INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

Bell & Howell Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

STUDIES ON DISPERSAL OF A NATIVE PARASITOID *ERETMOCERUS EREMICUS* AND AUGMENTATIVE BIOLOGICAL CONTROL OF *BEMISIA TABACI* INFESTING COTTON

by

Gregory Sinclair Simmons

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF ENTOMOLOGY

In Partial Fulfillment of the Requirements
For the degree of

Doctor of Philosophy

In the Graduate College

THE UNIVERSITY OF ARIZONA

2000

THE UNIVERSITY OF ARIZONA ®
GRADUATE COLLEGE

As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Gregory Sinclair Simmons

entitled Studies on dispersal of a native parasitoid Eretmocerus eremicus and augmentative biological control of Bemisia tabaci infesting cotton

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy

David N. Byrne	4.12.00 Date
Reginald S. Chapman	4/12/2000 Date
Martha S. Hunter	4.12.00 Date
Stephen E. Naranjo	Date
	Date

Final approval and acceptance of this dissertation is contingent upon the candidate's submission of the final copy of the dissertation to the Graduate College.

I hereby certify that I have read this dissertation prepared under my direction and recommend that it be accepted as fulfilling the dissertation requirement.

Dissertation Director David N. Byrne — 4.12.00 Date

STATEMENT BY AUTHOR

This dissertation has been submitted in partial fulfillment of requirements for an advanced degree at the University of Arizona and is deposited in the University Library to be made available to borrowers under the rules of the library.

Brief quotations from this dissertation are allowable without special permission, provided that accurate acknowledgment of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the Graduate College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however, permission must be obtained from the author.

SIGNED: Gregory S. Simmons

ACKNOWLEDGEMENTS

I thank my committee: David Byrne; Reginald Chapman; Martha Hunter; and Steve Naranjo. David Byrne's work on whitefly migration inspired me to work on parasitoid dispersal and, by example, he demonstrated how to be an effective applied entomologist. Reg Chapman taught me to make the most of the data even when imperfect and that learning the simplest details of insect behavior and biology is the most important (and the most difficult) work of an entomologist. Martha Hunter was a valuable resource for questions about parasitoids and biological control, my only regret is that she joined my committee after I had left campus. I would have liked to have interacted more with her and her students. I relied on Steve Naranjo's breadth in applied entomology, biological control, modeling, and experimental design and statistics for guidance on almost all aspects of my research. All of the members of my committee helped me to think and write more clearly and their efforts made me a better scientist. I thank Dan Papaj and Martin Taylor for their early service on my committee. Oscar Minkenberg supported me with grant funds and his enthusiasm and commitment to biological control were an inspiration. Tom Tobin provided advice, the use of his lab, and taught me how to make beer. Peter Turchin at U. Connecticut and Andrew Corbett at U.C. Davis both spent many hours explaining diffusion models.

I learned much from fellow students in entomology. Tovi Lehmann got me started programming in SAS and offered friendship and wise counsel on most everything. I wish I'd had more time to drink Turkish coffee in the blackfly lab. With Jon Diehl I spent many hours discussing ecology and biological control, his intelligence and wry sense of humor were much appreciated. David Bellamy lent me data from his flight chamber studies to model *E. eremicus* dispersal. I benefited from interactions with Byrne post-docs: Jackie Blackmer; Athayde Tonhasca; and Robin Rathman. Entomology staff members: Carl Olsen; Rose Kilby; Justine Collins; Marylou Stengal; Erich Draeger; Doris; and Patricia Baldewicz supported me in many ways. Thanks to the scientists and staff at the USDA-ARS Honey Bee Research Center in Tucson and at the Irrigated Desert Research Station in Brawley especially Eric Erickson and Louie Gaska in Tucson; and Steve Castle, C.C. Chu and Cliff Brown in Brawley. Advice was provided by Eric Natwick at the University of California and Dick Weddle of the Imperial County Office of the Agricultural Commissioner. Arizona cotton growers, Leon Conner, Gary John, and Mark Skausen kindly let me tromp through their fields tracking *Eretmocerus* dispersal. The Whitefly Management Committee provided financial support. Koppert B.V. provided parasitoids.

My supervisors in APHIS, Ted Boratynski and Bob Staten gave me the freedom to use data from my work for APHIS in my dissertation and gently prodded me to finish writing. Colleagues Kim Hoelmer, Bill Roltsch, and Charlie Pickett were founts of information on biological control and whitefly and parasitoid biology. They were also good models on how to succeed as a professional entomologist.

Let me finish with thanks to Tucson friends Jim Malusa, Wayne Vanvoorhies, Laurie Abbott, Sonya Norman, Ellinor Michel, Ellen Livingston, Elaine Lowry, Rick Hudson, Rob Rachowiecki, Scott Brill, George Ferguson, Greg Krukonis, Tim Lowe and Nelson Caldwell whose friendships made Arizona a pretty nice place to be.

TABLE OF CONTENTS

TABLES OF CONTENTS CONTINUED---*Continued*

TABLES OF CONTENTS CONTINUED---*Continued*

LIST OF TABLES

LIST OF TABLES –*Continued*

LIST OF FIGURES

LIST OF FIGURES---*Continued*

ABSTRACT

In the mid-1980s, a new biotype of *Bemisia tabaci* was introduced into the southern U.S. causing extensive damage to agricultural crops throughout the region. An augmentative biological control project was initiated using a native parasitoid, *Eretmocerus eremicus* to determine its efficacy against *B. tabaci* infesting cotton in the desert areas of California and Arizona. A series of experiments were conducted in 1992-1995: release rate studies in cages and open fields; parasitoid dispersal within fields to determine movement rates after point release; and experiments to determine the parasitoid-host spatial relationship.

Cage release rate studies, demonstrated that rates of parasitism could be increased 61 to 79% in the highest release treatments, with reductions in whitefly densities of 80 to 100% relative to control treatments. Cotton yields in the high release treatments peaked at 2.5 bales/ac and were 2.6 to 4.2 times greater than in control treatments. Effective release rates were estimated to be equivalent to 770,000 to 1.1 million parasitoids/ha.

Field releases equivalent to 7.9 million parasitoids/ha resulted in a peak rate of parasitism of 42% but there were no statistical differences in parasitism, whitefly densities, or cotton yield; relative to no-release plots. High levels of whitefly immigration from surrounding crops, and parasitoid dispersal from release plots, diluted the effects of release. In a second field release rate study, releases equivalent to 2.0 to 3.0 million parasitoids/ha increased levels of the percentage of discovered leaves to greater than 80%.

These studies suggested that releases equivalent to 770,000 to 3.0 million parasitoids/ha would result in high rates of parasitism and reductions in whitefly densities. An economic

analysis suggested this would cost at least $5,914/ha , >8 times the highest amount growers currently spend on whitefly control.

Parasitoid dispersal was analyzed with mark-recapture experiments and data were fit to a diffusion model. One female wasp flew 82 m in one day though the majority of wasps flew a few meters or less. Estimated diffusion rates and median dispersal distances were 0.40 to 0.71 m^2/min and 2.4 to 4.4 m/(4 to 8) days respectively. Analysis of dispersal data suggested that releases on 20 m centers would provide effective coverage within a field.

Density independent parasitism was common at the spatial scale of leaves and plants. There was positive density dependence for the percentage of discovered leaves suggesting that parasitoids aggregate to high density patches of whitefly but fail to achieve high levels of parasitization possibly due to egg limitation or mutual interference.

