

Received 24 January 2008; accepted 23 October 2008

Abstract

Transgenic crops producing toxins from \textit{Bacillus thuringiensis} (Bt) can be planted in the same field for many years, and many insects exploiting such crops must disperse to other habitats to persist. Accordingly, effects of transgenic crop farming could accumulate through time and affect insect populations across agricultural landscapes. We monitored the population density of seven ant genera and beetle families and of rare ants and beetles in 84 non-cultivated sites abutting agricultural fields in Central Arizona. We assessed the short-term (during planting year) and long-term (over 5–6 years) landscape effect of farming Cry1Ac cotton on ant and beetle density in non-cultivated sites, in addition to several local and regional variables. Landscape variables (e.g., sequence of crops planted in neighbouring fields, crop diversity, and abundance) were more frequently associated with insect density than local variables (e.g., plant productivity and diversity in non-cultivated sites). In the short-term, use of Bt relative to non-Bt cotton in neighbouring fields was positively associated with density of one ant and two beetle groups in non-cultivated sites. However, acreage of Bt cotton located within 1 km from non-cultivated sites had more negative effects than acreage of non-Bt cotton on density of one ant and one beetle group. In the long-term, the proportion of years that Bt cotton was planted in neighbouring fields was positively associated with ant density but not beetle density. Results suggest that the farming of Bt cotton in neighbouring fields frequently resulted in positive short- and long-term landscape effects on ants and beetles in non-cultivated sites, while Bt cotton planted farther away had less frequent negative short-term impacts.

© 2009 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.

Zusammenfassung

Transgene Kulturpflanzen, die Toxine von \textit{Bacillus thuringiensis} (Bt) produzieren, können über viele Jahre auf denselben Ackerflächen angebaut werden, so dass viele Insekten, die diese Kulturpflanzen nutzen, in andere Habitate ausweichen müssen, um zu überleben. Infolgedessen könnten sich die Auswirkungen des Anbaus von transgenen Kulturpflanzen über die Zeit akkumulieren und Insektenpopulationen in Agrarlandschaften beeinflussen. Wir untersuchten die Populationsdichten von sieben Ameisenarten und Käferarten und

*Corresponding author. Tel.: +1 520 626 8329; fax: +1 520 621 1150.
E-mail address: ycarrier@ag.arizona.edu (Y. Carrière).

1439-1791/5 - see front matter © 2009 Gesellschaft für Ökologie. Published by Elsevier GmbH. All rights reserved.
doi:10.1016/j.baae.2008.10.010

**Keywords:** Bacillus thuringiensis; Landschaftseffekte; Herbicide tolerance; Nontarget arthropods; Regional effects; Remote sensing; Risk assessment; Transgenic crops

### Introduction

Most transgenic crops engineered to produce toxins from Bacillus thuringiensis (Bt) or tolerance to herbicides (Ht) are grown as annual plants in monocultures. Such monocultures represent ephemeral and simplified environments that often do not allow for persistence of insect species (Duelli & Obrist 2003; Tscharntke, Klein, Krüssel, Steffan-Dewenter, & Thies 2005; Tscharntke, Rand, & Bianchi 2005). Because many insects exploiting transgenic crops must disperse to other agricultural fields or non-cultivated habitats to persist within and between cropping seasons, fields of transgenic crops may affect insect populations locally as well as across agricultural landscapes. Yet, most studies assessing impacts of transgenic crops on insect biodiversity have focused on in-field effects (Marvier, McCreedy, Regetz, & Kareiva 2007).

Non-cultivated habitats embedded in agro-ecosystems can be important sources of biodiversity for agricultural fields (Duelli & Obrist 2003; Tscharntke, Klein et al. 2005). Nevertheless, highly productive agricultural fields may also represent significant sources of arthropods for less productive non-cultivated areas. Accordingly, agricultural fields may alter biodiversity of non-cultivated habitats through spillover effects, defined as movement of agriculturally subsidised insects to non-cultivated habitats (Rand, Tylanakis, & Tscharntke 2006; Tscharntke, Klein et al. 2005; Tscharntke, Rand et al. 2005). While theory suggests that spillover effects often have negative impacts on biodiversity, empirical investigations are needed to understand how they affect non-cultivated habitats (Hunter 2002; Tscharntke, Klein et al. 2005).

