THE ROLE OF SURROUNDING VEGETATION AND REFUGES: INCREASING THE EFFECTIVENESS OF PREDATORS AND PARASITOIDS IN COTTON AND BROCCOLI SYSTEMS

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INTRODUCTION

The direct effects of cultural practices on insect pests have been extensively evaluated (Rabb *et al.*, 1984; Herzog and Funderburk, 1986; Dent, 2000). The majority of these practices have been designed to modify crop production to lower pest densities through sanitation, destruction of alternate habitats or hosts used by the pest, tillage, crop rotation or fallowing, manipulation of planting and harvesting dates, trap cropping, and manipulation of vegetational diversity. How cultural practices can be used to increase the effectiveness of natural enemies of insect pests has been less studied (Schellhorn *et al.*, 2000). Cultural practices can affect natural enemy population density and species diversity, and manipulation of these practices can provide the foundation for conservation biological control.

Many studies demonstrate that cultural practices affect natural enemies. Trap crops (Corbett *et al.*, 1991), rotation crops (Xia, 1994), creation of hedge rows (Coombes and Sotherton, 1986; Wratten and Thomas, 1990; Dennis *et al.*, 2000), and manipulation of noncrop habitat can enhance natural enemy abundance (Banks, 1955; Perrin, 1975; Andow, 1991; Schellhorn and Sork, 1997; Landis *et al*, 2000). However, the majority of these studies are descriptive and usually compare only the abundance of natural enemies in one production system or habitat to another. In order to develop predictions about how particular cultural practices change the abundance or effectiveness of predators and parasitoids, it is necessary to understand the underlying population processes, such as movement, reproduction, and longevity (Corbett and Plant, 1993; Prasifka *et al.*, 1999; Schellhorn *et al.*, 2000).

Here we report on the use of novel marking techniques to monitor the movement of natural populations of insect predators and parasitoids at the landscape and whole farm levels. We conducted studies in two distinct systems in Australia: cotton in New South Wales and broccoli in South Australia. The cotton (*Gossypium hirsutum* L.) system was characterized by a summer crop that grows for six months, followed by bare soil for six months, requiring that pests and natural enemies colonize each field anew at the beginning of the cropping season. The broccoli *(Brassica oleracea* L. *var. "marathon")* system was characterized by Mediterranean climate (hot, dry summers and cool, wet winters), where brassica vegetables are in continuous production year round. This results in resident populations of the major pest and its parasitoids.

MATERIALS AND METHODS

Cotton System

Insect predator abundance in crops and noncrops. Insect samples were taken from vegetation in the Namoi Valley in northern New South Wales, Australia. Sampling focused on three cotton fields, one on each of three farms, which were within a 4 km radius of each other. At each site we employed a standardized sampling technique of running a suction sampler across random 20 m sections of vegetation, repeated five times. The details of this method including the types of vegetation sampled are outlined in Silberbauer and Gregg (2002).

Movement of insect predators. To determine whether insect predators were moving among the different types of vegetation, a subsample (n=199) of insects collected were examined for pollen. Insect specimens were prepared for scanning electron microscopy (SEM) by breaking them into four or five pieces, and then adhering them to SEM stubs using double-sided poster tape. SEM stubs were placed in a low-temperature oven (40-60 °C) for at least 12 hours prior to sputter coating with gold. Each piece of insect was then examined under at least 500x magnification.

Any pollen found was examined under at least 1000x magnification and identified using Peter Gregg's pollen SEM photographic library (unpublished) and Jones *et al.* (1995). As many pollen species as possible were identified to species or family level. Because the descriptions of Australia's pollen flora is still incomplete, many of the pollen grains could not be identified, and thus were just labelled with numbers. All pollen species found were photographed and given identifying numbers.

Broccoli System

The Adelaide plains is the main vegetable producing area of South Australia. Brassica vegetables are in continuous production year round, which results in a resident population of the major pest, the diamondback moth, *Plutella xylostella* (L.), and its most abundant parasitoid, *Diadegma semiclausum* (Hellén).

