Effects of a Nicotinic Insecticide, Imidacloprid, and Vegetation Diversity on Movement of a Common Predator, Coccinella septempunctata

J. E. BANKS*1 AND J. D. STARK2
1Environmental Science, University of Washington, Tacoma
1900 Commerce Street, Tacoma, WA 98402, USA
2Department of Entomology, Puyallup Research and Extension Center
Washington State University
7612 Pioneer Way E., Puyallup, WA 98371, USA

Biopest. Int. 7(2): 113-122 (2011)

ABSTRACT We conducted a field experiment aimed at elucidating the effects of a selective pesticide on the behavior of a common agroecosystem predator, the seven-spotted ladybird beetle (Coccinella septempunctata L.) in plots with different background vegetation. We released individual seven-spotted ladybird beetles that had been sprayed with either (i) pesticide, or (ii) water, into experimental plots consisting of broccoli plants surrounded by either (i) weedy margins, or (ii) bare ground, and recorded their movement behavior. We analyzed the resulting trajectories by fitting observed ladybird movement to a correlated random walk (CRW). Neither beetles sprayed with pesticides nor those sprayed with water conformed entirely to the CRW model; however, beetles sprayed with pesticide exhibited marked differences in behavior depending on margin type. In particular, the CRW grossly underestimated the dispersal ability of pesticide-sprayed beetles that were surrounded by bare ground margins compared with beetles surrounded by weedy margins. These sublethal effects of pesticides on predator movement may have critical consequences for biological control, especially in integrated pest management schemes.

KEY WORDS: Diffusion model, habitat heterogeneity, ladybird beetles, movement behavior

INTRODUCTION

A central concern of integrated pest management is the integration of biological control with chemical and cultural controls of insect pests. Most protocols for determining the toxicity and compatibility of insecticides with biological control focus on natural enemy mortality or reduced reproduction (Hassan, 1992; Stark and Banks, 2001, 2003; Cabral et al., 2008). However, insecticides may affect behavior of natural enemies in the field to the extent that it alters their foraging ability, thereby affecting insect pest control (Stapel et al., 2000). Furthermore, vegetation background and diversity can play a central role in bolstering populations of natural enemies and reducing pest colonization (Root, 1973; Risch et al., 1983; Vandermeer, 1989; Andow, 1991; Banks, 1998, 1999, 2004, Altieri, 2004). Vegetation diversity has long been regarded as important in insect population regulation, especially for herbivorous insects (Elton, 1958; Root, 1973;...
Cromartie, 1975; Bach, 1980; Kareiva, 1987; Andow, 1990; Perfecto, 1992; Banks, 1998, 2000, 2004; Doak, 2000, Altieri, 2004). Increased plant diversity in agroecosystems has been frequently touted as a means of reducing herbivore populations, either by diminishing herbivore colonization and tenure-time on host plants, or through bolstering natural enemy populations (Root 1973; Vandermeer 1989). However, vegetation diversification alone remains an unreliable means of controlling pest insects: surveys of hundreds of experiments testing the effects of increased vegetation diversity on herbivore populations have revealed modest effects (Risch et al. 1983; Andow 1991; Tonhasca and Byrne 1994, Bommarco and Banks, 2003). This underscores the importance of understanding how various components of integrated pest management, including limited pesticide use, may act in concert to reduce pest loads.

In response to increasing global concerns over environmental and public health risks associated with the use of traditional, broad spectrum pesticides, a new suite of more selective pesticides are being developed for insect pest control. These new biorational pesticides are designed to target only certain insect taxa, leaving the remainder of biological communities largely intact. Of particular interest to biological control applications is the possibility of incorporating arthropod natural enemies into integrated pest management schemes. Studies have demonstrated that combinations of two or more natural enemies may act on pest populations in an additive fashion (Chang 1996), in a sub-additive fashion (Rosenheim et al. 1993), or synergistically (Losey and Denno 1998). Similarly, combinations of pesticides and natural enemies have been known to act synergistically on insect pests (Johnson and Gould 1992). Notably, past studies have typically measured interactions between biological control and other means of pest control (e.g., Quintela and McCoy, 1998) in terms of reductions in pest populations, rather than documenting the direct effects of other chemical or cultural methods on the biological control agents.