Here, we compared landscape effects of transgenic and non-transgenic cotton on insect density in non-cultivated habitats embedded in a patchwork of agricultural fields in the Sonoran Desert of Central Arizona. We focused on ground-dwelling ants and beetles, because their life history provides a useful contrast to understand landscape effects, they are major contributors to biodiversity, and play important roles in structuring communities (Duelli & Obrist 2003; Roth & Perfecto 1994). Single-season spillover effects were evaluated by comparing population density of ants and beetles in non-cultivated sites contiguous to non-Bt, Bt, or BtHt cotton fields. Impacts of transgenic and non-transgenic cotton located in more distant parts of the landscape were also investigated. Cumulative spillover effects were investigated by assessing ant and beetle density in non-cultivated sites adjacent to cotton fields, where Bt cotton had been used at different frequencies over several years.

### Materials and methods

#### Study area and sampling

Non-cultivated sites directly adjacent to cotton fields were arbitrarily selected within a region of ca. 6600 km² in Central Arizona. The region was delimited by frames of Landsat Enhanced Thematic Mapper Plus (ETM +) satellite images overlaid on Geographical Information System (GIS) maps with the position and identification (non-Bt or Bt cotton) of all cotton fields grown in the region (Cattaneo et al. 2006). GIS and remotely sensed...
data were co-registered to Universal Transverse Mercator (UTM) projection and the North American Datum of 1983 (NAD83), respectively.

Forty-four and 40 different non-cultivated sites were sampled in 2002 and 2003, respectively (see Appendix A). Sampling was conducted during the monsoon season (August–mid-September), when arthropod diversity peaks in the Sonoran Desert (Cattaneo et al. 2006). Two transects separated by 100 m were established with pitfall traps at each site (Fig. 1). Pitfall traps were 2 oz. polypropylene jars with 48 mm openings half-filled with 50% water and 50% propylene-glycol (Sierra et al. 2003). Rain shelters (18 cm Styrofoam plates held up with skewers) were placed ca. 20 cm above each trap. Transects started in non-cultivated sites 20 m from the border of a cotton field and extended 140 m inside non-cultivated areas. Pitfall traps were placed 20 m apart for a total of 14 in each site, and mapped via a Global Positioning System (GPS). Traps left in the ground for a period of 48 h without significant precipitation were brought to the laboratory for specimen identification.

Specimen identification

Insects used in this study were a subset of insects identified previously (see Cattaneo et al. 2006): in this study, 15,331 ants were distributed in nine morphospecies, four species groups, and 23 species, and 2947 beetles were distributed in 14 morphospecies, five species groups, and 71 species. Many taxa were only present at a few sites. For analyses, we identified the seven most common ant genera and seven most common beetle families, and pooled morphospecies, species groups, and species in those taxa. We pooled the remaining rare ants and beetles into two “Rare” groups. The common ant genera analysed were Dorymyrmex, Forelius, Myrmeccystus, Pheidole, Pogonomyrms, Solenopsis, and Tetramorium. The rare ant group comprised 10 genera (Aphaenogaster, Cardioconydia, Campanotus, Crematogaster, Hypoponera, Messor, Monomorium, Paratrechina, Tapinoma, and Temnothorax). The common beetle families analysed were Anthicidae, Carabidae, Lathridiidae, Mycetophagidae, Salpingidae, Staphylinidae, and Tenebrionidae. The rare beetles comprised 11 families (Bruchidae, Chrysomelidae, Cleridae, Coccinelidae, Curculionidae, Elateridae, Histeridae, Melyridae, Nitidulidae, Ptinidae, and Scarabaeidae).

Site characterisation

Insect biodiversity is locally affected by above ground plant productivity and other water-energy variables, altitude, and plant diversity (Hawkins et al. 2003; Kaspari, O’Donnell, & Kercher 2000). Altitude of sites was obtained via GPS. Meteorological stations across the study area (Cattaneo et al. 2006) were used to measure accumulated precipitation from January 1st to initiation of sampling at each site.

Landsat images from June 5th, July 7th, and August 24th in 2002 and May 15th, July 2nd, and August 3rd in 2003 were converted to surface reflectance, using an atmospheric correction model (Chavez 1996) and used to calculate the Normalised Difference Vegetation Index (NDVI) in each non-cultivated site. NDVI is positively associated with chlorophyll content and reflects above ground plant productivity (Anderson, Hanson, & Haas 1993). NDVI was measured in an area of $300 \times 240 \text{m}^2$ centred on the two transects of pitfall traps (Fig. 1). Values from the three images in each year were averaged to obtain a single estimate of NDVI, for each non-cultivated site.