CHAPTER 1

INTRODUCTION

1.1 The Problem

In the mid-1980s, a new biotype of the sweetpotato whitefly (B-biotype), *Bemisia tabaci* (Gennadius) (also referred to as *Bemisia argentifolii* Bellows and Perring, see Bellows et al. 1994b), was introduced into the southern U.S. (Hoelmer et al. 1991, Perring et al. 1993a, Perring et al. 1993b, Hoelmer et al. 1994) causing extensive damage to agricultural crops throughout the region (Faust 1992, Gruenhagen et al. 1993, Henneberry et al. 1993, Perring et al. 1993a, Gerling and Mayer 1996). Crops affected include cotton, alfalfa, vegetables, melons and ornamentals (Perring et al. 1993a, Perring et al. 1993b, Henneberry and Toscano 1997). Current estimates of this whiteflies' economic impact exceeds $500 million a year (Henneberry et al. 1997). In the Imperial Valley of California alone, crop losses during the period 1991-1995 were estimated at $100 million annually (Henneberry et al. 1997.) Increasingly, *B. tabaci* has also become a worldwide pest affecting agricultural production throughout most of the world's tropical and subtropical regions as well as greenhouse production in temperate areas (Gerling and Mayer 1996).

In cotton (*Gossypium* spp.), *B. tabaci* causes yield reduction by phloem sap feeding and lint contamination from the production of honeydew and associated sooty molds (Natwick 1993, Blua and Toscano 1994, Henneberry et al. 1995, Hendrix et al. 1996). There is also growing concern about the rising incidence of B-biotype vectored gemini viruses of cotton

that are a threat to US and worldwide cotton production (Bedford et al. 1994, Brown and O'Leary 1994).

Chemical control of whitefly infesting cotton has been inconsistent because of the rapid development of resistance to pesticides (Byrne et al. 1990, Dittrich et al. 1990, Prabhaker et al. 1992, Denholm et al. 1996, Dennehy and Williams 1997, Williams et al. 1997) and the difficulty in applying pesticides so that they provide good coverage on the underside of leaves where the nymphs occur (Bellows and Arakawa 1988, Gerling and Kravchenko 1996).

Because of difficulties with resistance and the efficacy of pesticide application there is much interest in developing alternatives to chemical control. One of the most important reasons for reducing chemical use is to increase the useful life of those materials that are still efficacious. Indeed, there are university extension recommendations and label restrictions that recommend or require the limited use of certain materials in order to manage whitefly resistance to these materials (Horowitz and Ishaaya 1994, Denholm et al. 1996, Simmons et al. 1997a, Sivasupramaniam et al. 1997). Furthermore, there are benefits to reducing the use of pesticides so as to conserve the natural enemies of whitefly and other key pests in the cotton system (e.g., pink bollworm, *Pectinophora gossypiella* (Sanders), lygus bug, *Lygus* spp., boll weevil, *Anthonomus grandis* Boeheman, cotton aphid, *Aphis gossypii*, Hagler and Naranjo 1994, Horowitz et al. 1994, Denholm et al. 1996). Natural enemy conservation is becoming an increasingly important consideration as a number of new selective materials have been developed for use against whitefly that have limited impact on natural enemies (e.g. insect growth

regulators, such as buprofezin and pyriproxyfen; and the nitromethylene analog imidacloprid, see Horowitz and Ishaaya 1996). If whitefly control should become upset due to the development of resistance to the selective materials and more frequent applications of broad spectrum materials were required (e.g. pyrethroids, endosulfan) this would upset the natural control of the other cotton pests. Another factor of importance is the increasing regulatory burden of using pesticides and the reduction in the use of certain materials due to the new federal Food Quality Production Act. Increased regulations and changes in the allowed use of regulated materials makes it more likely that growers will seek other options to control pests due to rising costs and the availability of fewer pesticides (Heinz et al. 1993).

Because of the above concerns, biological control is considered an important component for control of whitefly in desert regions of California and Arizona. There are many instances where whitefly species have been successfully controlled biologically (Summy et al. 1983, Gerling 1986, van Lenteren 1986, Onillon 1990, Rose and Debach 1991-1992, Gould et al. 1992, Hoelmer et al. 1994, Hoelmer 1996). Other evidence that supports the importance of natural control, are the observations that whitefly pest status has often changed from secondary to primary status after frequent pesticide applications for other pests (Byrne et al. 1990). This may occur because of the elimination (or disruption) of the complex of whitefly natural enemies that were present before pesticide applications were made (Byrne et al. 1990, Sundaramurthy 1992).

When this project began in 1992, there was an extensive worldwide effort to locate new species of natural enemies (Hoelmer 1996), but none of these were available for release in

the U.S. at that time. Therefore, the prospects for augmentative biological control using a native parasitoid *Eretmocerus eremicus* Rose and Zolnerowich were investigated. This parasitoid was the most common parasitoid recorded attacking the previous biotype (A-biotype) of *B. tabaci* infesting cotton in the desert areas of California and Arizona, and was observed attacking the new biotype (Gerling 1967, Bellows and Arakawa 1988, Bellows et al. 1994a, Hoelmer 1996).

Augmentative biological control is the use of mass-reared natural enemies to provide control where natural enemies are absent or are too rare to provide adequate control of the target pest (Stinner 1977, Debach and Rosen 1991, van Driesche and Bellows 1996). It can be divided into two strategies: 1) Inoculative biological control (van Lenteren et al. 1996) , where initial releases of small numbers of organisms are made early in the season to inoculate the crop and season long control is provided by the action of the progeny of the released organisms (Stinner 1977, van Driesche and Bellows 1996, van Lenteren et al. 1996); 2) Inundative biological control, where the activity of the released organisms is expected to provide immediate control (Stinner 1977, van Driesche and Bellows 1996, van Lenteren et al. 1996). For season-long control, repeated releases are often required (Stinner 1977, van Driesche and Bellows 1996). In reality, there exists a continuum between the two methods; e.g. in some systems multiple releases of natural enemies are required to initially establish the agent while long-term control is dependent on both the action of released parasitoids and the activity of their progeny (c.f. van Lenteren et al. 1996). Some key factors that determine which kind of system can be used are the crop duration, value, and damage threshold; the synchrony of the natural enemy with the pest;

the presence of a favorable environment for natural enemy reproduction; the frequency of insecticide applications; and the severity of the pest infestation. In the cotton system, it was considered that given the relatively low value of the crop and the current cost of the parasitoids; that only an inoculative approach was feasible.

Augmentative releases were considered the most promising strategy because of the lag time between when the pest arrived and naturally occurring parasitoids immigrated into the field. Because past records of parasitism of *B. tabaci* by *Eretmocerus* spp. (earlier references to *Eretmocerus* spp. reared from *B. tabaci* in southeastern California are thought to have been *E. eremicus*, see Rose and Zolnerowich 1997) indicated that high levels of parasitism did not occur until near the end of the season (Gerling 1966, Gerling 1967, Bellows and Arakawa 1988), it was reasoned that to achieve effective control, the parasitoid would have to be introduced directly into the field to attack whitefly before the pest entered an exponential population growth phase. It is not clear why naturally occurring parasitoids do not colonize cotton earlier in the season to provide control, but the general lack of non-crop vegetation in desert cotton and vegetable production areas is considered an important factor (Roltsch and Pickett 1994, Roltsch and Simmons 1997). The model for the use of an inoculative release strategy against *B. tabaci* comes from the system used for greenhouse biological control of the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) with the parasitoid *Encarsia formosa* Gahan. This system, termed seasonal inoculative biological control, has been used successfully for greater than 20 yr in European greenhouse vegetable production (van Lenteren 1986, van Lenteren and Noldus 1990, van Lenteren et al. 1997). At the start of each new crop, parasitoids are introduced to inoculate the system.

Thereafter, few or no subsequent releases are made (van Lenteren 1986). Lastly, it was determined that the same rearing system developed for *E. formosa* could be adapted for use in mass-rearing of *E. eremicus*. This made it possible to obtain enough insects to test the feasibility of the augmentative release of parasitoids as a new control method.

In the remainder of sections that follow in this chapter, I will first present a section titled "Experimental Approach and Preview" that will outline the work presented in this dissertation giving a brief overview of work that was performed. The next section titled, "The Natural History of the System" describes the biology of both the pest and the natural enemy. A final required section in this chapter is titled "Explanation of the Dissertation Format".