Pest and parasitoid abundance in mature broccoli. Monitoring the movement of natural populations of insects in fields involves three steps. First the density of insects of interest must be sufficiently high to allow the use of mark and capture techniques. Second, the mark must be identifiable on the species of insects that are to be evaluated. Third, the capture methods must not cause any cross contamination or removal of the mark, and any biases should be known.

To monitor the movements of *P. xylostella* and *D. semiclausum* from mature to young broccoli , we created conditions favouring rapid insect population growth by withholding irrigation for two weeks and insecticides for five weeks. In addition, because the experiment took place on a grower's property, we arranged for the grower to withhold insecticides on all adjacent broccoli bays (long narrow adjacent fields, usually 210 m x 10 m, separated by a 1.5 m alley) for ten days (Fig. 1), even though Dipel® was the only product used on the property over the previous three months and was used infrequently. To determine if the mature bay of broccoli had sufficiently high insect densities to successfully employ spray of a fluorescent dye as a marker, we sampled a bay (210 x 10 m) for insects using a suction sampler at 30 row meters of plants, replicated ten times. From past experience we had determined that the density of a species needed to be ca one per row metre to have enough individuals in a field to mark and monitor movement.

Movement of pest and parasitoids. The experiment to assess moth and parasitoid movement was conducted in Virginia, South Australia, on a property that had eleven bays of broccoli in production (each bay measuring 210 x 10 m with a 1.5 m alley), and bare cultivated soil surrounding the cropped field (Fig. 1). We used a novel marking technique and sprayed a nontoxic, fluorescent resin-based dye (SARDI Fluorescent Pigment) in the broccoli field to mark natural populations of *P. xylostella* moths and their main larval parasitoid, *D. semiclausum*. In a prior experiment, we established that our dye marked the moth and the parasitoid, and that we were able to capture these species with suction sampling and yellow-sticky-bucket traps (inverted 9 liter buckets, with a 5 cm wide ring made of

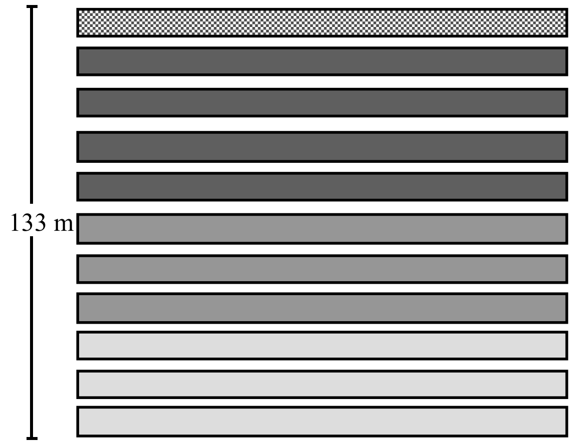

Figure 1. Layout of experimental field at Newman and Sons property, Virginia, South Australia, Australia. The checked rectangle represents the bay of broccoli sprayed with fluorescent dye. Each additional rectangle represents a bay of broccoli from mature to young differing in age by one week.

particle board around the base; all coated with tangle trap) placed 20 cm from the ground. At the time of our experiment, the youngest bay of broccoli was three weeks from harvest and the three most mature bays of broccoli were no longer suitable to harvest, so plants in all bays were similar in the amount of vegetative growth.

To determine if *P. xylostella* and *D. semiclausum* move from mature to young broccoli before cultivation we sprayed 120 liters of the dye mixture on the entire 210 x 10 m bay of broccoli. Next, we placed four yellow-sticky-bucket traps 20 cm from the ground per bay in each of five alternating bays, plus the bay that was sprayed with the fluorescent dye. Forty-eight hours after spraying the dye, we used a suction sampler in each of the 10 bays not treated with dye (the dyed bay was excluded because suction sampling on plants with the resin picks up fluorescent dye and cross contaminates samples) to sample 30 m sections at three locations per bay. Five days after spraying the dye, we removed the yellow-sticky-bucket traps from all plots and subsampled two alternate quadrats of the traps for moths and parasitoids. To determine whether diamondback moth and *D. semiclausum* move from mature to younger broccoli when there is a disturbance from cultivation, we proceeded in the same manner as above. After spraying the dye and placing the yellow-sticky-buckets in the bays of broccoli, the dyed bay was cultivated (the usual practice after broccoli is harvested) leaving only bare soil, thus forcing the mobile insects from the broccoli. The yellow-sticky-buckets were removed from all bays three days after setting them up.

