

Farm-scale evaluation of the impacts of transgenic cotton on biodiversity, pesticide use, and yield

Manda G. Cattaneo*, Christine Yafuso*, Chris Schmidt*†, Cho-ying Huang‡, Magfurar Rahman‡, Carl Olson*, Christa Eilers-Kirk*, Barron J. Orr‡, Stuart E. Marsh‡, Larry Antilla§, Pierre Dutilleul¶, and Yves Carrière*||

*Department of Entomology, University of Arizona, 410 Forbes Building, P.O. Box 210036, Tucson, AZ 85721-0036; †Graduate Interdisciplinary Program in Insect Science, University of Arizona, 225 Life Sciences South, Tucson, AZ 85721; ‡Arizona Remote Sensing Center, Office of Arid Lands Studies, University of Arizona, 1955 East Sixth Street, Tucson, AZ 85710; §Arizona Research and Protection Council, 3721 East Weir Avenue, Phoenix, AZ 85040-2933; and ¶Department of Plant Science, Macdonald Campus, Raymond Building, McGill University, St-Anne-de-Bellevue, QC, Canada H9X 3V9

Edited by May R. Berenbaum, University of Illinois at Urbana-Champaign, Urbana, IL, and approved April 6, 2006 (received for review September 22, 2005)

Higher yields and reduced pesticide impacts are needed to mitigate the effects of agricultural intensification. A 2-year farm-scale evaluation of 81 commercial fields in Arizona show that use of transgenic *Bacillus thuringiensis* (Bt) cotton reduced insecticide use, whereas transgenic cotton with Bt protein and herbicide resistance (BtHr) did not affect herbicide use. Transgenic cotton had higher yield than nontransgenic cotton for any given number of insecticide applications. However, nontransgenic, Bt and BtHr cotton had similar yields overall, largely because higher insecticide use with nontransgenic cotton improved control of key pests. Unlike Bt and BtHr cotton, insecticides reduced the diversity of nontarget insects. Several other agronomic and ecological factors also affected biodiversity. Nevertheless, pairwise comparisons of diversity of nontarget insects in cotton fields with diversity in adjacent noncultivated sites revealed similar effects of cultivation of transgenic and nontransgenic cotton on biodiversity. The results indicate that impacts of agricultural intensification can be reduced when replacement of broad-spectrum insecticides by narrow-spectrum Bt crops does not reduce control of pests not affected by Bt crops.

agricultural sustainability | environmental impact | transgenic crops

The increasing world population and changes in consumption patterns may necessitate significant agricultural intensification in the next 50 years (1, 2). Unless crop yield is improved and release of fertilizers and pesticides from croplands is reduced, such intensification could augment contamination and perturbation of managed and natural ecosystems, ultimately harming biodiversity and public health (1–4). It was proposed that transgenic *Bacillus thuringiensis* (Bt) crops could be valuable tools for increasing agricultural productivity while minimizing the environmental impacts of agriculture (1, 2). However, the potential effects of transgenic crops on nontarget arthropods have caused concern, especially in regions where agricultural land is important to sustain biodiversity (5–7).

Although Bt crops are grown extensively worldwide (8), no large-scale studies had been performed to simultaneously test whether they have favorable agricultural effects and minimal impacts on nontarget arthropods. Here, we report results of a 2-year farm-scale evaluation of the effects of transgenic cotton on biodiversity, pesticide use, and yield. We studied 81 commercial fields in a region of 6,600 km² in Arizona, where Bt cotton represented 48% and 62% of the cotton planted in the first and second year of the study, respectively. Forty fields were planted to nontransgenic (nonTr) cotton, 21 fields to transgenic cotton producing the Bt toxin Cry1Ac (Bt), and 20 fields to cotton with Bt protein and herbicide resistance (BtHr). Bt cotton with Cry1Ac controls the pink bollworm (*Pectinophora gossypiella*), a major insect pest of cotton (9, 10).