1.2 Experimental Approach And Preview

The investigations reported in this dissertation combine empirical and theoretical approaches to evaluate the efficacy of *E. eremicus* as an augmentative biological control agent. The first goal was to determine if field releases of mass-reared *E. eremicus* into cotton could be used to increase parasitism and suppress whitefly populations. This was the first release in a field crop of commercially-produced parasitoids for use against the new biotype of *B. tabaci*. Positive results would demonstrate the feasibility of the approach and initiate further work on refining the technique. Specifically, there were five questions:

1. Can augmentative releases of *E. eremicus* suppress populations of *B. tabaci* infesting desert cotton (appendix A, chapter 2, and chapter 3)?
2. At what rate should parasitoids be released (appendix A, chapter 2, and chapter 3)?
3. If multiple releases are required how many parasitoids should be employed in a single release (chapter 3)?
4. At what spacing should parasitoids be released (chapter 4)?
5. What is the parasitoid-host spatial relationship between *E. eremicus* and *B. tabaci* (chapter 5)?

In appendix A (and chapter 2) I report on the first series of experiments conducted in field inclusion cages during the cotton season of 1992. These were replicated studies with parasitoids released at two rates compared against no-release treatments. Cages were large enough to enclose about 50 cotton plants and approximately 5.5 m^2 of area. Weekly releases of parasitoids were made for 8 wk while changes in rates of parasitism

and whitefly densities were estimated. At the end of the experiment, yield information was collected. A preliminary estimate of an effective release rate was made. Some limitations of working in field cages are discussed.

In Chapter 3, I describe continued experimentation in 1993 using field inclusion cages and a series of open field releases. The first experiment was a parasitoid release rate experiment in inclusion cages. Release rates were an increasing geometric progression that included rates above and below the release rates used in the 1992 trial. Data on parasitism and whitefly densities were analyzed with repeated measures least squares regressions and with a series of nonlinear regression analysis. Yield data were analyzed with least squares regression. From these analyses, I was able to estimate minimum parasitoid release rates with more precision than in the 1992 study.

Also in chapter 3, I report the results of an open field release experiment in small plots of cotton. Despite releasing parasitoids at a rate about 3.5 higher than in the highest cage treatment (in 1993), there were no significant differences in levels of parasitism nor whitefly densities between the release plots and the control plots. This result (coupled with the observation that levels of parasitism in both release and control plots were higher than in nearby untreated fields) suggested that there was parasitoid emigration from the release plots into the control plots contaminating the control treatment. This was the first indication that the size or the distance between experimental plots in this system could be an important factor. This observation led to changes in the design of the subsequent release experiments.

The last experiment described in chapter 3 was a release rate experiment in an open field. There were five release rates (and a control). Again, release rates were made in a geometric progression. Small release plots were embedded within a much larger field and separated from other plots by large swaths of cotton. This design was an attempt to eliminate the effect of the interaction of parasitoid movement with small plot size that had appeared to cause the 1993 open release experiment to fail. In this experiment, only a single release was made and the effects of the release were monitored for several days after the release using a sentinel host technique. The goal of this experiment was to estimate the number of parasitoids needed in a single release. To produce the sentinel hosts, cotton plants were infested with whitefly in a parasitoid free greenhouse. Once young nymphs had developed, leaves with nymphs were excised and tied to cotton plants in the release areas in uniform arrays. The sentinel host technique was used because it was reasoned that it might better reflect patterns of parasitism resulting from a single release than samples of cotton leaves from the field. Leaf samples from the cotton field are exposed to naturally occurring parasitoids for varying periods of time making it more difficult to interpret the results of a single release. The sentinel leaf data were analyzed with least squares regression and daily parasitism rates were recorded. Unfortunately, high levels of parasitism occurred in the no-release plots (caused by naturally occurring parasitoids), which resulted in a poor fit of the regression model and no statistical differences were found. Therefore, it was difficult to draw many conclusions from these data. There was a non-statistically significant trend of a decreasing slope of the

parasitism curves with time, which suggested that parasitoids were either leaving the release area, dying, or becoming egg-depleted over the three day sample period.

Analyses of samples from cotton plants in the release area were also conducted. There were no statistically significant effects on levels of parasitism for any of the release rates. This result was not surprising given the problem with naturally occurring parasitism. Analysis of the patterns of leaves that had one or more parasitized whitefly was more informative. This variable may be more sensitive to the effects of a single release than the percentage parasitism. Analyses with least squares regression and nonlinear regressions revealed significant effects of increasing release rates. It was possible with these data to estimate the effects of a single release. Chapter three closes with a summary of all of the release studies and a preliminary economic analyses on the use of augmentative releases for whitefly control. Prospects for the future use of augmentative biological control in cotton and future research needs are discussed.

In chapters four and five, I describe attempts to apply recent theory concerning the evaluation of biological control agents to the *E. eremicus-B. tabaci* system. This theory suggests that the most effective natural enemies are those that discover and reduce prey populations early in the season when pest populations are low and their distribution is patchy (Kareiva and Odell 1987, Kareiva 1990a, Kareiva 1990b). Typically, most studies provide high numbers of prey to measure killing rates without consideration of the speed and rate and which they discover prey (Kareiva 1990b). Experiments take place on spatial scales ranging from petri dishes, caged leaves or branches, whole plant cages, to large field cages (e.g. Huffaker et al. 1976, Stinner 1977, Luck 1988) which

provide unrealistically favorable conditions designed to favor natural enemy performance (Kareiva 1990b, O'Neil 1990, Andersen and Kareiva 1993). Small scale experiments are often a necessary first step because of limited resources. Important information can be gained from such efforts, e.g. a natural enemy that failed to kill large numbers of prey when provided large quantities in a petri dish would fare poorly in the field (Kareiva 1990b). However, small scale experiments fail to include a critical factor, the ability to search out and kill prey that are scarce and have heterogeneous distributions across several spatial scales (Anderson and Kareiva 1993).

While it was beyond the scope of this dissertation to directly test this theory, two of its elements were considered. In chapter four, I describe a series of parasitoid mark-recapture experiments conducted during the 1994-95 cotton seasons. With these data I was able to fit a diffusion model that allowed me to estimate median dispersal distances and to estimate diffusion coefficients, which provide a standard measure of the mobility of dispersing wasps. With these results, practical questions, such as how far apart to place parasitoid release points, can be answered. I also discuss the potential of using estimates of diffusion coefficients to evaluate natural enemy performance in the field, and suggest how estimates of movement rates could be of value in the assessment of potential non-target effects of biological control.

In chapter five, I describe a series of experiments designed to characterize the spatial relationship between *E. eremicus* and *B. tabaci* and relate these results to theory regarding the importance of parasitoid aggregation to host density to augmentative biological control. I determined that the predominant patterns of this parasitoid-host

relationship were either density independent or of weak inverse density independence at the scale of both leaves and plants. Possible reasons for these findings and suggestions for improvements of future experiments are discussed. In particular, I argue that these experiments should be repeated in an experimental setting where host density is explicitly manipulated to estimate the strength of aggregation over a broad range of host densities.

In closing with chapter six, I summarize the major conclusions of the previous chapters and suggest how my findings could be used to develop augmentative biological control strategies for managing *B. tabaci*. I also describe areas where knowledge is lacking and where more research is needed to improve the prospects for biological control.

1.3 Natural History of the Insects

1.3.1 Biology of *Bemisia tabaci.*

There is controversy over the taxonomic status of the two biotypes of *B. tabaci* as a new proposed species designation of *B. argentifolii* for the B-biotype (see Bellows et al. 1994b) is not accepted by all researchers (Brown et al. 1995, Byrne et al. 1995). This has become complicated by the discovery that the B-biotype occurs in regions all over the world. It is now becoming clear that there are an assortment of biological and biochemical differences between the two biotypes and there is an emerging consensus that the systematics of the *B. tabaci* group are complex and perhaps involve numerous species or incipient species (Brown et al. 1995) with several different "non-B" biotypes now discovered (Bedford et al. 1994, Brown et al. 1995). Therefore, for the rest of this section (and this dissertation), I will refer to both the A and B-biotypes simply as *B. tabaci* when discussing characteristics that are common to both, and only refer to the different biotypes when it is necessary to distinguish a difference between them.