Imidacloprid is a nicotinic insecticide that was designed to be effective in the selective control of economically important crop pests (e.g., Aphididae) with minimal impacts to non-target organisms. In the past decade, however, a suite of studies have revealed that imidacloprid can in fact have deleterious effects on non-target insects, including parasitoids (Smith and Krischik, 1999; Rogers and Potter, 2003; Rogers et al., 2007). In some cases, it has been shown to interfere with biological controls by reducing fitness and mobility in coccinellids (Smith and Krischik, 1999). Effects on movement may be especially important for biological control, as recent behavioral studies have shown that the details of how beetles move along branches searching for aphids can affect foraging efficiency (Girling et al., 2007).

We describe a field study in which we quantified the movement behavior of the seven-spotted ladybird beetle (Coccinella septempunctata L.) subject to differing spray intensities of a selective pesticide, imidacloprid. We conducted these field trials in plots with two different types of margins (weedy or bare) in order to further our understanding of how mixtures of chemical and cultural controls mesh with biological control.

MATERIALS AND METHODS

Study System

C. septempunctata, the seven-spotted ladybird beetle, is a common aphidophagous predator that was introduced to the United States in the 1950s and has since become ubiquitous (Angalet et al., 1979; Elliott et al., 1996). C. septempunctata will consume aphids and other insects, but is also attracted to pollen and nectar (Hagen, 1962). Individuals typically fly among patches of vegetation searching for likely prey habitat, then switch to a walking behavior once they have alighted and begin foraging.

Experimental Design

We established plots of broccoli (Brassica oleracea, var. Emperior F1, Zenner Bros., Oregon) surrounded by either bare ground or weedy vegetation at Washington State University's
Puyallup Research and Extension Center Experimental Farm Five, 70km south of Seattle in Puyallup, Washington, U.S.A. Broccoli were established in square plots measuring 2.5 × 2.5 m, surrounded in each case by 1 m wide margins of either (a) bare ground, or (b) weedy vegetation. Broccoli were spaced 0.5 m apart; each plot contained 16 (4 × 4) broccoli plants. The most prevalent species in weedy margins were *Amaranthus powellite* (S. Watson), *Chenopodium album* (L.), *Echinochloa colona* (L.), and *Echinochloa crus-galli* (L.). All plots were separated from other plots by a minimum of 5.5 m in order to ensure that insect movements within plots were independent of neighboring plots in the field. Broccoli was used in this experiment because it can be grown in homogenous stands of relatively uniform plants. Weedy vegetation in-between plots and within broccoli areas of each plot was regularly removed by tractor and hand cultivation, respectively, throughout the duration of the experiments.

Broccoli were grown from seed in planting flats and kept in the greenhouse until large enough to transplant into the field; plant plots were established in the field during early summer (June) and allowed to grow for one month before behavioral observations were conducted. Because broccoli attracts a suite of herbivorous aphids in western Washington, including *Myzus persicae* (Sulzer) and *Brevicoryne brassicae* (L.), all broccoli plants in experimental plots were sprayed with the selective pesticide Pirimor (active ingredient pirimicarb) 5 day before observational data were collected. We sprayed in order to remove prey so that beetle behavioral experiments could be conducted without prey as a confounding factor in beetle response to vegetation manipulations, *C. septempunctata* has demonstrated a strong tendency to move towards aphid prey (termed "preytaxis") in past behavioral studies (Kareiva and Odell, 1987). In order to minimize possible effects of residuals on experimental ladybirds, we waited 5 days after sprays before doing any movement behavioral experiments, even though pirimicarb has been shown to have negligible soil toxicity and minimal impacts on *C. septempunctata* (Unal and Jepson, 1991). Subsequently, plants were checked throughout the duration of the experiment to ensure that no aphids were evident on any of the broccoli plants.

**Behavioral Experiments**

We released seven-spotted ladybird beetles (*C. septempunctata*) and observed their movement in broccoli plots surrounded by weedy or bare ground margins.