Results and Discussion

Effects of Transgenic Cotton on Pesticide Use. Transgenic cotton was treated with fewer broad-spectrum insecticides than nonTr

cotton (Table 1, which is published as supporting information on the PNAS web site). In the first year of the study, the average number of insecticide applications in nonTr cotton was 6.6, which was significantly higher than in Bt (3.4) and BtHr (2.8) cotton (Fig. 1*a*). In the second year, the difference in insecticide use in nonTr cotton (6.8) compared with Bt (5.1) and BtHr (4.7) cotton was smaller but still significant (Fig. 1*b*). Insect growth regulators (IGRs), which are considered less harmful to nontarget arthropods than broad-spectrum insecticides, are used in Arizona for controlling the sweet potato whitefly (*Bemisia tabaci*) in cotton (11–14). Use of IGRs did not differ significantly between transgenic and nonTr cotton (Table 2, which is published as supporting information on the PNAS web site). The average number of herbicide applications, including or excluding glyphosate, did not differ significantly among nonTr, Bt, and BtHr cotton (Table 2 and Table 3, which is published as supporting information on the PNAS web site). Thus, lower use of broad-spectrum insecticides but similar use of herbicides occurred in transgenic cotton compared with nonTr cotton.

Effects of Transgenic Cotton on Yield. Average yield of cotton types ($P = 0.17$) and yield differences among cotton types ($P = 0.33$) did not vary between years. Least-squares means of yields for nonTr, Bt, and BtHr cotton were estimated with an analysis of covariance model, after controlling for the significant effects of insecticide use and seeding rate (see below). There was no significant difference between yields of Bt and BtHr cotton ($P = 0.88$). However, transgenic cotton produced more lint than nonTr cotton (one-tailed contrast, $t = 1.87$, $df = 58$, $P = 0.033$). The combined yield increase (least-squares mean \pm SE) in Bt and BtHr cotton was 130.0 ± 69.5 kg·hectare (ha)⁻¹, which represented 8.6% of the least-squares mean yield of nonTr cotton ($1,509.0 \pm 47.9$ kg·ha⁻¹).

Yield was positively associated with the number of broad-spectrum insecticide applications (log-transformed) (slope = 446.96, $t = 3.24$, $df = 58$, $P = 0.002$). However, no significant interaction occurred between cotton type, insecticide use, and year ($P = 0.14$) or between cotton type and insecticide use ($P = 0.20$), showing that yield of the cotton types was affected similarly by insecticide applications. Thus, yield of the cotton types increased at a slower rate with each additional insecticide application. Nevertheless, yield gain over the range of insecticides applied per field (0–16) was 550.0 kg·ha⁻¹, 4.2 times greater than yield gain caused by use of transgenic cotton.

Conflict of interest statement: No conflicts declared.

This paper was submitted directly (Track II) to the PNAS office.

Freely available online through the PNAS open access option.

Abbreviations: Bt, *Bacillus thuringiensis*; BtHr, Bt protein and herbicide resistance; nonTr, nontransgenic; IGR, insect growth regulator; ha, hectare; NDVI, Normalized Difference Vegetation Index.

||To whom correspondence should be addressed. E-mail: ycarriere@ag.arizona.edu.