Statistical analysis. The Kolmogorov-Smirnov goodness-of-fit test was used to determine the difference in the distribution of the parasitoids and moths before and after cultivation. A sign test was used to detect the difference in direction of the pattern of dispersal for the parasitoid and moth, before and after cultivation.

RESULTS

Cotton System

Insect predator abundance in crops and noncrops. There were six species of abundant generalist insect predators extracted from the samples: transverse ladybird, *Coccinella transversalis* (Fabricus); minute two-spotted ladybird, *Diomus notescens* (Blackburn); a damsel bug, *Nabis (Tropiconabis) kinbergii* Reuter; red and blue beetle, *Dicranolaius bellulus* (Guerin-Meneville); a green lacewing, *Mallada signatus* (Schneider); and a brown lacewing, *Micromus tasmaniae* (Walker). The average number of individuals of these six species summed that were collected from each type of vegetation through the season varied (Fig. 2). Cotton, lucerne, and wheat had the highest densities of adult insect predators in spring; cotton, sorghum, and sunflower had the highest densities in early summer; and by mid summer the highest populations were in sorghum. By late summer the abundance of insect predators dropped to almost zero, with a few remaining in cotton, sorghum, pasture, or lucerne.

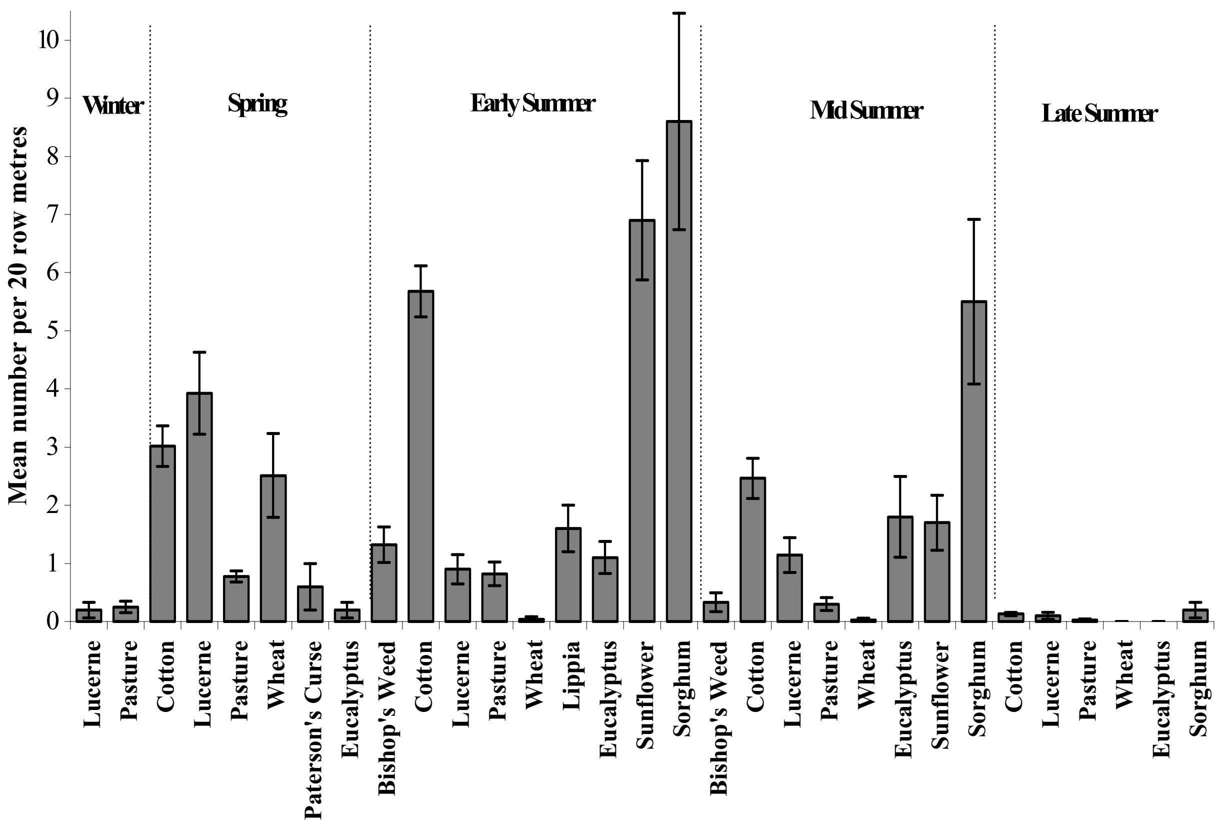

Figure 2. Mean number (± 1 SE) of the six most abundant insect predators summed in each type of vegetation per seasonal period.

Movement of insect predators around cotton landscape. Of the 199 individuals examined, 170 (85%) carried pollen, and all six predator species had individuals that carried pollen. Of the individuals with pollen, 151 (89%) carried more than one type ("species") of pollen. This pattern was similar for the two most abundant species captured in cotton; 72% of *D. notescens* (n = 47) and 82% of *M. tasmaniae* (n = 35) carried more than one type of pollen. The pollen types carried most frequently by

M. tasmaniae were Bishop's weed, *Ammi majus*, (L.), cotton, other Malvaceae and *Eucalyptus* spp., and by *D. notescens* were other Malvaceae, Bishop's weed and *Eucalyptus* spp. For those individuals captured outside of cotton, 90% of *M. tasmaniae* (n = 10), carried more than one type of pollen and 75% carried cotton pollen; and for *D. notescens* (n = 14) 57% carried more than one type of pollen and 40% carried cotton pollen.