The sweetpotato whitefly, *B. tabaci* Bellows (Gennadius) feeds on plant phloem (Byrne and Bellows 1991). Both adults and nymphal forms feed. The life cycle includes the egg, four nymphal instars and the adult. Except for the first instar crawler, all of the nymphal stages are sessile feeders (Gill 1990, Byrne and Bellows 1991). Individuals are usually located on the underside of the leaf. The late fourth instar is usually referred to as a pupa although this term is technically incorrect as whiteflies do not have holometabolous development (Byrne and Bellows 1991). Some authors reserve the term pupa for the last non-feeding stage of the fourth instar when wing development occurs (Byrne and Bellows

1991). Body color of the nymphs ranges from transparent to opaque, their color can be attributed to a combination of waxes and the color of their host, usually a green to green yellow color in the pupa (Gill 1990). The adult body length is about 0.9 mm with a wing length of approximately 2 mm; males are slightly smaller than females (Byrne and Bellows 1991). The wings of the adult have a generally white appearance due to wax secretions. Other body parts, with the exception of the eyes, are a light yellow color (Gill 1990, Byrne and Bellows 1991).

Both nymphs and adults are coated with wax, and adults have a specific waxing behavior, using their hind and forelegs to distribute the wax produced from wax plates located on the underside of the abdomen (Byrne and Hadley 1988, Gill 1990, Byrne and Bellows 1991). The wax has been hypothesized to serve several functions including protection from desiccation and solar radiation (Byrne and Hadley 1988); defense against natural enemies (Gerling 1990); and protection from pathogens (Byrne and Hadley 1988, Fransen 1990). Adults oviposit and feed preferentially on the undersurface of the youngest leaves of the host plant (Ohnesorge et al. 1980, van Lenteren and Noldus 1990), which results in a vertical stratification of nymphs within the plant (Von Arx et al. 1984). For plants with stratified and uniform growth (such as cotton), this feature has important implications for sampling whitefly (Von Arx et al. 1984, Naranjo and Flint 1994), and for assessing parasitism. Because *E. eremicus* preferentially oviposit under younger nymphs (Headrick et al. 1996), but parasitism is not apparent until the 4th instar, to assess parasitism, it is important to select leaf samples from the strata (or leaf node) that contain 4th instar nymphs.

The fecundity of the B-biotype has been reported to be greater than the A-biotype (Bethke et al. 1991) though this difference may be host plant dependent (Costa and Brown 1991, Enkegaard 1993). Other life-history traits concerning the differences between the two bio-types have either not been reported or are similar. Estimates from laboratory studies of the maximum number of eggs produced on cotton as a rearing host range from about 43 to 253 eggs per female (Powell and Bellows 1992a). This is apparently dependent on temperature as fecundity generally decreases with increasing temperature (Powell and Bellows 1992a). This appears to be an effect of decreased adult longevity at higher temperatures (Powell and Bellows 1992a, Enkegaard 1993). Field-measured fecundities on cotton range from 48 to 394 eggs per female (Byrne and Bellows 1991) and the time for egg to adult development ranges from 17 to 65 days (Butler et al. 1983). Adult longevity ranges from about 13 to 43 days (Bethke et al. 1991, Powell and Bellows 1992a). *B. tabaci* have an estimated 7-9 generations per year in the low elevation desert regions in southeastern California (Zalom and Natwick 1987).

The B-biotype of *B. tabaci* is often reported to have a broader host plant range than the A-biotype (Brown 1992, Perring et al. 1993b). While no reliable estimates about the magnitude of this difference are available, it seems clear that in the southwestern U.S., the B-biotype attacks crops not seriously affected by the A-biotype. These include melons, cole crops, and alfalfa (Perring et al. 1993b). Thus in these areas, an insect that was previously mainly a pest of cotton is now a year round pest. The problem has been particularly severe in desert cotton and vegetable production regions in Arizona and California. These areas have favorable temperatures most of the year providing

abundant host crops. This allows the exponential growth of large populations of *B. tabaci*, which develop during the spring through fall seasons of each growing year. Coupling favorable climates with a wide variety of host plants, a high fecundity and short generation time, and the migratory ability of this pest (Byrne et al. 1990, Byrne et al. 1996); leads to near continuous pest pressure on most of the major crops grown in these areas.

1.3.2 Biology of *Eretmocerus eremicus*

Eretmocerus eremicus Rose and Zolnerowich is an aphelinid parasitoid that attacks *B. tabaci* occurring in the desert areas of Arizona and California (Rose and Zolnerowich 1997). It is a solitary, nymphal ecto-endoparasitoid (Gerling 1966) and in culture has a 50:50 sex ratio (Simmons and Minkenberg 1994). The female parasitoid is synovigenic, and laboratory estimates of fecundity range from approximately 30 to 149 eggs (Vet 1980, Powell and Bellows 1992b). A recent field estimate of the realized fecundity of *E. eremicus* attacking whitefly on cantaloupe was about 33 parasitoid progeny per female (Hoelmer 1998). Given that the present study was conducted in the Imperial Valley of California during the hot summer months, this is probably a close approximation to the wasp's expected fecundity when attacking whitefly in cotton. Laboratory longevity estimates of females range from 5 to 24 d at 20° C and 3 to 8 d at 29° C (Powell and Bellows 1992b).

It is not known if *E. eremicus* is attracted to hosts from a distance, but attraction to whitefly infested leaves has recently been shown for other *Eretmocerus* species (Heinz

and Parrella 1998). Once *E. eremicus* discovers a host infested leaf, it has a density dependent arrestment response to increasing quantities of whitefly honeydew (Shimron et al. 1992, as *Eretmocerus* sp. since confirmed to be *E. eremicus*, Dan Gerling, Tel Aviv University, Israel, personal communication). Female parasitoids oviposit into all nymphal stages of *B. tabaci* but there is a preference for the first through third instar (Headrick et al. 1995, Headrick et al. 1996). The female deposits an egg under the nymph, which lies quiescent until the whitefly host reaches the fourth instar (Gerling 1990, Gerling et al. 1990). After hatching, the first instar parasitoid chews a hole and enters the host through the venter. It completes its development inside the host, from which the adult wasp emerges (Gerling 1966, Gerling et al. 1990). Host feeding has been observed in *E. eremicus* and is an additional source of whitefly nymphal mortality (Gerling 1990, Headrick et al. 1996).

The taxonomic status of many species in the genus *Eretmocerus* has been unclear, including those found in southern California. Previous studies of the parasitoids of *B. tabaci* found in Imperial County, California, refer to the species variously as *Eretmocerus haldemani* Howard, *Eretmocerus californicus* Howard, *Eretmocerus* nr. *haldemani*, *Eretmocerus* nr. *californicus* Howard and *Eretmocerus* sp. (e.g. Gerling 1966, Gerling 1967, Gerling 1986, Bellows and Arakawa 1988). It is probable that all of the previous references to these *Eretmocerus* species reared from *B. tabaci* in the southern desert areas of California should be regarded as one species, *Eretmocerus eremicus* Rose and Zolnerowich (Rose and Zolnerowich 1997).

1.4 Explanation of Dissertation Format

The publication by Simmons and Minkenberg (1994), is included as part of this dissertation as appendix A. This research was a collaborative effort between myself and Dr. Oscar Minkenberg, my research assistantship supervisor my first two years of work on this dissertation. Dr. Minkenberg provided monetary and logistical support. He also provided useful guidance and critiques including editorial advice concerning the publication. My contribution to this publication included: creating the experimental design and protocol, sewing the field cages, performing the field and laboratory work, data analysis, and writing the paper.

CHAPTER 2

PRESENT STUDY

My work on this dissertation began in 1992, when I conducted my first experiments on the use of *E. eremicus* as an augmentative biological control agent against *B. tabaci*. The methods, results, and conclusions of this study are presented in the form of a published paper appended to this dissertation. The paper, Simmons and Minkenberg (1994), can be found in appendix A. The following is a summary of the major findings described in Simmons and Minkenberg (1994).