© 2006 by The National Academy of Sciences of the USA

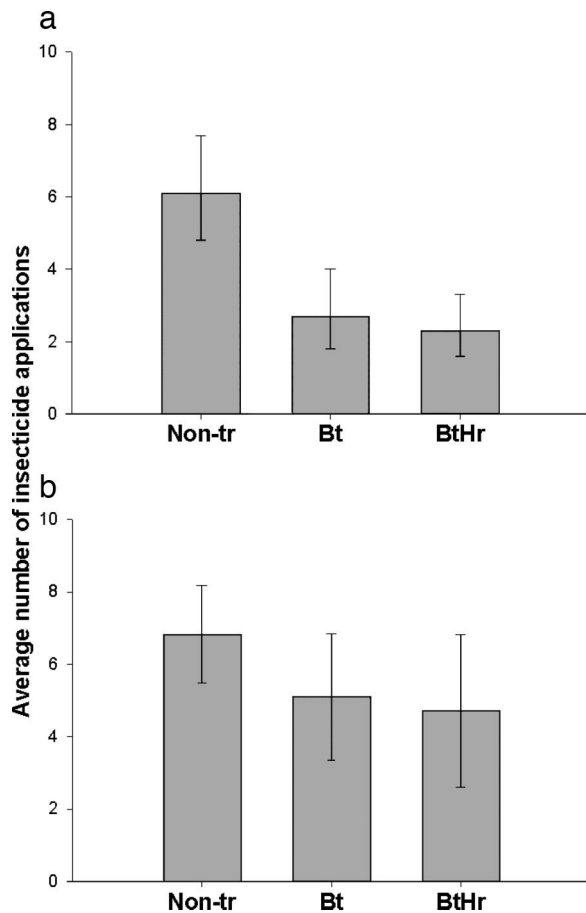


Fig. 1. Average number of broad-spectrum insecticide applications in nonTr, Bt, and BtHr cotton (with 95% confidence intervals). The number of insecticide applications was significantly higher in nonTr than in transgenic cotton in 2002 (one-tailed contrast, $t = 4.13$, $df = 72$, $P < 0.0001$) (a) and in 2003 (one-tailed contrast, $t = 1.99$, $df = 72$, $P = 0.025$) (b). The number of insecticide applications was higher in 2003 than in 2002 ($P = 0.058$), although differences in insecticide applications among cotton types did not vary between years ($P = 0.47$).

Seeding rate had a positive effect on yield (slope = 41.20, $t = 2.45$, $df = 58$, $P = 0.017$), but did not differ among nonTr, Bt, and BtHr cotton ($P = 0.36$). A yield gain of 288.4 kg·ha⁻¹, twice the yield gain caused by use of transgenic cotton, occurred over the range of seeding rates (9.0–16.8 kg·ha⁻¹).

The positive effect of broad-spectrum insecticides on yield demonstrates their key role in controlling major cotton pests. About 90% of broad-spectrum insecticides applied in Arizona cotton target the key pests *B. tabaci*, the western tarnished plant bug *Lygus hesperus*, and *P. gossypiella* (14). Bt cotton does not kill *B. tabaci* and *L. hesperus*. Thus, the rise in insecticide use in transgenic cotton in the second year of the study (Fig. 1) probably reflects an increasing need to control these two pests.

Because Bt cotton is resistant to *P. gossypiella*, transgenic cotton had higher yield than nonTr cotton for any given number of insecticide applications (see above). However, no overall yield difference occurred among nonTr, Bt, and BtHr cotton ($P = 0.96$). Based on the above analysis of covariance model, the higher use of insecticides in nonTr than in transgenic cotton (Fig. 1) increased yield by 137 and 51 kg·ha⁻¹ in 2002 and 2003, respectively. Such yield gains compensated for gains caused by the use of transgenic cotton (i.e., 130.0 kg·ha⁻¹). Thus, the similar yields in nonTr and transgenic cotton likely occurred because the additional insecticides applied in nonTr cotton

significantly reduced damage caused by pests not killed by Bt cotton.

Effects of Transgenic Cotton on Biodiversity. At least one edge of each cotton field was directly adjacent to noncultivated vegetation. To compare impacts of cultivation of nonTr and transgenic cotton on nontarget arthropods, we used pairwise comparisons of ant and beetle diversity in each type of cotton field with diversity of these taxa in adjacent noncultivated sites.

A total of 17,255 ants grouped in 9 morphospecies, 3 species groups, and 27 species were found in cotton fields and adjacent noncultivated sites. Species richness was higher in beetles than ants, as 10,444 beetles grouped in 23 morphospecies, 4 species groups, and 91 species were found at the same sites.