Non-cultivated vegetation consisted primarily of desert flats with diverse, but often perturbed vegetation cover containing perennial grasses and grasslike plants and scattered forbs, bushes, and cacti. Plant diversity was evaluated in non-cultivated sites by counting the number of “vegetation types” present. Nine common types were recorded: cacti, creosote bush (Larrea tridentata), flowering weeds, grasses, mesquite (Prosopis velutina), paloverde (Cercidium floridum), salt cedar (Tamarix ramosissima), rabbit-brush (Chrysothamnus nauseosus), and “other brush”.

Neighbouring agricultural fields

In 2002, about 75% of the non-cotton crop fields within 2 km from the border of the non-cultivated sites were identified on the ground and digitally mapped (Cattaneo et al. 2006). A remote sensing classification technique that combined supervised and unsupervised algorithms (Schowengerdt 1997) was used to classify the remaining 25%. Combining the GIS data and remote sensing classification provided an overall accuracy of...
crop coverage of 92%. In 2003, 99.7% of the crop fields at a distance of 2 km from the non-cultivated sites were identified on the ground and mapped.

**Landscape parameters**

Questionnaires were distributed to cotton producers to obtain information on cropping history of the fields adjacent to non-cultivated sites (Fig. 1). This allowed us to identify the type of cotton (i.e., non-Bt, Bt, or BtHt) grown in fields adjacent to non-cultivated sites at the time of sampling, and the crops grown in the five years preceding the year of sampling. Overall, seven Bt or BtHt cultivars producing the toxin Cry1Ac, and 12 non-Bt cultivars were used by farmers during 2002 and 2003. Cropping history was categorised as follows: 1 if cotton was planted for 4–5 years; 2 if cotton was planted for 2–3 years and other crops in other years; 3 if cotton was planted once or less and other crops in other years; and 4 if the field remained fallow for 4–5 years. For fields categorized as 1, we used GIS maps available since 1998 and information from cotton producers to determine the proportion of years that Bt cotton had been planted before sampling.

The total area of non-Bt and Bt cotton planted around each non-cultivated site and the total area of agricultural fields (TAF) was quantified with GIS in eight rectangular areas of increasing size, with the smallest and largest, respectively, extending 250 and 2000 m from the border of the non-cultivated sites. FRAGSTATS software (McGarigal & Marks 1995) was used to estimate the Shannon’s Diversity Index ($H'$) of agricultural fields (including fallow fields) in each rectangular area. $H'$ measures landscape diversity of agricultural fields, and is calculated as $\sum p_i \ln p_i$, where $p_i$ is the proportion of the landscape in field type $i$. $H'$ increases when the number of field types increases (keeping evenness constant), or the area occupied by each field type becomes more even (keeping the number of field types constant).

**Spatial and statistical analyses**

**Scale of regional effects**

Two analyses were performed to determine an appropriate scale for investigating regional effects. In the first, we computed the Pearson correlation coefficient between density of each ant and beetle group ($\log(X+1)$ transformed) and the variables measured in increasing areas away from the non-cultivated sites (i.e., area of Bt cotton ($\log(X+1)$ transformed), area of non-Bt cotton ($\log(X+1)$ transformed), $H'$, and TAF). We assumed that the dimension of the area corresponding to a minimum (when coefficients were negative) or maximum value of the coefficients indicated the scale at which density of a group responded most strongly to a particular variable (Holland, Fahrig, & Cappuccino 2004). For each of the four variables, we averaged the scale of maximum response for the eight ant or beetle groups. For ant and beetle groups, the average scale of maximum response was, respectively, 1150, 893, 1000, and 750 m and 1027, 1187, 964, and 1100 m for the area of Bt cotton, area of non-Bt cotton, $H'$, and TAF. In the second analysis, we used multiple regressions to assess the association between density of each ant and beetle group and the four variables measured in areas of increasing dimension. We assumed that the area corresponding to a maximum value of the coefficient of determination ($R^2$) indicated the scale at which a group responded most strongly to the variables. The scale of maximum response was averaged for the eight ant or beetle groups and was, respectively, 969 and 1250 m for ants and beetles. Based on these results, we used the area extending 1000 m from the edge of a non-cultivated site as a common scale to investigate effects of the four variables.

**Effects of factors not related to transgenic cotton**

Stepwise regression (mixed procedure; JMP 2001) was used to identify factors associated with density of each ant and beetle group, and with the density of all ants and beetles (densities were $\log(X+1)$ transformed), in non-cultivated sites. Factors included in the model were not related to effects of transgenic cotton, which was the primary focus of this study. Such factors were altitude, accumulated precipitation, NDVI, cropping history, number of plant types, $H'$ in an area extending 1000 m from the border of the non-cultivated sites ($H'_{-1000}$), TAF in an area extending 1000 m from the border of the non-cultivated sites (TAF-1000), and year.