Immediately prior to the movement experiments, we collected live *C. septempunctata* individuals from vegetation adjacent to the experimental plots and placed them individually in cardboard containers. Beetles in containers were kept in a cool and shady area of the field before being released in broccoli plots. Special care was taken not to keep beetles in containers for more than 1 hr before using them in behavioral observations in order to reduce biases in their behavior due to being held captive too long (Gui and Boitane, 2010); in most cases, beetles were kept no longer than 15–20 min before being released and observed. Each beetle was sprayed with either pesticide (imidacloprid) or water (control) before release and observation. Each individual was released on the ground in the center of a randomly chosen weedy or bare ground margin plot and its subsequent movement behavior was recorded; beetles were transferred from containers directly to the ground in order to minimize effects of being handled (Gui and Boitane, 2010). As soon as a beetle began moving, we began marking its positions at 5 s intervals, placing thin pieces of wire with numbered flags on the ends into the ground at each position. After ten 5-second steps were completed, we measured net squared displacements and the step lengths and turning angles from each previous direction for all steps. As beetle movement was restricted to the ground, trajectories were constrained to two dimensions. We recorded a total of 20 trajectories (10 pesticide sprayed, 10 water sprayed, with 5 of each per vegetation treatment).

**Analysis: Predator Movement Experiment**

We used a diffusion model to compare the
movement behavior of beetles that had been sprayed with pesticide with that of beetles that had been sprayed only with water in the two different margin type treatments. We used a random walk model that assumes that beetles move randomly but with some tendency to move in the same direction from one step to the next. The model, a modified simple diffusion model originally developed by Goldstein (1951), may be described in closed form as:

$$\frac{\partial^2 p}{\partial t^2} + \frac{2}{T} \frac{\partial p}{\partial t} = v \frac{\partial^2 p}{\partial x^2}$$

where \( p \) is the particle (or beetle) density, \( v \) is the speed, and \( T \) is a characteristic step correlation time (Okubo and Grünbaum 2001). This model, known as the telegraph equation or more generally as a correlated random walk (CRW) model, has been employed in several other insect movement studies, including those modeling beetles (Kareiva and Shigesada, 1983; Wallin and Ekbom, 1988; Turchin, 1991, 1998; Crist et al., 1992; Chapman et al., 2007; Jackson et al., 2009). Because this model allows for some correlation in direction of successive moves, it is a more realistic description of the behavior of many foraging insects than a simple random walk. Furthermore, it is also mathematically more realistic for terrestrial insects, predicting a finite dispersion velocity rather than an infinite velocity characteristic of simple random walks (Okubo and Grünbaum, 2001).

In order to test the fit of observed beetle movement to the CRW model in the two treatment settings (pesticide versus water spray), we compared mean observed net squared displacements, \( \bar{\xi} \), for each beetle to predicted \( \bar{\xi} \) and 95% confidence intervals generated by 10,000 runs of a bootstrap simulation described by Turchin (1998). This method consists of repeatedly sampling with replacement turning angles and step lengths from the observed data for beetles subjected to each treatment type in order to generate simulated trajectories. We compared observed and simulated mean net squared displacements for beetles sprayed with pesticide in both weedy and bare ground margin experimental plots. Autocorrelation in turning directions was tested by developing contingency tables based on correlated subsequent turn directions: left-left, left-right, right-right, right-left for bare ground and weedy plots for pesticide- and water-sprayed beetles. Autocorrelation in step lengths was analyzed by calculating Pearson product-moment correlation coefficients between subsequent lengths for bare ground and weedy plots for pesticide- and water-sprayed beetles.

RESULTS

Predicted mean net squared displacements for beetles sprayed with pesticide underestimated observed beetle movement in both bare ground and weedy plots, although the CRW was an accurate description of beetle movement in weedy plots after the first movement step (Fig. 1). In contrast, the CRW model was rejected for both bare ground and weedy plots for beetles that had been sprayed with water (Fig. 2), underestimating beetle movement for the entire ten steps in the bare ground plots and for the first three steps in weedy plots.

Autocorrelation analysis indicated that step lengths were correlated with previous step lengths for both sprayed and control (water sprayed) beetles. In particular, autocorrelations were significant for sprayed beetles in bare ground plots (Pearson = 0.869, \( P < 0.001, n = 40 \)); for sprayed beetles in weedy plots (Pearson = 0.80, \( P < 0.001, n = 40 \)); for beetles sprayed with water in bare ground plots (Pearson = 0.664, \( P < 0.001, n = 40 \)); and for beetles sprayed with water in weedy plots (Pearson = 0.551, \( P < 0.005, n = 27 \)).

There was no autocorrelation in the direction of beetle movement in either bare ground or weedy margin plots for either pesticide or water sprayed treatments (pesticidal bare ground: \( \chi^2 = 0.75, P > 0.1, n = 6 \); pesticidal weedy: \( \chi^2 = 0.073, P > 0.1, n = 19 \); water-sprayed bare ground: \( \chi^2 = 0.186, P > 0.1, n = 19 \); water-sprayed weedy: \( \chi^2 = 0.363, P > 0.1, n = 13 \)).