Ant density declined significantly from noncultivated vegetation to cotton fields (Fig. 2a). The average density decline was similar in nonTr, Bt, and BtHr cotton ($P = 0.54$). As expected (15), the density reduction in paired habitats was associated with a decline in ant species richness (one-tailed test; slope = 0.028, $t = 1.70$, $df = 76$, $P = 0.047$). However, the average reduction in species richness did not differ among cotton types ($P = 0.44$) (Fig. 2b). In contrast, beetle density increased from noncultivated vegetation to cotton fields (Fig. 2c). The average density increase did not differ among nonTr, Bt, and BtHr cotton ($P = 0.69$). Increased density in cotton fields was associated with a rise in beetle species richness (one-tailed test; slope = 0.33, $t = 3.14$, $df = 76$, $P = 0.0012$), whereas the average change in species richness did not differ among nonTr, Bt, and BtHr cotton ($P = 0.69$) (Fig. 2d). Thus, cultivation of transgenic and nonTr cotton had similar effects on diversity of nontarget insects.

Several factors affecting ant and beetle diversity may have differed among nonTr, Bt, and BtHr cotton. Accordingly, the similar changes in ant and beetle diversity in the cotton types (Fig. 2) could have masked negative effects of transgenic cotton on these taxa. To assess this possibility, we used path analyses to evaluate the impacts of broad-spectrum insecticides, IGRs, transgenic cotton, and other factors (see *Materials and Methods*) on ant and beetle diversity in cotton fields. BtHr cotton reduced ant density compared with Bt cotton (Fig. 3a). However, a path analysis evaluating the effects of nonTr and BtHr cotton on ant density (results not presented) showed no significant difference between nonTr and BtHr cotton ($P = 0.12$). Furthermore, comparisons between nonTr and transgenic cotton did not reveal any significant impacts of transgenic cotton on ant diversity (Fig. 3a) and beetle diversity (Fig. 3b). In contrast, broad-spectrum insecticides significantly reduced ant and beetle species richness. Moreover, application of IGRs positively affected beetle density but negatively affected beetle species richness, resulting in an overall negative impact on beetle species richness (Fig. 3b).

Characteristics of cotton fields (sand soil content and seeding rate) and noncultivated sites [Normalized Difference Vegetation Index (NDVI) and vegetation diversity] and accumulated precipitation significantly affected ant and beetle diversity (Fig. 3). Accumulated precipitation, NDVI, and seeding rate did not differ among cotton types ($P > 0.31$). Sand content (percentage \pm SE) differed significantly among nonTr (44.0 \pm 2.6), Bt (49.1 \pm 3.6), and BtHr (55.5 \pm 3.5) cotton [$F(2,78) = 3.47$, $P = 0.036$]. The average number of vegetation types (\pm SE) in noncultivated sites differed significantly among nonTr (3.22 \pm 0.16), Bt (2.61 \pm 0.22), and BtHr (2.50 \pm 0.22) cotton [$F(2,77) = 8.54$, $P = 0.016$]. The significant differences in the number of vegetation types contributed in increasing beetle species richness in nonTr cotton (Fig. 3), thereby compensating for the negative impacts on beetle species richness of the higher use of broad-spectrum insecticides in nonTr cotton. However, as with the impacts of broad-spectrum insecticides, the significant differ-