**Landscape effects of transgenic cotton**

Significant factors ($P<0.05$) retained in the stepwise regression procedure were included as covariates in a multiple regression model, which assessed the effect of cotton type in adjacent cotton field, area of Bt cotton in an area extending 1000 m from the border of non-cultivated sites (BT-1000, $\log(X+1)$ transformed), and area of non-Bt cotton in an area extending 1000 m from non-cultivated sites (NON-1000, $\log(X+1)$ transformed), on density of each ant and beetle group, or density of all ants and beetles. In each case, linear contrasts of least square means (JMP 2001) were used to further assess impacts of the cotton types in fields adjacent to non-cultivated sites. A Tukey–Kramer test was also used to compare homogeneity of slopes associated with BT-1000 and NON-1000 (Sokal & Rohlf 1981). A significantly more negative slope for BT-1000 than NON-1000 would indicate larger negative effects of Bt cotton on density of a group compared to effects of non-Bt cotton (and vice versa).
To assess whether short-term spillover effects generally affected ants or beetles, for each of the eight ant and beetle groups, we calculated the difference between the least squares means corresponding to the effect of Bt and non-Bt cotton, or the effect of BtH and non-Bt cotton. A one-sample \( t \)-test was used to assess whether the mean of such differences was significantly different from 0. A positive and significant mean difference would indicate that spillover effects of transgenic cotton generally increased ant or beetle density in non-cultivated areas, while a significantly negative difference would indicate negative impacts. Similarly, we used a one-sample \( t \)-test to assess whether the mean of the differences between the slope associated with Bt-1000 and NON-1000 was different from 0. A positive and significant mean difference would indicate positive impacts of Bt cotton located in more distant parts of the landscape on ant or beetle density, while a significantly negative difference would indicate negative impacts of Bt cotton.

To evaluate cumulative spillover effects of Bt cotton, we analysed data of the non-cultivated sites near fields mainly planted to cotton over the five years before sampling (\( N = 22 \)). Simple linear regression was used to assess the association between the proportion of years that a field was planted to Bt cotton and density of the ant and beetle groups in neighbouring non-cultivated sites. To assess generality of the long-term spillover effects, a one-sample \( t \)-test was used to evaluate whether the mean of the slopes obtained for the ant or beetle groups differed significantly from 0. A positive mean significantly different from 0 would indicate positive cumulative spillover effects of Bt cotton on ant or beetle density, while a significantly negative mean would indicate negative long-term impacts.

**Correlations among variables related and not related to transgenic cotton**

To assess whether intercorrelations between explanatory variables might influence observed patterns, we used pairwise Pearson correlation analyses to evaluate relationships among the continuous explanatory variables (i.e., altitude, accumulated precipitation, NDVI, number of plant types, \( H' \)-1000, TAF-1000, Bt-1000, and NON-1000).

**Results**

**Neighbouring agricultural fields**

Cotton fields adjacent to non-cultivated sites averaged 32.4 ha (95% confidence interval (CI) = 26.7, 39.3) and 20.8 ha (17.1, 25.3) in 2002 and 2003, respectively. The average closest distance between non-cultivated sites and agricultural fields of a different type than adjacent cotton fields (Fig. 1) was 103.7 m (59.1, 180.9) and 99.9 m (64.6, 154.6) in 2002 and 2003, respectively. Thus, cotton fields adjacent to non-cultivated sites are expected to have an overriding influence on ant and beetle density, compared to crop fields farther from non-cultivated sites. The average percentage of cultivated area within a distance of 1000 m from non-cultivated sites was 39.8% (35.4, 44.2) and 42.3% (38.2, 46.4) in 2002 and 2003, respectively. In 2002, agricultural fields near the non-cultivated sites were cotton (51%), alfalfa (16%), fallow fields (12%), small grains (12%), corn (4%), and other crops (5%). Crops pooled under the category “other crops” (melon, orchards, potato, pasture, and vegetables) represented <1% of the cultivated area. In 2003, agricultural fields were cotton (52%), small grains (16%), fallow fields (14%), alfalfa (13%), corn (3%), and other crops (2%: same crops as in 2002). Percentage of cotton producing the toxin Cry1Ac within a distance of 1000 m from non-cultivated sites was, respectively, 42% and 62% in 2002 and 2003.