Because of the severity of an outbreak of a new biotype of *B. tabaci* in cotton and vegetables in southeastern California, and the lack of effective chemical controls, in 1992 a study of augmentative biological control with a native parasitoid was initiated. The parasitoid *E. eremicus*, was tested in field inclusion cages to make preliminary estimates of effective release rates and to determine if parasitoid release could be an effective means of control. If this initial work proved successful, it would lead to further experimentation in open fields and the development of the methods needed for the implementation of a new pest control method for whitefly in cotton.

Previous work on the old whitefly, *B. tabaci* biotype A, had indicated that naturally occurring populations of *E. eremicus* could sometimes achieve high rates of parasitism on whitefly infesting cotton. Naturally occurring rates of parasitism as high as 80 to 90% had been observed on late season cotton. However, this parasitoid had never been released as an augmentative control agent, nor mass-produced, so it was unknown if earlier season suppression could be achieved by releasing parasitoids. Furthermore, it

was unknown if this native parasitoid could control the new more virulent biotype of whitefly.

A replicated inclusion cage study was conducted with 1.8 x 3 m cages placed over small plots of cotton within a larger cotton field. These cages were constructed and placed in the field in late May before large populations of whitefly had developed. The experimental design was randomized complete blocks with three treatments, low parasitoid release, high parasitoid release and no-release. There were a total of eight replications. Parasitoids of *E. eremicus*, reared on the greenhouse whitefly, *Trialeurodes vaporariorum*, were mass-produced by a beneficial insect producer. Weekly releases of parasitoid pupae began on 2 June and continued for 8 wk. Sampling was conducted every two weeks and continued until the cotton was defoliated. All pupal whitefly, emerged whitefly, parasitoid pupae, and emerged parasitoids were counted. Percentage of parasitism was calculated by dividing the sum of parasitoids (pupae and emerged parasitoids) by the sum of whitefly and parasitoids. At the conclusion of the experiment, cotton lint was picked by hand from each cage to estimate the effect of each treatment on yield. Parasitism data were arcsine transformed and whitefly counts were log transformed. Analyses were made with ANOVA and were found significant, so mean separation tests were performed.

The results indicated that augmentative release of *E. eremicus* increased rates parasitism and suppressed whitefly densities. The mean percentage of parasitism reached a high of 61% in the high release treatment and was significantly higher than both the low release and the no-release cages. Some individual samples from single

cages had rates of parasitism as high as 88%, which indicated that high rates of parasitism were possible. The density of whitefly pupae on leaves in the high release treatment was significantly lower at about one-fifth the density of the other treatments. Seed cotton yield was significantly higher and was more than twice as high in the high release treatment as compared with the low release and control treatments at 0.21 versus 0.10 and 0.08 kg/m^2 respectively.

Taken together these results suggested that it was possible to achieve high rates of parasitism and suppress whitefly densities with parasitoid release. The native parasitoid achieved rates of parasitism on the new biotype equal to those reported for the old biotype. The number of parasitoids needed to achieve these results was estimated to be between 113 to 367 parasitoid adults per m^2 or 1.1 to 3.7 million per ha. These results were promising, because it demonstrated the potential of a new whitefly control method by release of a native parasitoid. However, because the cages altered the growing environment of the cotton and restricted movement of both the whitefly and the parasitoid, it was reasoned that experimentation in open fields was needed to determine if economic suppression of whitefly could be achieved.

CHAPTER 3

RELEASE RATE STUDIES

3.1 Introduction

The results from the 1992 cage release rate study demonstrated that releases of mass-reared *E. eremicus* could increase parasitism and suppress *B. tabaci* populations infesting cotton (Simmons and Minkenberg 1994, Appendix A). This was an important result as it was the first field demonstration of the potential for the native *E. eremicus* to attack the new biotype of *B. tabaci*. Although an extensive worldwide search for new species of parasitoids was underway, the fact that a native parasitoid could attack the new biotype and perform well during the severe summers of southeastern California was a promising result.

The next step was to try and determine the minimum number of parasitoids needed to provide sufficient levels of control. While this may appear to be a simple question, Andow and Prokym (1991) have divided this into three questions: 1) How often should parasitoids be released? 2) How far apart should release locations be from each other? 3) How many parasitoids should be released per location? Question 2 was addressed by the movement studies in chapter 4. An inclusion cage experiment using several release rates was conducted in 1993 as an extension of the cage study in 1992 in an attempt to more precisely answer question 3. Question 1 was not addressed in this study.

Because of the inherent problems of working within cages in this system (Simmons and Minkenberg 1994, Appendix A,) open field studies were conducted in 1993 and 1995. In an effort to determine if a high release rate of parasitoids could provide sufficient control, a replicated release experiment was conducted in small plots of cotton in 1993. The high release treatment was compared to no-release control plots. If adequate control was achieved in releases outside of cages, future work could focus on

estimating minimum effective release rates. Because of concerns about obtaining spurious results from work in small plots (Sterling et al. 1992), a different approach was taken in the 1995 release rate studies. The key difference in this experiment was that the release areas were embedded within a larger area of cotton (to avoid the problem of working with artificially small plot sizes). The 1995 study was designed to more fully answer question 3: how many parasitoids should be released at a given release location? The inclusion cage experiment in 1993 was designed to determine how many parasitoids in total should be released in order to achieve sufficient suppression of whitefly populations. While this approach demonstrated the feasibility of using releases *E. eremicus* to control *B. tabaci* and provided a starting point for initiating release rate studies in open fields, it was not intended to estimate the effects of a single release. A positive result in the 1995 release rate studies could provide the basis for future large scale studies in open fields.

3.2 General Techniques for Sampling and Estimation of Parasitism and Whitefly Densities.

For the series of experiments described in this chapter, the same general methods were used for sampling and to count whitefly and parasitoids. On each sample date, mainstem leaf samples were collected from randomly selected plants to estimate whitefly densities and rates of parasitism. These leaves were collected from the region of the plant that was most infested with whitefly pupae (MIL). As described in Simmons and Minkenberg (1994) (Appendix A), this area was determined by the inspection of several plants on each sample date, but generally was between mainstem leaf nodes 6 to 9 (the terminal leaf being 1).

Sample leaves were examined using dissecting microscopes at 30x and counts were made of all *B. tabaci* pupae, emerged adult *B. tabaci*, emerged *E. eremicus*, *B. tabaci* parasitized by *E. eremicus*, and *B. tabaci* parasitized by *Encarsia* spp. Counts were made of the entire leaf or of a 5.1 cm^2 leaf disk taken from the third distal sector of the cotton leaf with the edges of the disk tangent to the leaf veins that mark the third sector. The switch to counting only leaf disks occurred when total counts (whitefly stage + parasitoids) exceeded 100. This was done to reduce counting time as populations increased as the season progressed. All counts were transformed to a per cm^2 basis by dividing by the area of the leaf or the disk. The percentage of parasitism was calculated by dividing the number of emerged and unemerged *E. eremicus* pupae, by the sum of

emerged *B. tabaci* pupae, emerged and unemerged *E. eremicus* pupae, and emerged and unemerged *Encarsia* spp. pupae.

3.3 Cage Release Rate Experiment in 1993

3.3.1 Methods

As a continuation of the inclusion cage study in 1992, a similar cage experiment was conducted in 1993 to study a greater range of release rates. Two test plots were planted with short staple cotton ('Deltapine 5461') on 3 April at the USDA-ARS Irrigated Desert Research Station in Brawley, CA. Plots measured about 11 m wide (12 rows wide) by 18 m long with 1-m row spacing and were maintained using standard agronomic practice for cotton grown in the area. On 28 May, 14 cages measuring 1.83 m wide by 3 m long and 1.83 m high were erected in the two plots; seven cages per plot. The cages were built over two rows of cotton and contained a mean (± SEM) of 47.8 ± 3.0 plants. The cages were covered with polyester organdy (70 mesh) with a Velcro closure at one end. A randomized complete block design was used, with seven treatments per block and two replicate blocks. Treatments consisted of the following series of release rates: 0, 9, 18, 36, 72, 144, and 288 parasitoids per m^2. The high release rate of 288, and the lower release rates of 18 and 32 parasitoids per m^2; were meant to approximately correspond to the high and low release rates in the 1992 study of 295 to 755 and 14 to 59 parasitoids per m^2 respectively.