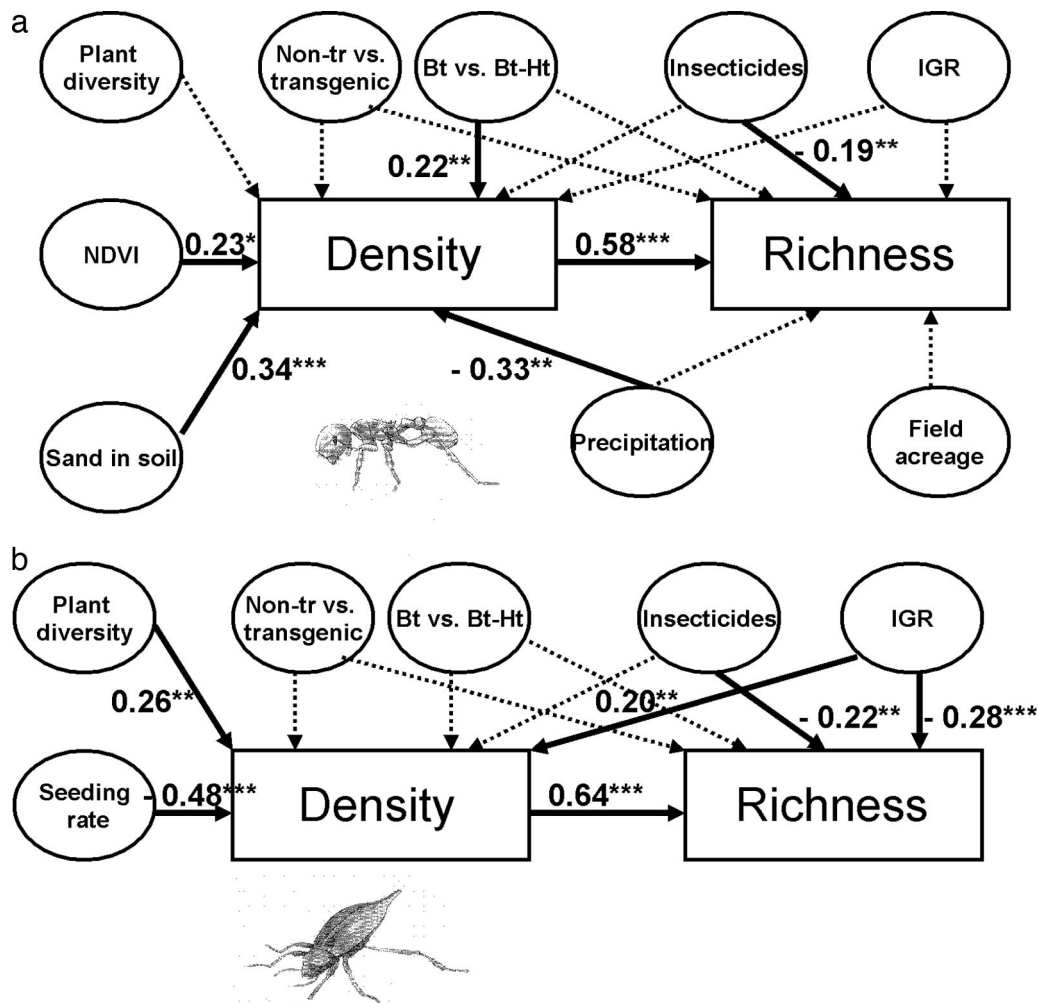


Fig. 3. Path analyses evaluating the effects of nonTr and transgenic cotton on density and species richness of ants and beetles (see *Materials and Methods* for details). Path coefficients not significantly different from zero are represented by dotted arrows; significant path coefficients are represented by continuous arrows. *, $P < 0.07$; **, $P < 0.05$; ***, $P < 0.01$. (a) For ants, variables included in the analysis were the orthogonal contrast between nonTr and the weighted average of Bt and BtHr cotton (nonTr vs. transgenic), the orthogonal contrast between Bt and BtHr cotton (Bt vs. BtHr), number of broad-spectrum insecticide applications before insect sampling (insecticides), whether a field was treated with an IGR before insect sampling (IGR), number of plant types in noncultivated sites (plant diversity), NDVI of noncultivated sites, percentage of sand in soil (sand in soil), accumulated precipitation before insect sampling (precipitation), and field acreage. The compound path coefficients linking species richness to Bt vs. BtHr, NDVI, sand in soil, and precipitation were 0.13 (revealing lower species richness in BtHr than Bt cotton), 0.13, 0.20, and -0.19 , respectively. (b) For beetles, the first five variables considered for ants and seeding rate were included in the path analysis. The compound path coefficients linking species richness to plant diversity and seeding rate were 0.17 and -0.31 , respectively. The total correlation between IGRs and species richness was -0.15 .

