REPRODUCTION AND CROP COLONIZATION BY THE CONVERGENT LADY BEETLE, *HIPPODAMIA CONVERGENS*

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Abstract

As an annual crop, cotton depends on colonization by predatory and parasitic arthropods for biological pest suppression. These natural enemies help control pests at no cost to cotton producers and should be increasingly effective with the anticipated reduction in overall pesticide loads as the systematic boll weevil eradication programs advance. Knowledge of how predators and parasitoids colonize cotton may allow for habitat manipulations that better use existing populations of beneficial insects. We investigated colonization of cotton fields and subsequent reproduction by *Hippodamia convergens* (the convergent lady beetle) in an agricultural system where two other crops, corn and grain sorghum, are produced concurrently with cotton. Carbon stable isotopes served as natural marks for predators feeding in these crops and allowed investigation of (1) the time period over which lady beetles colonized cotton from nearby grain sorghum and corn, and (2) if these external crops contributed resources used to produce *H. convergens* eggs in the cotton crop. Results indicated a four to five week period of concerted movement into cotton by lady beetle adults, with a few individuals continuing to move into cotton late, even as the *H. convergens* populations in cotton declined. Analysis of egg masses suggested that prey resources in corn and grain sorghum contributed to the production of egg masses in cotton during the six-week period of *H. convergens* egg production. Further, it was observed that the weeks when strong immigration of adults occurred were coincident with the greatest collections of egg masses produced by adults feeding in corn and grain sorghum. Data from this and other studies emphasize the importance of diverse and continually available resources for predatory insects, and the potential for other crops to contribute to cotton pest control.

Introduction

Like all annual crops, cotton acquires natural enemies (predators and parasitoids) by immigration from outside its field borders. Colonizing natural enemies provide natural biological control of a variety of crop pests at no cost to the producer. Additionally, they reduce cotton producers' need for repeated applications of pesticides, which themselves often necessitate further pesticide use through outbreaks of secondary pests. However, with the ongoing elimination of the boll weevil, *Anthonomus grandis* Boheman, as a pest over millions of hectares, decreased pesticide applications in cotton should allow for natural enemies to play even greater roles in pest management programs. In the event that boll weevil eradication continues to succeed, one obvious route to improving cotton pest suppression is by understanding the colonization of cotton by key natural enemies and exploring possible ways to improve the abundance and timing of immigrating beneficial insects.

Crop colonization by beneficials may occur in at least five different ways (Wratten and Thomas, 1990), and in agricultural systems where two or more crops are produced during overlapping growing seasons, one crop may provide a source of predators or parasitoids for a neighboring crop type. Because crops planted early in the year are often implicated as sources of predators and parasitoids for crops planted later in the season, differing crop phenologies are sometimes suggested as responsible for these movements (Fye, 1972; Wratten and Thomas, 1990). Several studies in agricultural systems including cotton as a component have suggested that other crops act as sources of natural enemies for cotton fields (Wille, 1951; Fye, 1971; Fye and Carranza, 1972; Robinson et al, 1972a,b; Lopez and Teetes, 1976; Wu, 1986; Corbett et al, 1991; Prasifka et al, 1999), including alfalfa, peanuts, corn, and grain sorghum.

The studies implicating intercrop movement of beneficial arthropods have used a variety of methods, but direct evidence from marked and recaptured individuals has been suggested to be the preferable method (Hagler and Jackson, 2001). However, applications of physical or chemical marks to natural enemies may fail to provide sufficient marked insects (relative to abundances of unmarked natural populations) to allow for robust, interpretable recapture data (e.g. Kieckhefer and Olson, 1974). One solution to the problems with marking insects in relatively simple agricultural systems is to use a natural marking technique. Stable carbon isotopes represent one such technique. Because C_3 plants (e.g. cotton, alfalfa, wheat) and C_4 crops (e.g. grain sorghum, corn) fix CO_2 using differing pathways, the heavy isotope of carbon, ^{13}C, is represented differentially in these groups of plants. C_3 plants generally have ^{13}C (a change in ^{13}C:^{12}C in parts per thousand relative to a reference, with units abbreviated as $^o/_{oo}$) values between −32 and −22 $^o/_{oo}$, while C_4 plants range from −23 to −9 $^o/_{oo}$ (Rounick and Winterbourn, 1986). Conveniently, the $\delta^{13}C$ values of the plants are conferred to insects feeding on the plants with little distortion, and this phenomenon also extends to predators consuming the herbivores. In cases where individuals change diets from C_3 to C_4 (or vice versa), isotope values also change to the more recent food source, with most of the change for arthropod predators coming within seven days of the change in diet (Ostrom et al, 1997). Therefore, in simple systems of both C_3 and C_4 plants, isotope ratios of herbivores and their predators indicate the feeding habits of individuals, and can also be used to indicate recent movement.