DISCUSSION

The results of this experiment highlight the
potential for unpredictable effects on biological control stemming from the interaction between selective pesticide disturbances and the presence of weedy vegetation in and around host plants. In related field experiments, we have found synergistic interactions between pesticides and vegetation diversity (Banks and Stark, 2004; Adams et al., 2005), suggesting combined approaches (typical of IPM) may be a means of reducing reliance upon chemical control. The difference in beetle behavioral responses to pesticide sprays in bare ground vs. weedy plots in the current study high-

![Graph](image)

**Fig. 1.** Comparison of observed (solid lines w/open circles) and predicted (solid lines w/closed circles) beetle mean squared displacement for beetles sprayed with pesticide in (a) bare ground margin plots and (b) weedy margin plots. Predicted mean squared displacement and 95% confidence interval (dotted lines) were obtained by simulations.
lights the need for further detailed studies of the sublethal effects of pesticides in conjunction with cultural biological controls.

The results of our behavioral experiments with ladybird beetles are consistent with a related study we performed in which we tested beetle movement behavior at different vegetation spatial scales. In that study, we found that the CRW did not describe the movement of unsprayed beetles in plots absent of prey with the same spatial dimensions as the ones in the current experiment (Banks and Yasnak, 2003). However, a more careful examination of the details of the results of the current experiment reveals some potential mechanisms underlying beetle response to the experimental treatments. Although the movement of neither beetles sprayed with pesticide nor those sprayed with water conformed completely to the CRW model.
(Figs. 1, 2), in both cases beetle movement in bare ground plots markedly exceeded the mean net squared displacement predicted by the model. This pattern was especially striking for beetles that had been sprayed by pesticide, in which the model underestimated beetle movement for eight steps in bare ground plots (Fig. 1a) but underestimated beetle movement for only one step in weedy plots (Fig. 1b). There was a correlation among step lengths, but the autocorrelation was significant for all treatment combinations, minimizing the likelihood that detected treatment differences were affected by this bias. Taken together, our results indicate that for a given landscape treatment, beetles exposed to pesticide moved more directly out of plots than beetles exposed to water only. This difference in movement behavior, indicating potential shifts in foraging strategies mediated by the interaction between habitat heterogeneity and pesticide exposure, may have important implications for the compatibility of biological control and pesticide use in agricultural settings.

Extrapolating from our results to larger spatial and temporal scales can be risky due to the potential for temporally and spatially varying behavior (Cain, 1991; Morales and Ellner, 2002); furthermore, the relatively small number of observations upon which this result is based mandates caution in over interpreting the significance of these results. The time intervals used for each step in beetle movement observations may also bias results, though a lack of autocorrelation in turning angles suggests that beetle movement was not oversampled (Turchin, 1998). The number of steps (ten) chosen for each observation were enough for beetles to move to and beyond broccoli plants within each plot, and therefore enable us to draw conclusions about beetle movement behavior in the different habitat treatments (Cain et al., 1985). Apart from pesticide exposure and vegetation background, prey density and hunger level of predatory beetles may also influence beetle foraging behavior and subsequent prey consumption (Wallin and Ekborn, 1994; Yasuda and Ishikawa, 1999), and species composition of coccinellid prey may alter beetles’ susceptibility to insecticides (Kalushkov, 1999). However, our data illustrate that further work exploring the mechanistic underpinnings of how pesticides affect natural enemy movement and foraging behavior are sorely needed.

Overall our results indicate that beetles moved farther away from their point of origin when in bare ground plots than in weedy plots, with this difference exacerbated when beetles were exposed to pesticide. These results may have important consequences for biological control, as they suggest that beetles may represent less of a predation force in bare ground plots than in plots with weedy margins — a tendency that is exacerbated by the action of pesticides. Furthermore, these results suggest that pesticides may interfere with beetles’ ability to orient towards host-plants in the absence of well-defined plots borders (i.e. weedy margins); clearly further investigation of how beetles perceive host-plant vegetation and habitat boundaries is warranted.

Acknowledgments. Thanks to S. Hopkins, C. Yasenak, D. Matheson and R. Schwinkendorf for assistance in the field. This project was supported by a USDA CSREES PMAP grant (97-04104) to J.E.B. and J.D.S.

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Accepted 8 November 2011