can react differently to agricultural perturbations. Ant and beetle diversity could have been affected negatively through direct exposure to Cry1Ac (e.g., after ingestion of plant residues, plant tissues, or prey containing a Bt toxin) or through changes in resources or shelters (e.g., lower abundance of prey or weeds) caused by the use of transgenic cotton (6, 16, 20). Nevertheless, greater impacts of transgenic cotton than nonTr cotton were not evident (Figs. 2 and 3). However, IGRs, broad-spectrum insecticides, and several other agronomic and ecological factors significantly affected ant and beetle diversity. Further experiments are needed to determine whether BtHr cotton has greater negative impacts on ant density than Bt cotton because of reduced biomass or diversity of weeds (6, 21). Recently published experimental (22–25) and large-scale (26) studies confirm that impacts on insect communities are much greater for broad-spectrum insecticides than for Bt crops.

Generalizations about environmental impacts of transgenic crops are difficult because farmer management practices influ-

ence such impacts and constraints on management practices vary regionally (6, 16–18). Nevertheless, our findings indicate that Bt crops could be useful to reduce environmental impacts of agricultural intensification, especially where replacement of insecticides by Bt crops will not reduce the control of pests unaffected by Bt proteins.

Materials and Methods

Sampling Protocol. To select experimental fields in western Pinal County, Arizona, we used Geographical Information System (GIS) maps that identified with high accuracy the location of cotton fields and the type of cotton planted in each field (27). Fields were arbitrarily selected within a region of $\approx 6,600$ km², which was delimited by the frames of Landsat-7 Enhanced Thematic Mapper Plus satellite images overlaid on the GIS maps. For each year of the study, the percentage of Bt cotton in the region was determined from analyses of the GIS maps (27).

Each selected field had at least one border adjacent to

A. Ali, K. Dell, G. Fugate, J. Harms, and D. Overton provided field and laboratory assistance. S. Cover, J. Ellington, and D. Wheeler helped with specimen identification. M. Sisterson, B. Tabashnik, and K. Walker

provided comments on the manuscript. Environmental Protection Agency Cooperative Agreement X-82974701-O provided support for this study.