Weekly shipments of *E. eremicus* reared on *Trialeurodes vaporariorum* were provided by an insectary operated at the University of Arizona. Four releases were made at approximately 12 d intervals between 3 June and 7 July. On each release date, the parasitoid pupae needed for each treatment were counted and split into four equal

portions. These were placed into 0.47 liter paper cups, protected from predators with Tanglefoot™, and placed in the appropriate cage underneath the plants.

To estimate the total number of wasps released into each cage, 1 wk after each release all containers were collected and all the parasitoid pupae released in each cage were counted to determine the number of wasps that had emerged during the interval. The number of parasitoids put out for subsequent releases was adjusted based on the estimate of the actual number of wasps released in the previous interval, so that by the last release, the intended release rates were achieved as near as possible.

Sampling for whitefly densities and percentage parasitism started on 3 June and continued at approximately 2 wk intervals until the conclusion of the experiment on 18 August. On each sample date, 40 MIL mainstem leaves were collected from the plants in each cage, one leaf per plant.

Parasitism data were analyzed with nonlinear regression analysis (PROC NLIN, Freund and Littell 1991) using the following equation:

$$\text{Percentage of parasitism} = a(1 - \exp[-bx]) * 100 \qquad (3.1)$$

Where x is the number of E. *eremicus* released per cage, a is a scaling factor, and b is a proportionality parameter. This exponential model has the characteristic of reaching an asymptote with increasing values for x. If this model fits the parasitism data well, the shape of the curve can be used to estimate the release rate above which no significant increases in parasitism are achieved. Eq.3.1 also has the characteristic of having a value

of 0 when x is 0, which is the expected result if no parasitoids were released. However, because naturally occurring parasitoids were present at low levels in the cages, there were non-zero values of parasitism in the no-release cages. To account for this, the parasitism data was transformed by subtracting the averaged values for parasitism of the two no-release cages from the values for parasitism from all other cages. This transformation had the effect of providing a much better fit of the model to the data than analysis with the non-transformed data.

Whitefly densities were analyzed with nonlinear regression (PROC NLIN, Freund and Littell 1991) using the following equation:

$$\text{No. of } \textit{Bemisia} \text{ pupae} = a(\exp[-bx]) \tag{3.2}$$

Where x is the number of E. *eremicus* released per cage, a is the number of *Bemisia* pupae when x = 0, and b is a proportionality parameter. This empirical equation has the property of decreasing exponentially to an asymptote of 0 with increasing values of x, which is the expected result with increasing numbers of parasitoids released. As for the analysis of parasitism data with Eq.3.1, where there is a close fit of the model to the data, the shape of the curve can be used to estimate the point where further releases of parasitoids are ineffectual.

Seed cotton yield was estimated by hand picking all the lint from all plants within the cages. Yield data were analyzed with least squares linear regression (PROC REG, Freund and Littell, 1991) and then transformed to a per ha basis.

3.3.2 Results

Emergence of *E. eremicus* from pupae varied from 68 to 90% for the four release intervals. The total number of parasitoids released in each cage was less than intended because of variation in release rates (Table 3.1). There were a total of 12 different release densities ranging from 6 to 223 parasitoids/m^2 (Table 3.1). Values for parasitism for the two no-release cages were averaged so there were a total of 13 points to be fit by regression.

The percentage of parasitism peaked on 20 July in the higher release cages of greater than 104 parasitoids per m^2 (Fig. 3.1). The highest level of parasitism of 79% was achieved in the highest release cage where 223 parasitoids per m^2 were released (Fig. 3.1). Rates of parasitism declined in the higher release cages after 20 July, but continued to increase until the end of the experiment in the lower release cages, where $\leq$ 66 parasitoids per m^2 were released (Fig. 3.1).

Nonlinear regressions of the parasitism data were highly significant and had reasonably high R^2 values for all dates except for the first sample date, which was not analyzed because of missing values for parasitism (Table 3.2). The highest R^2 values were 0.76 and 0.77 on 6 July and 20 July respectively (Table 3.2, Fig. 3.2). By inspection of the regression curves for each date, it appears that by the last sample dates (4 & 18 August) an asymptote in the rate of parasitism occurs at the release rate of about 36 parasitoids per m^2 (Fig. 3.2).

Table 3.1. Number of parasitoids released on each release date in 1993 cage trial.

Treatment[a]	No. released on each release date					
Block 1	6-3	6-15	6-25	7-8	Total	Total No./m^2
(9)	8	11	6	9	34	6
(18)	11	9	5	98	123	22
(36)	116	2	0	65	183	33
(72)	67	5	54	165	291	53
(144)	270	80	34	186	570	104
(288)	364	163	72	60	659	120
Block 2						
(9)	7	12	3	26	48	9
(18)	15	6	21	53	95	17
(36)	91	38	4	75	208	38
(72)	95	108	12	146	361	66
(144)	164	32	226	240	662	121
(288)	435	28	21	738	1222	223

[a] Numbers in parentheses were the target number of parasitoids/m^2 to release in each cage.