**Effects of factors not related to transgenic cotton**

Landscape variables were more frequently associated with ant density in non-cultivated sites than local variables (Table 1). NDVI and altitude were the only local factors significantly associated with ant density. In contrast, cropping history of agricultural fields, regional diversity of agricultural fields (\( H' \)-1000), and regional abundance of agricultural fields (TAF-1000) was significantly associated with density of three, three, and one of the eight ant groups, respectively (Table 1). Cropping history and \( H' \)-1000 was also significantly associated with the density of all ants.

More frequent planting of crops other than cotton was associated with increased density of *Myremecocystus* and rare ants (Fig. 2). However, density of *Tetramorium* was greatest when neighbouring agricultural fields had been left uncultivated for 4–5 years, before the year of sampling. The density of all ants tended to increase when neighbouring agricultural fields were frequently farmed with crops other than cotton or were fallow for 4–5 years (Fig. 2). There was a significant positive association between \( H' \)-1000 and the density of three ant groups or of all ants (Table 1). Thus, increased regional diversity of agricultural fields apparently had positive effects on ant density. On the other hand, increased TAF-1000 was negatively associated with density of rare ants (Table 1), indicating that rare ant species may be lost as an increased proportion of the available land area is farmed.

In contrast to ants, density of beetles in non-cultivated sites was less frequently associated with
landscape variables (Table 1). Cropping history was only associated significantly with density of the Anthicidae, which were apparently negatively affected by farming of neighbouring fields (Table 1, Fig. 2). There was a positive association between TAF-1000 and density of the Staphylinidae in non-cultivated sites (Table 1). Analyses on all beetles revealed that none of the variables, unrelated to transgenic cotton, were influential (all $P \leq 0.1$).

### Landscape effects of transgenic cotton

During the year of planting, use of transgenic cotton in fields adjacent to non-cultivated sites was more frequently associated with density of beetles (Table 2: three of eight groups) than ants (Solenopsis). Density of Solenopsis was greatest near fields cultivated with Bt or BtHt cotton compared to non-Bt cotton (Table 2). In two of the three beetle groups where spillover effects were apparent, density was greatest near fields cultivated with transgenic cotton rather than non-Bt cotton (Table 2). In the third case (Staphylinidae), density was significantly higher in non-cultivated sites adjacent to Bt rather than BtHt cotton. These results indicate that short-term spillover effects of cultivation of transgenic cotton were fairly uncommon (i.e., 25% of groups), but generally positive.

Density of ants and beetles was rarely affected by Bt and non-Bt cotton located within 1000 m of

---

**Table 1.** Impacts of factors unrelated to transgenic cotton on density of ants and beetles in non-cultivated sites.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Ants</th>
<th>Beetles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group affected</td>
<td>Slope or means ($\pm$ SE)$^a$</td>
</tr>
<tr>
<td><strong>Local variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Year (2002 or 2003)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Altitude</td>
<td>Rare</td>
<td>$-0.0092 (0.0040)^*$</td>
</tr>
<tr>
<td>NDVI</td>
<td>Dorymyrmex</td>
<td>4.63 (1.29)$^\dagger$</td>
</tr>
<tr>
<td></td>
<td>All ants</td>
<td>2.31 (0.92)$^*$</td>
</tr>
<tr>
<td><strong>Landscape variables</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cropping history</td>
<td>Myremecystus</td>
<td>Fig. 2$^*$</td>
</tr>
<tr>
<td></td>
<td>Rare</td>
<td>Fig. 2$^\dagger$</td>
</tr>
<tr>
<td></td>
<td>Tetramorium</td>
<td>Fig. 2$^*$</td>
</tr>
<tr>
<td></td>
<td>All ants</td>
<td>Fig. 2$^*$</td>
</tr>
<tr>
<td>H$^\prime$-1000</td>
<td>Dorymyrmex</td>
<td>0.52 (0.16)$^\dagger$</td>
</tr>
<tr>
<td></td>
<td>Pheidole</td>
<td>0.35 (0.16)$^*$</td>
</tr>
<tr>
<td></td>
<td>Pogonomyrmex</td>
<td>0.72 (0.20)$^\dagger$</td>
</tr>
<tr>
<td></td>
<td>All ants</td>
<td>0.36 (0.14)$^*$</td>
</tr>
<tr>
<td>TAF-1000</td>
<td>Rare</td>
<td>$-0.0031 (0.0009)^*$</td>
</tr>
</tbody>
</table>

Only factors selected by stepwise regression that remained statistically significant in multiple regression models considering factors unrelated and related to transgenic cotton are reported.