1. Tilman, D., Fargione, J., Wolff, B., D'Antonio, C., Dobson, A., Howarth, R., Schindler, D., Schlesinger, W. H., Simberloff, D. & Swackhamer, D. (2001) *Science* **292**, 281–284.
2. Khush, G. S. (2001) *Nat. Rev. Genet.* **2**, 815–822.
3. Pimentel, D. S. & Raven, P. H. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 8198–8199.
4. Huang, J., Rozelle, S., Pray, C. & Wang, Q. (2002) *Science* **295**, 674–677.
5. Hails, R. S. (2002) *Nature* **418**, 685–688.
6. Squire, G. R., Brooks, D. R., Bohan, D. A., Champion, G. T., Daniels, R. E., Haughton, A. J., Hawes, C., Heard, M. S., Hill, M. O., May, M. J., *et al.* (2003) *Philos. Trans. R. Soc. London B* **358**, 1779–1799.
7. O'Callaghan, M., Glare, T. R., Burgess, E. P. J. & Malone, L. A. (2005) *Annu. Rev. Entomol.* **50**, 271–292.
8. Lawrence, S. (2005) *Nat. Biotechnol.* **23**, 281.
9. Tabashnik, B. E., Patin, A. L., Dennehy, T. J., Liu, Y.-B., Carrière, Y., Sims, M. A. & Antilla, L. (2000) *Proc. Natl. Acad. Sci. USA* **97**, 12980–12984.
10. Carrière, Y., Eilers-Kirk, C., Sisterson, M., Antilla, L., Whitlow, M., Dennehy, T. J. & Tabashnik, B. E. (2003) *Proc. Natl. Acad. Sci. USA* **100**, 1519–1523.
11. Dennehy, T. J. & Williams, L. (1997) *Pest. Sci.* **51**, 398–406.
12. Ellsworth, P. C. & Martinez-Carillo, J. L. (2001) *Crop Protec.* **20**, 853–869.
13. Naranjo, S. E., Ellsworth, P. C. & Hagler, J. R. (2004) *Biol. Contr.* **30**, 52–72.
14. Carrière, Y., Sisterson, M. S. & Tabashnik, B. E. (2004) in *Insect Pest Management: Field and Protected Crops*, eds. Horowitz, A. R. & Ishaaya, I. (Springer, New York), pp. 65–95.
15. Gotelli, N. J. & Colwell, R. K. (2001) *Ecol. Lett.* **4**, 379–391.
16. Carpenter, J., Felsot, A., Goode, T., Hammig, M., Onstad, D. & Sankula, S. (2002) *Comparative Environmental Impacts of Biotechnology-Derived and Traditional Soybean, Corn, and Cotton Crops* (Council for Agricultural Science and Technology, Ames, IA).
17. Quaim, M. & Ziberman, D. (2003) *Science* **299**, 900–902.
18. Fitt, G. P., Wakelyn, P. J., Stewart, J., James, C., Roupakias, D., Hake, K., Zafar, Y., Pages, J. & Giband, M. (2004) *Global Status and Impacts of Biotech Cotton: Report of the Second Expert Panel on Biotechnology of Cotton* (International Cotton Advisory Committee, Washington, DC).
19. Huang, J., Hu, R., Rozelle, S. & Pray, C. (2005) *Science* **308**, 688–690.
20. Zwahlen, C. & Andow, D. A. (2005) *Environ. Biosafety Res.* **4**, 113–117.
21. Brooks, D. R., Bohan, D. A., Champion, G. T., Haughton, A. J., Hawes, C., Heard, M. S., Clark, S. J., Dewar, A. M., Firbank, L. G., Perry, J. N., *et al.* (2003) *Philos. Trans. R. Soc. London B* **358**, 1847–1862.
22. Dively, G. P. (2005) *Environ. Entomol.* **34**, 1267–1291.
23. Naranjo, S. E. (2005) *Environ. Entomol.* **34**, 1193–1210.
24. Naranjo, S. E. (2005) *Environ. Entomol.* **34**, 1211–1223.
25. Whitehouse, M. E. A., Wilson, L. J. & Fitt, G. P. (2005) *Environ. Entomol.* **34**, 1224–1241.
26. Head, G., Moar, W., Eubanks, M., Freeman, B., Ruberson, J., Hagerty, A. & Turnipseed, S. (2005) *Environ. Entomol.* **34**, 1257–1266.
27. Carrière, Y., Eilers-Kirk, C., Kumar, K., Heuberger, S., Whitlow, M., Antilla, L., Dennehy, T. J. & Tabashnik, B. E. (2005) *Pest Manag. Sci.* **61**, 327–330.
28. Schumacher, A. & Whitford, W. G. (1976) *Southwest. Nat.* **21**, 1–8.
29. Brown, P. W. & Russell, B. (1995) *Weather Data and On-Line Documentation* (Arizona Meteorological Network, Tucson).
30. Brown, P. W., Silvertooth, J. F. & Watson, T. F. (1992) in *Cotton: A College of Agriculture Report*, ed. Silvertooth, J. F. (University of Arizona College of Agriculture and Life Sciences, Tucson), pp. 421–451.
31. Jensen, J. R. (2000) *Remote Sensing of the Environment: An Earth Resource Perspective* (Prentice-Hall, Upper Saddle River, NJ).
32. Vaudor, A. (1991) PISTE (Université de Montréal, Montreal), Version 3.0.
33. Sokal, R. R. & Rohlf, F. J. (1995) *Biometry: The Principles and Practice of Statistics in Biological Research* (Freeman, New York).