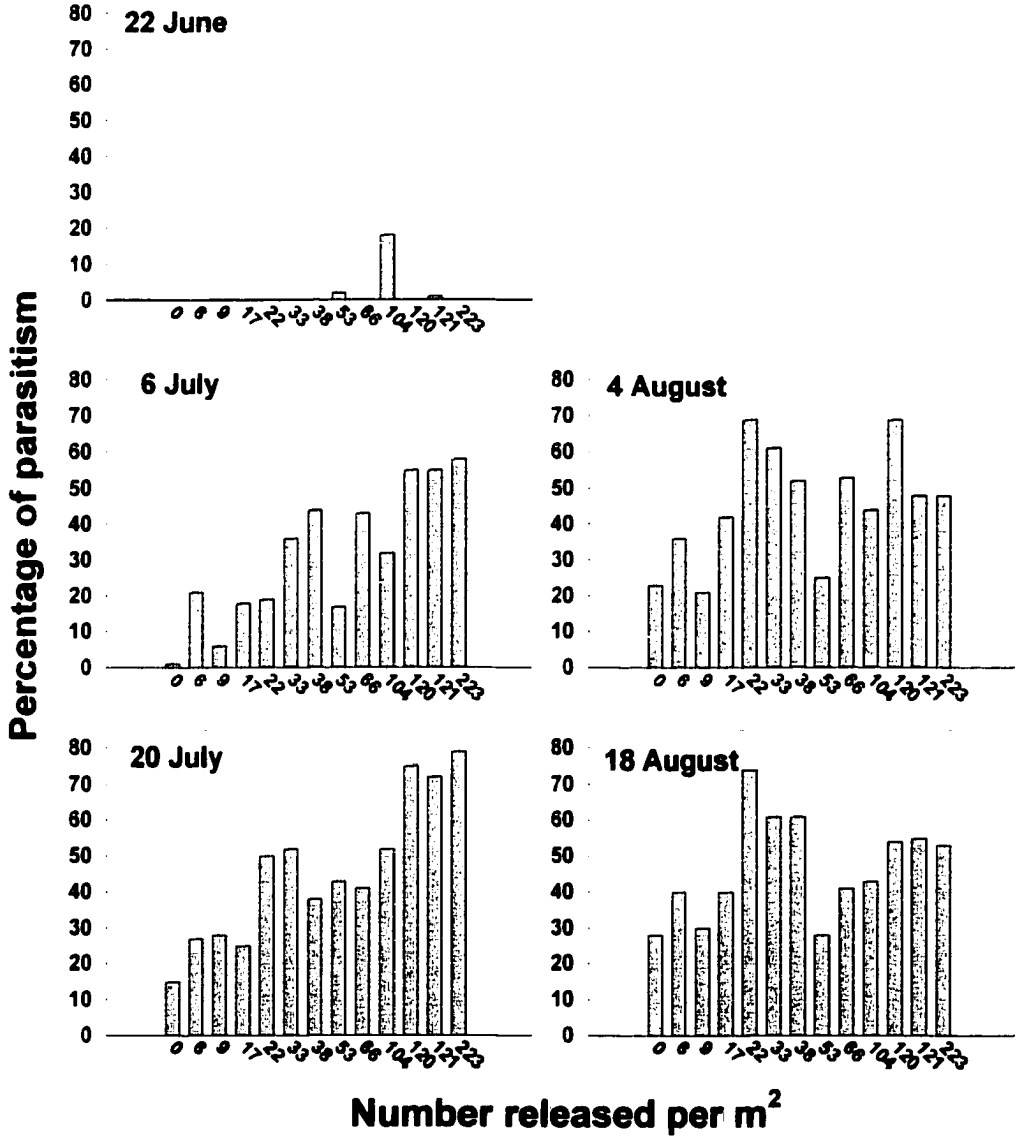


Fig.3.1. Mean percentage of parasitism on each sample date versus the number of released *E. eremicus* in the 1993 cage release rate experiment.

Table 3.2. Nonlinear regressions of the percentage of parasitism on leaves from the cotton plant samples versus the number of released *E. eremicus* in the 1993 cage release rate experiment. Values for parasitism were transformed by subtraction of the value of parasitism in the no-release cages from all values (see text).

Sample Date	R^2	F	df	P	$a \pm$ SE	$b \pm$ SE
6 July	0.73	29.4	1, 11	0.0002	52.8 ± 7.9	0.004 ± 0.002
20 July	0.79	41.9	1, 11	0.0001	64.1 ± 10.8	0.003 ± 0.001
4 August	0.36	6.3	1, 11	0.03	28.2 ± 5.0	0.020 ± 0.010
18 August	0.27	4.1	1, 11	0.07	22.7 ± 4.6	0.020 ± 0.020

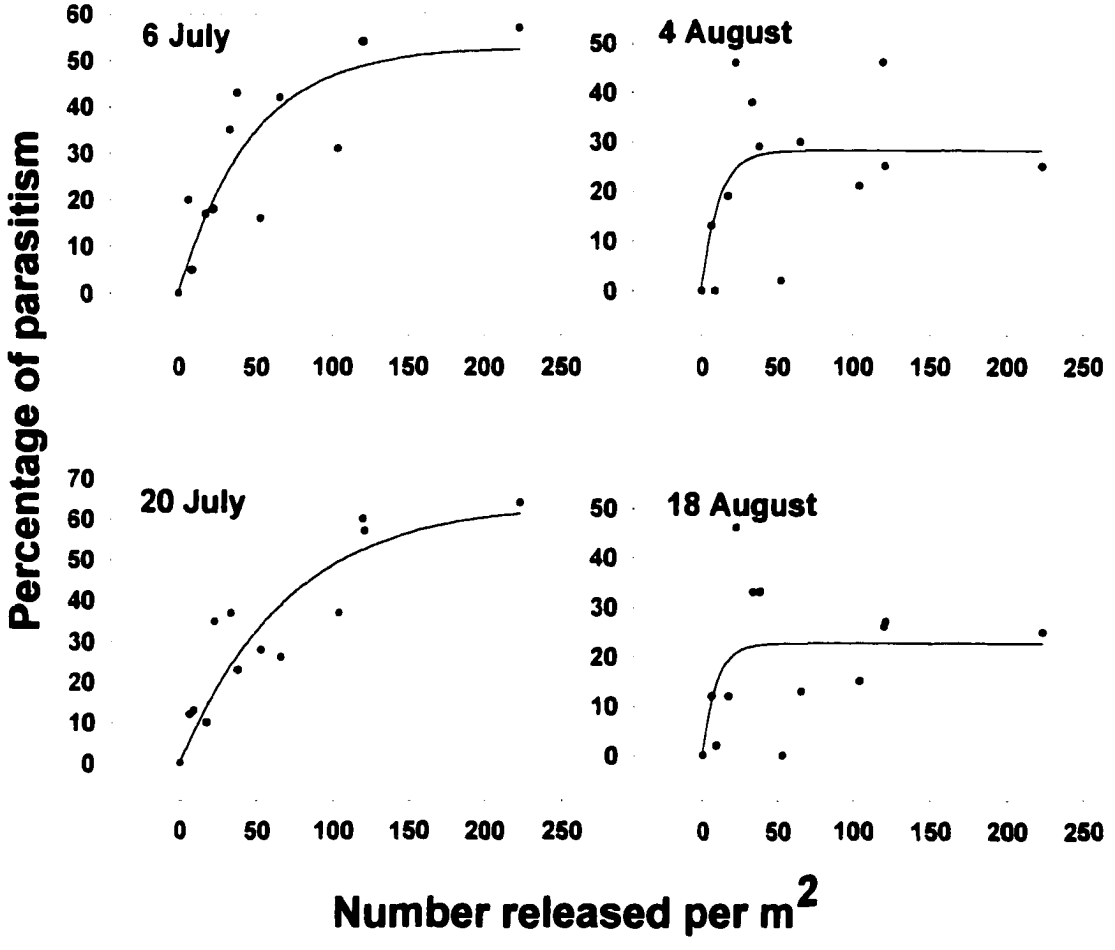


Fig. 3.2. Percentage of parasitized *B. tabaci* on each sample date versus the number of released *E. eremicus* in the 1993 cage release experiment. Parasitism values are transformed by subtraction of parasitism values in no-release cages. The solid lines are fitted regression lines (Eq. 3.1).

The number of whitefly pupae per cm^2 declined in all cages over the course of the experiment (Fig. 3.3). The highest density of whitefly occurred on 20 July in the no-release cages with a mean of 2.76 pupae/cm^2 of leaf (Fig. 3.3). Relative to the no-release cages, the percent reduction in whitefly ranged from 92 to 100% in the highest release cage of 223 parasitoids per m^2 and from 18% to 73% in the mid-range release cages of about 36 parasitoids per m^2 (cages with release rates of 33 to 38 parasitoids per m^2, Fig. 3.3). The number of whitefly pupae ranged from 0.001 to 0.02 pupae per square cm in the pre-release sample on 4 June (Fig. 3.3). An ANOVA indicated that there was a significant difference in the number of whitefly pupae between blocks at the start of the experiment with a block 1 versus block 2 mean of 0.009 and 0.002 whitefly pupae per cm^2 respectively ($F = 23.2$; df = 1, 12; $P = 0.0004$). Although highly significant, this amounts to a difference of about 0.5 whitefly per leaf that was unlikely to affect the outcome of this experiment given the high fecundity of *B. tabaci*.

Fitting the whitefly pupal density data to Eq. 3.2, resulted in significant regressions on most sample dates (Table 3.3, Fig. 3.4). The highest R^2 was 0.55 on 20 July (Table 3.3, Fig. 3.4). By inspection of the fitted regression curves, it appears that releases greater than about 110 parasitoids per m^2 did not result in further reductions of whitefly (Fig. 3.4).