In this study we used stable isotope methods to investigate movement and reproduction by the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, in an agricultural system where food sources with distinct carbon isotope ratios were available to the coccinellid predator. Building on previous work (Ostrom et al, 1997) successfully using this technique in agroecosystems, our objectives were to determine; (1) the time period over which colonization and mixed-feeding occurs by the convergent lady beetle and (2) if external crops contribute resources used in production of lady beetle larvae in the cotton crop. Ideally, information on these aspects of lady beetle behavior will not only prove interesting from a basic perspective on predator behavior, but can be combined with future and existing knowledge to help design agricultural systems that maximize the benefits of existing predators.

Materials and Methods

Research was conducted near the town of Taylor, TX (Williamson County) in a system consisting of four crops grown annually. In this area, cotton, corn, and grain sorghum are cultivated in partially overlapping periods of spring and summer, with corn harvested first and cotton last. In addition to these warm-season crops, winter wheat is also grown in this area.

The four sites selected for study were large, paired cotton and grain sorghum fields oriented with parallel rows and sharing one lengthwise border. Each site had four plots flagged in cotton at 10, 30, 50 and 70 m from the cotton – grain sorghum interface. Plots were one row in width, of varied length (due to difference in site dimensions), but always inset 100 m from field edges. One or more corn fields were always located near the selected sites, but these fields did not always share an edge with selected cotton fields.

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Trips to all sites were made once weekly after the emergence of cotton seedlings in late April, and cotton fields were inspected for the presence of *H. convergens* adults or eggs. Upon discovery of the first adult ladybird beetles in cotton, collecting trips were scheduled for each subsequent Monday until beetle density declined below detectable levels.

In trips subsequent to the initial discovery of *H. convergens*, all 16 plots (four sites × four plot distances) were searched for *H. convergens* adults and egg masses by manual inspection of individual plants, with an effort of approximately 20 person-minutes expended per plot. Ladybird beetle adults were collected using a double-chambered aspirator, while eggs were taken still attached to the plant material on which they were found. Additionally, samples of leaf tissue were taken once at each site from both cotton and grain sorghum fields, and from the corn field nearest to each site. After collection, all samples were frozen in dry ice for preservation in the field, and later placed into freezers in the Biological Control Facility at Texas A&M University until preparation for analysis.

Before analysis of isotopic composition, samples required appropriate preparation. All samples were washed twice in reverse-osmosis treated water, dried for 2-3 days at 60 °C and massed to an accuracy of ± 1 μg before being tightly packaged into tin sample capsules. While isotopic analysis usually requires the homogenization of material, often by grinding solids to a fine powder and subsampling to accurately represent large samples (Boutton 1991), this was not always necessary. Adult lady beetles were analyzed as whole animals or as halves created by slicing along the anterior-posterior axis with a surgical scalpel, while egg masses were analyzed as whole clusters of eggs as discovered in the field. Samples of plant tissues (leaves) were large enough to require homogenization and were pulverized to a powder with a Wig-L-Bug mill (Spex Certiprep, Metuchen, NJ) before enclosing a subsample into the tin sample capsule.

After preparation, samples were shipped to the University of Georgia's Institute of Ecology Stable Isotope Laboratory for analysis by a combustion – gas chromatography – mass spectrometry series process. Succinctly stated, samples were flash-combusted and the resulting gases (CO_2 and others) sent to a gas chromatograph, where CO_2 was separated out and sent to the mass spectrometer. The mass spectrometer separated CO_2 molecules based on a charge-to-mass ratio, giving the ratio of ^{13}C to ^{12}C in the sample. We expressed carbon isotope ratios ($\delta^{13}C$) of individual samples as:

$$\delta^{13}C = [(R_{sample}) - (R_{standard})] \times 1000$$

where R_{sample} and $R_{standard}$ values are the ratios of ^{13}C to ^{12}C atoms in the experimental sample and standard reference (Ehleringer and Rundel, 1988), and units for $\delta^{13}C$ are shown as $^o/_{oo}$.

Data analysis relied on classification of individual samples as members of either of two groups: those having carbon isotope ratios like those expected for predators in cotton, and those with isotope ratios like sorghum or corn. A 95% confidence interval (CI) was created for the range of values expected to be shown by adults and egg masses of *H. convergens* in the event that predator had been exclusively in the cotton crop. Because shifts between trophic levels fall into a characteristic range, with the average change in $\delta^{13}C$ value from an animal to its diet being $+0.8 \pm 1.1\,^o/_{oo}$ (DeNiro and Epstein, 1978), the CI was generated by adding $0.8\,^o/_{oo}$ to the mean $\delta^{13}C$ value of cotton, then adding an additional 0.8 ± 1.96(SD). This represents a change of two trophic levels (plant to herbivore, and herbivore to predator), but error is only introduced in the second level, because the error is a between-individual measure. That is, since aphidophagy and egg predation necessitate the consumption of many individual prey items, the shift at the herbivore level should converge to the group average.