Broccoli System

Pest and parasitoid abundance and movement from mature to young broccoli. Immediately before spraying fluorescent dye on the mature broccoli bay (and resident insect populations), the adult *P. xylostella* and *D. semiclausum* densities were 0.75 ±0.57 (SD) and 1.01 ± 0.50 (SD) per row meter, respectively. Based on results from suction sampling before cultivation, marked *P. xylostella* did not appear to move far as all marked individuals were captured within 36 m of the dyed bay. However, after cultivation, one marked *P. xylostella* was captured as far as 60 m from the dyed bay, yet there was no difference in their distribution before and after cultivation ($D = 0.10$, df = 9, $P = 0.666$). The result from the yellow-sticky-bucket traps was different than from the suction sampling due to the time and type of capture. The moth, *P. xylostella*, moved as far as 108 m (the farthest distance sampled in broccoli) from the dyed bay both before and after cultivation (two and one marked individuals, respectively), but their distribution did not differ ($D = 0.33$, df = 5, $P = 0.400$; Table1). When considering the pattern of dispersal found on yellow-sticky-bucket traps, the direction of the difference of marked *P. xylostella* captured was greater at each distance before cultivation compared to after cultivation ($C_{0.05\,(0)6}$, $P < 0.01$).

Table 1. Proportion of marked *P. xylostella* moths captured on yellow-sticky-bucket traps before and after cultivation. "0" metres is the source of marked insects.

	Distance from dyed broccoli (m)					
	0	12	36	60	84	108
Before cultivation (n=1128)	0.40	0.13	0.03	0.05	0.02	0.01
After cultivation (n=1137)		0.08	0.01	0.03	0.00	0.01

The dispersal pattern of *D. semiclausum* differed from that of *P. xylostella* adults. Based on results from suction sampling before cultivation, 5% of marked *D. semiclausum* were captured 60 m from the dyed bay (Fig. 3). After cultivation, the dispersal of marked *D. semiclausum* was greater ($D = 0.50$, df = 9, $P = 0.037$), and 7% of marked individuals were captured at 108 m from the dyed bay with greater than 50% of marked individuals dispersing farther than the closest bay of broccoli, 12 m from the dyed broccoli (Fig. 3).