$^a$Slopes (continuous explanatory variables) or least squares means (categorical variables) are from multiple regression models including variables listed in Tables 1 and 2. $^* P < 0.05; ^{**} P < 0.01; ^{\dagger} P < 0.005; ^{\dagger\dagger} P < 0.001$.

$^b$See Fig. 2 for values of least squares means.

---

**Fig. 2.** Density (least squares means of log-transformed density $\pm$ SE) of three ant groups (Myremecystus, Rare, Tetramorium), all ants, and one beetle family (Anthicidae) as a function of cropping history (see text for categorisation of crop history).
Table 2. Impacts of factors related to transgenic cotton on density of ants and beetles in non-cultivated sites.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ants</th>
<th>Beets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group affected</td>
<td>Slope or means (± SE)a</td>
</tr>
<tr>
<td>Crop type</td>
<td>Solenopsis</td>
<td>Bt: 1.70 (0.20)a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BtHt: 1.92 (0.24)a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-Bt: 1.21 (0.16)b</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT-1000</td>
<td>Solenopsis</td>
<td>−0.35 (0.14)b</td>
</tr>
<tr>
<td>NON-1000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Significant covariates included in multiple regression models are given in Table 1. Only groups significantly affected by transgenic cotton are reported.

aLeast squares means contrasts were used to compare impacts of Bt and non-Bt cotton, BtHt and non-Bt cotton, and Bt and BtHt cotton. Means with different letters were statistically different: 0.009 < P < 0.05.

bSlopes were significantly different from 0, P < 0.03.

Table 3. Slope of the association between the proportion of years fields were planted to Bt cotton and abundance of ants and beetles in adjacent non-cultivated sites.

<table>
<thead>
<tr>
<th></th>
<th>Ants</th>
<th>Slope (± SE)b</th>
<th>Beetles</th>
<th>Slope (± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorymyrmex</td>
<td>−0.024 (0.34)</td>
<td></td>
<td>Anthicidae</td>
<td>−0.083 (0.28)</td>
</tr>
<tr>
<td>Forelius</td>
<td>0.90 (0.50)*</td>
<td></td>
<td>Carabidae</td>
<td>−0.010 (0.47)</td>
</tr>
<tr>
<td>Myrmecocystus</td>
<td>0.41 (0.29)</td>
<td></td>
<td>Lathridiidae</td>
<td>0.069 (0.19)</td>
</tr>
<tr>
<td>Pheidole</td>
<td>−0.12 (0.38)</td>
<td></td>
<td>Mycetophagida</td>
<td>−0.45 (0.39)</td>
</tr>
<tr>
<td>Pogonomyrmex</td>
<td>0.19 (0.51)</td>
<td></td>
<td>Salpingidae</td>
<td>0.56 (0.39)</td>
</tr>
<tr>
<td>Solenopsis</td>
<td>0.22 (0.49)</td>
<td></td>
<td>Staphylinida</td>
<td>−0.076 (0.15)</td>
</tr>
<tr>
<td>Tetramorium</td>
<td>0.59 (0.33)**</td>
<td></td>
<td>Tenebrionida</td>
<td>0.54 (0.29)**</td>
</tr>
<tr>
<td>Rare</td>
<td>0.25 (0.32)</td>
<td></td>
<td>Rare</td>
<td>0.17 (0.22)</td>
</tr>
</tbody>
</table>

**P = 0.090; ***P = 0.095; ***P = 0.083.

The slope of BT-1000 was significantly more negative than the slope of NON-1000 in Solenopsis and the Anthicidae (Tukey–Kramer, P < 0.05), suggesting negative regional effects of Bt cotton farming on these groups. Analyses on the density of all ants and beetles did not reveal any significant effects of factors related to transgenic cotton (all P-values > 0.11).

Pooling results from individual analyses for ants or beetles, there was no general trend for significant short-term spillover effects of transgenic cotton, or effects of Bt cotton located within 1000 m of non-cultivated sites. In ants, the mean difference between the least square means density of groups in non-cultivated sites near Bt and non-Bt cotton (t = 0.75, P = 0.48) and near BtHt and non-Bt cotton (t = 0.20, P = 0.84) were not significantly different from 0. Also, the difference between the slope associated with BT-1000 and NON-1000 was not significantly different from 0 (t = 0.81, P = 0.44). Similarly in beetles, the mean difference between the least square means density of groups found near Bt and non-Bt cotton (t = 1.35, P = 0.21) and near BtHt and non-Bt cotton (t = 1.27, P = 0.24) were not significantly different from 0, nor was the difference between the slope associated with BT-1000 and NON-1000 (t = 0.76, P = 0.47).