Individual lady beetles and egg masses were compared to this confidence interval to determine if corn or sorghum resources were present in each sample. For lady beetle adults, a value outside the interval suggests the beetle recently immigrated into cotton from corn or grain sorghum. When females recently feeding in crops outside of cotton produce eggs masses, the eggs should show isotope ratios outside the confidence interval. Later, we pooled samples within dates to show the contributions of grain sorghum and corn to adult *H. convergens* populations in cotton and the production of egg masses by these adults.

Results and Discussion

H. Convergens Abundance and Population Trends

Active quantitative sampling for lady beetle adults and eggs was conducted for 11 weeks (May 8 – July 17), which resulted in the collection of 515 lady beetle adults and 41 aggregations of eggs. Adult lady beetles were collected during the first ten weeks, but an absence of adults resulted in the cessation of sampling after week eleven. The occurrence of egg masses, and by logical extension lady beetle reproduction, was limited to the first six weeks of sampling (May 8 – June 12). Lady beetle adult and egg mass density in all sampling trips were calculated to a per-plant mean for each cotton field with error expressed between sites (Figure 1). In general, adult lady beetle densities were about ten times those of egg masses (note x-axes, Figure 1).

Plant-Carbon Data and Preliminary Calculations

Plant samples collected in week seven (June 19) were analyzed with one sample each of cotton and grain sorghum from each site, and one sample from each nearest corn field. While this only showed inter-site, and not intra-site, variation in plant isotope levels, results indicated little range in plant $\delta^{13}C$ values. Corn and grain sorghum samples (n = 4, each crop) yielded means (± SE) of $-12.38 \pm 0.38\,^o/_{oo}$ and $-12.71 \pm 0.27\,^o/_{oo}$, respectively. The similarity of these crops indicated that high isotope levels relative to cotton generally could not be attributed to immigration from a single crop. Cotton values were distant from the other crops, with a mean and standard error of $-27.74 \pm 0.66\,^o/_{oo}$.

Preliminary calculations based the cotton $\delta^{13}C$ values and expected shifts between trophic levels (based on DeNiro and Epstein, 1978) were used to calculate the 95% confidence interval to detect immigrant lady beetles as described above. The resulting 95% CI was $-26.14 \pm 2.16\,^o/_{oo}$. This interval has an upper limit of $-23.99\,^o/_{oo}$, so any value above this established as the threshold value for detecting immigrant lady beetles in the adult samples collected.

Adult Lady Beetle Movement

By testing lady beetles in each sampling period versus the 95% confidence interval previously described (meant to encompass the range of values for lady beetles with recent feeding exclusively in cotton), an evaluation of lady beetle colonization over time is possible. Using the individual $\delta^{13}C$ values, the proportion of immigrants per site was determined for each sampling date, with a mean proportion of immigrants and standard error of this statistic also calculated (Figure 3).

These data show a period of strong lady beetle immigration during the first four to five weeks of *H. convergens* populations in cotton, as well as a small number of individuals entering the crop during a period of rapid decline in lady beetle abundance. This result contradicts the expectation of concerted immigration of adults, which is suggested by prior field experimentation and observation (Prasifka et al, 1999). Specific to the first objective of this study, carbon isotope detection of lady beetle immigration indicates that the movement of *H. convergens* into cotton was not a singular or "pulse" event; movement continued for a period of several weeks, not days.

***H. Convergens* Egg Production**

Given the wide range of adult $\delta^{13}C$ values and the commonness of recently immigrated adults in cotton, variation in the carbon isotope data was expected. That is, if eggs produced by female beetles represent recent feeding history, then some egg masses were expected to show varied values, particularly given the complete overlap of egg production with the heaviest periods of adult movement into cotton. Egg masses with $\delta^{13}C$ values above the upper limit of the 95% CI were interpreted as eggs from immigrant females, although we have no information on the expected difference in isotopic ratios between females and their eggs. As with adult samples, the proportion of egg masses produced by immigrants was calculated for each sampling date, with a mean proportion of immigrants and standard error of this statistic also calculated (Figure 3).