D. semiclausum was captured on the yellow-sticky-bucket traps as far as 108 m before and after cultivation, and there was no difference in their dispersal ($D = 0.50$, df = 5, $P = 0.208$; Fig. 4). However, the direction of the difference in dispersal of marked *D. semiclausum* was greater after cultivation than before cultivation–the opposite from that for diamondback moth ($C_{0.05\,(1)6}$, $P < 0.01$).

Although we have focused on dispersal before and after cultivation, it should be noted that dispersal over time maybe confounded with cultivation for the moths. This could have only been avoided if two colors of resin-based dye had been available (which we now have). Results from our

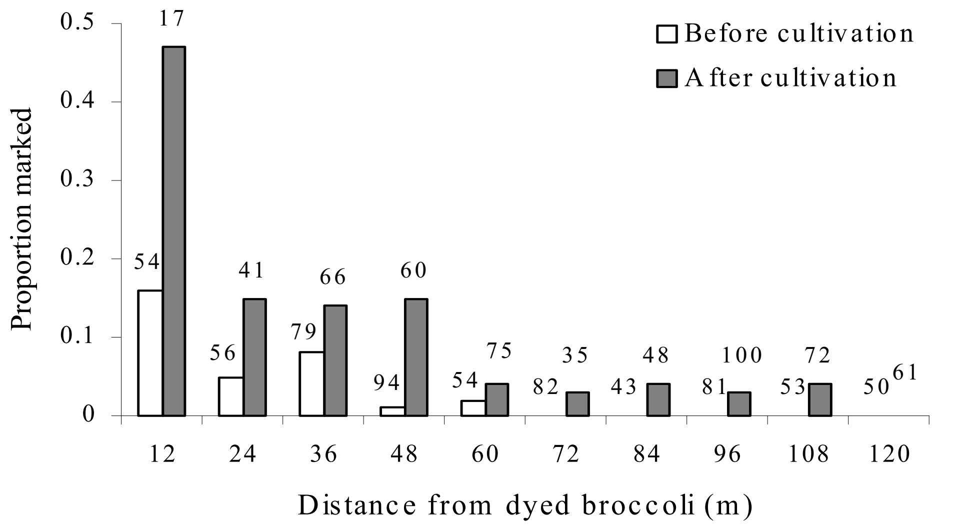

Figure 3. Proportion of marked *D. semiclausum* captured with a suction sampler (numbers above bars are numbers of wasps captured). "0" metres is the source of marked insects.

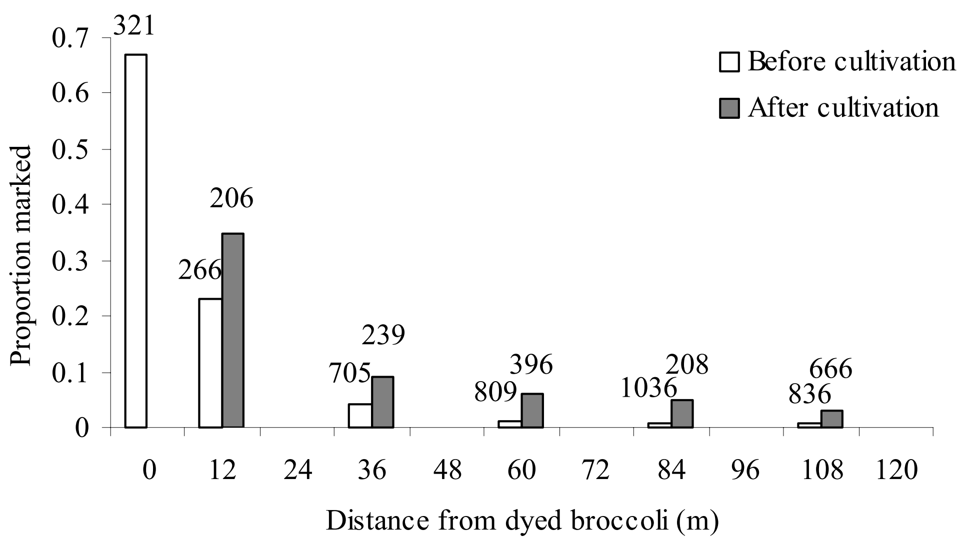

Figure 4. Proportion of marked *D. semiclausum* captured on yellow-sticky-bucket traps before and after cultivation, (numbers above bars are numbers of wasps captured). "0" metres is the source of marked insects.