The association between the proportion of years that adjacent fields were planted to Bt cotton and density of the ant groups was generally positive, although it was only marginally significant in Forelius and Tetramorium (Table 3). The associations were more variable in beetles, where a marginally significant positive slope was only detected in the Tenebrionidae. However, the mean slope of these associations was significantly different from 0 in ants (95% CI = 0.67, 0.27, t = 2.59, and P = 0.036) but not in beetles (t = 0.028, P = 0.97), indicating that more frequent planting of Bt cotton generally had cumulative and positive spillover effects on ant density, but did not consistently affect beetles.
Correlations among variables related and not related to transgenic cotton

Few of the local and landscape variables were significantly correlated across the non-cultivated sites in 2002 and 2003. Five pairwise correlation coefficients out of 28 were significantly different from zero ($P<0.05$) in both 2002 and 2003, and the average of the absolute value of the coefficients was, respectively, $0.17$ (95% CI $= 0.13$–0.22) and $0.16$ (0.12–0.21). Furthermore, different pairs of variables were significantly correlated in 2002 and 2003. This suggests that intercorrelations within and among local and landscape explanatory variables did not have a strong influence on the patterns detected in this study.

Discussion

Two types of studies can be used to assess impacts of transgenic crops on biodiversity of non-target arthropods. The first is motivated by the need to evaluate impacts on ecological services in agricultural fields (Romeis, Meissle, & Bigler 2006). Key assumptions are that local effects of transgenic crops occur in the short-term (during the growing season), are not masked by immigration from source habitats, nor distorted by regional sink effects (Sisterson, Carrière, Denney, & Tabashnik 2007). The second focuses on impacts affecting insect biodiversity across the agricultural landscape. The reason for such studies is that agricultural intensification may significantly reduce biodiversity and resilience of agro-ecosystems on a regional scale (Tscharntke, Klein et al. 2005; Tscharntke, Rand et al. 2005). Here, we specifically used a landscape perspective to evaluate the impacts of farming transgenic and non-transgenic cotton on ant and beetle density in non-cultivated habitats embedded in a patchwork of agricultural fields (Appendix A and Fig. 1). To our knowledge, this is the first study investigating non-target landscape effects of a transgenic crop.

Effects of factors not related to transgenic cotton

NDVI (Dorymyrmex and all ants) and altitude (rare species) were the only local variable associated with ant density, while regional crop diversity and abundance affected four of the eight ant groups (Table 1), and crop history affected three ant groups and ants in general (Fig. 2). In contrast, significant local and regional effects were rare in beetles (Table 1, Fig. 2). This suggests that ant density in non-cultivated sites was more strongly influenced by landscape effects than was beetle density.

Ant colonies often remain in the same general location for several years (Hölldobler & Wilson 1990). While foraging distances from nests depend on body size and are generally short (e.g., less than 50 m), ants may establish networks of ephemeral nests to increase their foraging space, and large ants may cover distances of a few hundred metres (Hölldobler & Wilson 1990). In contrast, beetles are more mobile and may frequently move more than 1000 m to track resources (Holland et al. 2004). Sessile colonies and central foraging are two characteristics expected to increase sensitivity of ants to long-term changes occurring in the vicinity of nests. Accordingly, cropping history of agricultural fields adjacent to non-cultivated sites was more frequently associated with density of ants than beetles (Table 1).

Frequent farming of crops other than cotton (mainly alfalfa and small grains) in agricultural fields adjacent to non-cultivated sites (Fig. 1) was associated with increased density in two of the three ant groups responsive to crop history and with a slight increase in the density of all ants (Fig. 2). We previously found that farming of transgenic and non-transgenic cotton equally reduces ant biodiversity, compared to adjacent non-cultivated sites (Cattaneo et al. 2006). Disturbance by flood irrigation, tillage, and insecticides may be largely responsible for declines in ant biodiversity in cotton fields (Cattaneo et al. 2006). Such disturbances are more common in cotton than in other crops in our study area, which could explain the positive association between the frequent farming of crops other than cotton and ant density (Fig. 2). Thus, crops such as alfalfa and small grains may represent important resources for ants in agro-ecosystems of Central Arizona. Fields left in fallow may grow dense patches of weeds. Such fields apparently provided valuable habitats for Tetramorium ants and Anthicidae beetles, as well as for ants in general (Fig. 2).