Whereas low sample sizes hinder interpretation, results show at least one aggregation of eggs produced by an immigrant female collected for each period where eggs were sampled, with the exception of the second trip (May 17), in which zero of four egg masses exceeded the upper limit of the confidence interval. Coincident with the two weeks of heaviest immigration by adult *H. convergens*, sampling periods three and four (May 22 and May 29) show the greatest proportion of egg masses falling above the upper limit of the 95% CI. With reference to the second objective, it appears that corn and grain sorghum contributed to the production of eggs in cotton over the entirety of the beetles' reproductive period in cotton. In general, contributions of crops other than cotton to beetle populations extended beyond the simple immigration of adults, but also to producing the next generation of predators.

Summary

This study is one of many to suggest the potential of specific composition of crops and cultural techniques of landscape design to conserve predators for improved cotton pest suppression. Yet to our knowledge, no landscape-scale habitat manipulations of this sort are being commercially used for cotton in the United States. This may be because researchers in predator conservation and landscape design have not provided convincing evidence of suppression using these techniques. This may be due to the considerable difficulty in establishing coordinated research on an area-wide scale, but we note that current regulations of transgenic crops have resulted in cooperative, organized planting programs over large areas. To make a convincing appeal for using landscape design and habitat manipulation, compelling results must be shown on an area-wide basis. Consequently, our future cotton research will be directed to an area-wide scope, in the hope the diversity of biotic resources in agriculture can be used beneficially.

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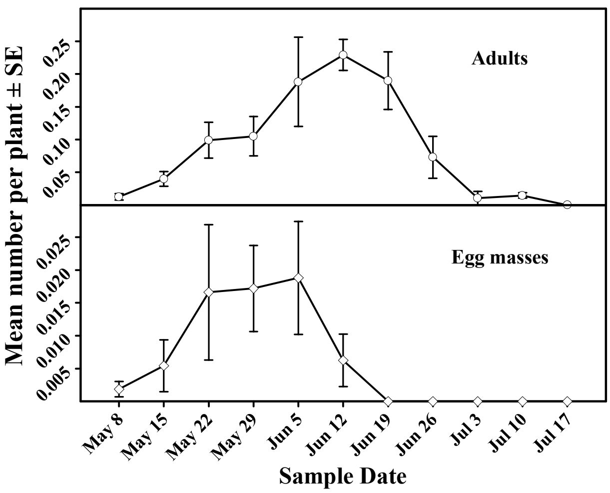

Figure 1. *H. convergens* adult and egg mass densities during eleven-week field collection period. Results are presented as per-plant measures, though sampling intensity was kept constant at 20 person-minutes per plot and error bars represent between-site variation. Note that scales for adults and egg masses differ by approximately ten-fold.

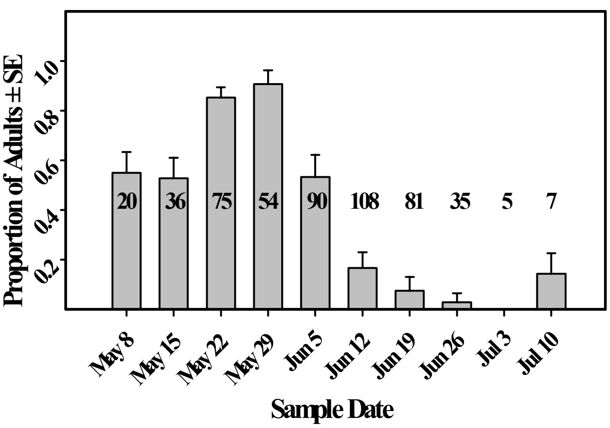

Figure 2. Mean proportion of recently immigrated *H. convergens* adults (± standard error) in four cotton fields over time. Immigrants determined by comparison with the upper bound of a 95% confidence interval for isotope ratios of lady beetles feeding in cotton. Numbers aligned with columns represent sample sizes for a collecting date.

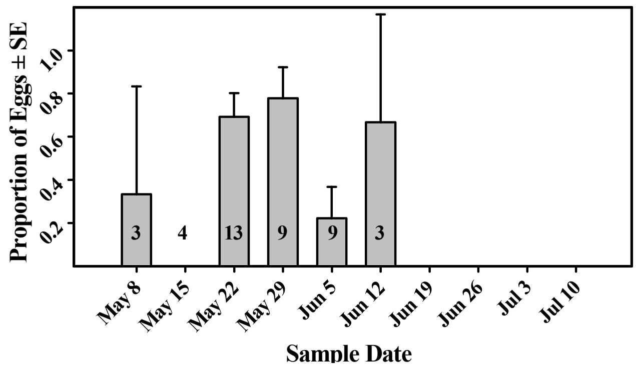

Figure 3. Mean proportion of egg masses produced by recently immigrated *H. convergens* adults (± standard error) in four cotton fields over time. Masses produced by immigrants determined by comparison with the upper bound of a 95% confidence interval for isotope ratios of lady beetles feeding in cotton. Numbers aligned with columns represent sample sizes for a collecting date.