laboratory experiments showed that 99.8% of *D. semiclausum* die within 5 days without a sugar source, and the average longevity is 2.9 days, and 99.8% of *P. xylostella* die within 11 days and the average longevity is 5.7 days (Schellhorn, unpublished data). There were five days between the cultivation experiments which suggests that the parasitoids were unlikely to have lived long enough for this issue to be important. However, *P. xylostella* is likely to have lived long enough, yet there was equal or less dispersal after cultivation which suggests that the issue was not important for the moths.

DISCUSSION

Our results from the cotton system show that insect predators of cotton pests are present in several types of vegetation throughout the year. In addition, we found that the two most abundant insect predators, *M. tasmaniae* and *D. notescens*, visit several types of vegetation before moving into cotton, and move back and forth between cotton and other types of vegetation. These findings suggest that particular types of vegetation on-farm or in the larger landscape may conserve and enhance local populations of insect predators.

Our results from the broccoli system show that the patterns of movement for *P. xylostella* adults and *D. semiclausum* before and after cultivation were different. For *P. xylostella* adults, the results from suction sampling suggest limited dispersal before and after cultivation, a finding similar to that of our preliminary experiments (Schellhorn, unpub.). Results from the yellow-sticky-bucket traps showed that *P. xylostella* dispersed to 108 m, but the majority of marked individuals dispersed to the adjacent broccoli before and after cultivation. However, for *D. semiclausum*, the majority of marked individuals dispersed further than the adjacent broccoli. Fewer *D. semiclausum* dispersed before cultivation than after cultivation, suggesting that disturbance increased parasitoid movement, which was not the case for *P. xylostella.* The difference in the degree of dispersal suggested by suction sampling versus yellow-sticky-bucket traps before cultivation was most likely caused by the difference in the sampling date: suction sampling was conducted 48 hours after the broccoli bay was first treated with dye, versus 96 hours for the yellow-sticky-bucket traps.

In large-scale monocultures, such as New South Wales cotton, planting of early-season annuals or early-flowering perennials may improve overwintering conditions, or increase colonization and subsequent population increase by *M. tasmaniae* or *D. notescens* before the occurrence of populations of summer pests. This appears to be happening in grapes in the western United States of America, where the solitary egg parasitoid *Anagras* spp., overwinters in French prune trees that harbor an alternative host (Doutt and Nakata, 1973; Kido *et al*., 1984). In the early spring, *Anagrus* spp. colonizes adjacent vineyards and plays a critical role in increasing parasitism and controlling populations of western grape leafhopper (Corbett and Rosenheim, 1996; Murphy *et al*., 1998). The next study in cotton will be to test particular annuals or perennials for improved overwintering and subsequent colonization of cotton.

In the broccoli system, production is continuous so natural enemies have to be maintained, disturbance minimized and population increase encouraged throughout the year without causing an increase in pests. Maintaining bays of harvested, uncultivated broccoli (a type of refuge) at 70 m intervals may allow parasitoid populations to build-up and move into adjacent, younger plantings. Disturbance, such as harvest or cultivation, can disrupt biological control (Schellhorn *et al*., 2002; Honk 1982; Carillo 1985). Maintaining on-farm refuges may reduce the effects of such disturbances on natural enemies and increase recolonization (van den Bosch *et al*., 1966; Mullens *et al*., 1996).

Pollen and resin-based fluorescent dye are excellent tools to monitor movement of field populations of natural enemies and pests. The data from this project show that information on species-specific behavior and population processes, particularly movement, are helpful to manage cultural practices intended to increase natural enemy abundance as part of a biological control program. By increasing our knowledge of natural enemies and pests in relation to habitat use, we should be able to make predictions about how to implement effective cultural practices to manage pest insects.

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