A positive association between insect density in non-cultivated sites and regional diversity of agricultural fields would be expected in generalist, mobile insects capable of exploiting resources in many agricultural fields (Jonsen & Fahrig 1997). However, diversity of agricultural fields ($H'\text{-1000}$) was frequently associated with density of ants, but never associated with density of beetles (Table 1). This finding was unexpected given the higher mobility of beetles than ants. Dispersal of ant, winged queens and males, can occur over several kilometres (Hölldobler & Wilson 1990). This suggests that the positive association between agricultural field diversity and ant density involved faster growth and reproduction of ant colonies in regions with greater diversity of agricultural fields. Higher diversity of agricultural fields could have increased regional habitat suitability, as cotton was a dominant crop that often was less suitable than other types of agricultural fields for ants (Fig. 2). In contrast to regional diversity of agricultural fields, total cropped area (TAF-1000) had mixed effects and was rarely associated with density of
ants and beetles (Table 1). Nevertheless, the negative association between density of rare ants and cultivated area indicates that the preservation of non-cultivated habitats could be valuable to sustain regional biodiversity of ants.

### Landscape effects of transgenic cotton

Effects of transgenic and non-transgenic cotton were investigated after correcting for the influence of other local and regional factors associated with ant and beetle density (Table 2). Analyses of pooled results for ants and beetles did not indicate general short-term spillover effects of the cotton types. Nevertheless, cotton type in adjacent fields was more frequently associated with density of beetles than ants (Table 2).

We previously found that density of beetles was generally lower in non-cultivated sites than in adjacent cotton fields (Cattaneo et al. 2006). For cotton fields directly adjacent to non-cultivated sites, insecticide sprays were about 60% less frequent in transgenic fields than in non-transgenic fields in 2002, and similar reductions of about 30% were observed in 2003 (Cattaneo et al. 2006). Seven classes of insecticides were used (organophosphate, pyrethroid, organochlorine, neonicotinoid, carbamate, oxadiazine, and insect growth regulators), although a significant reduction in the number of sprays applied to transgenic versus non-transgenic cotton was only apparent for organophosphates and pyrethroids (unpublished data). Higher use of insecticides, but not whether cotton was transgenic or not, was associated with reduced species richness of ants and beetles in cotton fields (Cattaneo et al. 2006). This suggests that spillover of the Anthicidae and Staphylinidae was smaller for non-Bt than Bt or BtHt cotton (Table 2). Hence, the increased abundance of the Staphylinidae in non-cultivated sites adjacent to Bt cotton compared to BtHt cotton (Table 2). More work is needed to understand these patterns.

Analyses of pooled results revealed that frequent planting of Bt cotton over a period of 5–6 years was generally associated with greater ant density in nearby non-cultivated sites, although it did not generally affect beetle density. In individual analyses, intensive use of Bt cotton apparently had greater positive effects on Forelius and Tetramorium ants and Tenebrionidae beetles (Table 3). As in the case of impacts of cropping history, the greater influence of frequent planting of Bt cotton on ants than beetles may be due to the higher sedentariness of ants, making them more responsive to long-term changes in landscape characteristics.

In summary, we investigated landscape effects of transgenic cotton producing Cry1Ac, by monitoring density of ants and beetles in non-cultivated habitats embedded in a patchwork of agricultural fields. Because sampling was restricted to the monsoon season, it is unclear whether the patterns documented in this study may apply during the rest of the year. Nevertheless, beetles appeared less affected than ants in the short-term by regional crop diversity (Table 1), and in the long-term by the succession of crops in fields adjacent to non-cultivated sites (Table 1, Fig. 2). Short-term (Table 2) and long-term (Table 3) spillover effects of transgenic cotton were relatively common and positive, while short-term regional effects of Bt cotton were rarer and negative (Table 2). This suggests that transgenic cotton mainly had positive landscape effects on ants and beetles in the Sonoran Desert of Arizona.

### Acknowledgements

We thank A. Ali, K. Dell, G. Fulgate, J. Harms, and D. Overton for field and laboratory assistance, S. Cover, J. Ellington, C. Olson, C. Schmidt, and D. Wheeler for help with specimen identification, and Dave Crowder, Shannon Heuberger, Kathleen Walker and four reviewers for comments on a draft of this manuscript.
Partial support was provided by Environmental Protection Agency Cooperative Agreement X-82974701-O.

Appendix A. Supplementary material

The online version of this article contains additional supplementary data. Please visit doi:10.1016/j.baae.2008.10.010.

References