Linear regression of cotton yield versus the number of released parasitoids was highly significant with a high R^2 ($R^2 = 0.84$; F = 56.2; df = 1, 11; $P < 0.0001$). Increases in the number of parasitoids released, resulted in significant increases in seed cotton yield with a high of about 2.2 kg cotton per cage (or on an area basis, 4007 kg per ha) in the highest

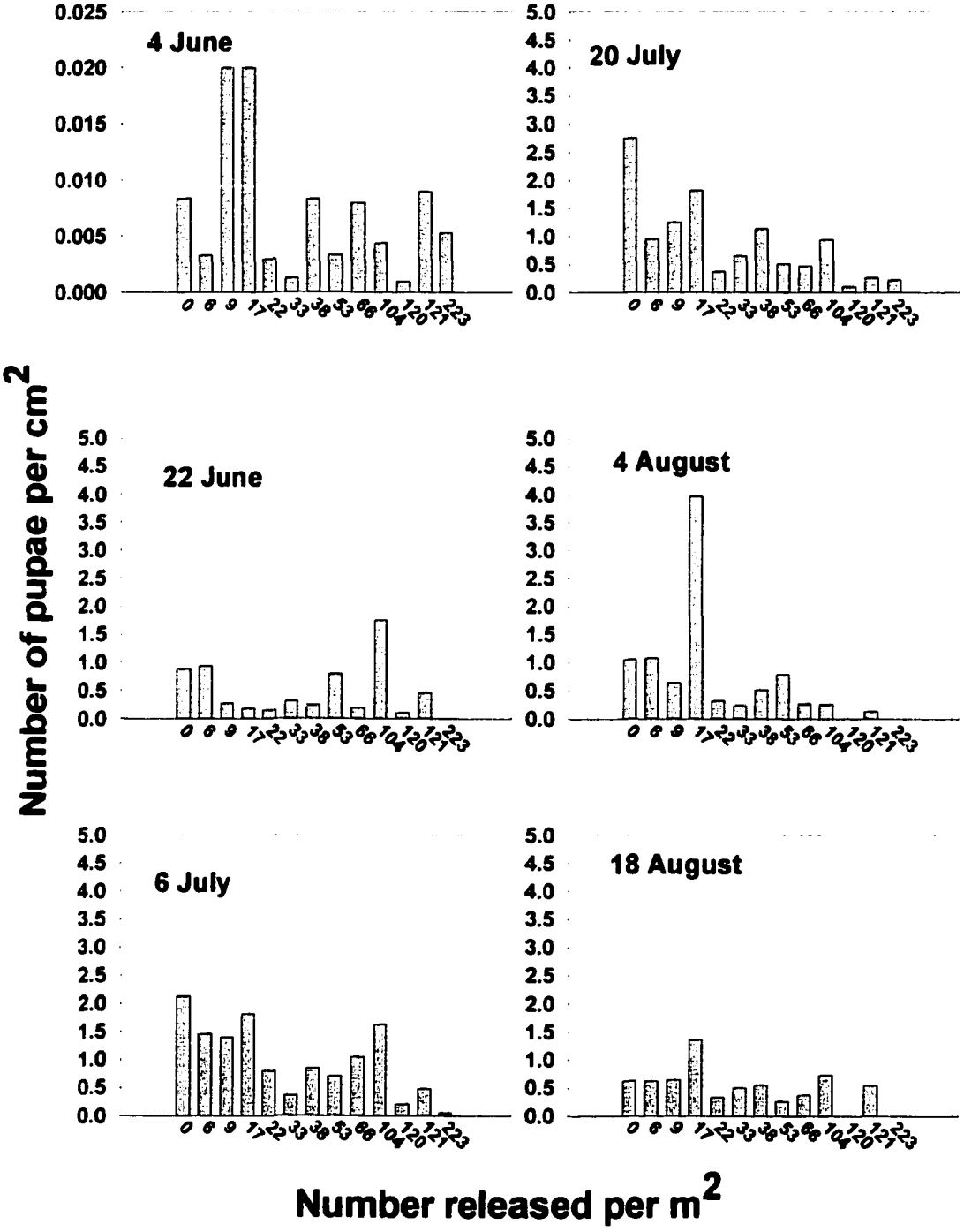


Fig. 3.3. Mean number of *B. tabaci* per cm^2 on each sample date versus the number of released *E. eremicus* in the 1993 cage release rate experiment.

Table 3.3. Nonlinear regressions of the density of *B. tabaci* on the leaves of the cotton plant samples versus the number of released *E. eremicus* in the 1993 cage release experiment. Data were fit to Eq. 3.2.

Sample Date	R^2	F	df	P	$a \pm$ SE	$b \pm$ SE
6 July	0.46	9.24	1,11	0.01	1.6 ± 0.3	0.002 ± 0.001
20 July	0.55	13.3	1,11	0.004	1.9 ± 0.4	0.004 ± 0.002
4 August	0.24	3.48	1,11	0.09	1.5 ± 0.6	0.003 ± 0.003
18 August	0.32	5.07	1,11	0.05	0.7 ± 0.2	0.001 ± 0.001

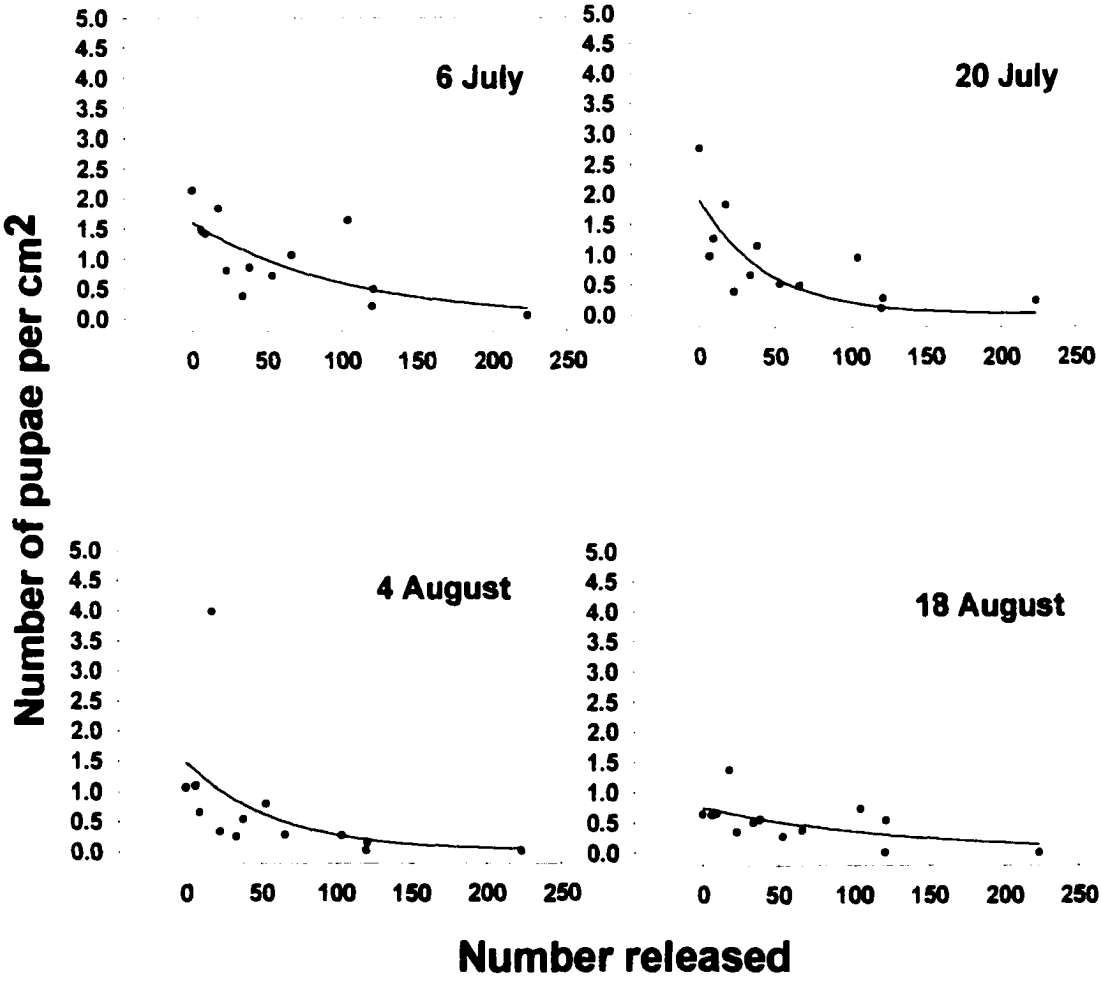


Fig. 3.4. Number of *B. tabaci* per cm^2 of leaf on each sample date versus the number of *E. eremicus* released in the 1993 cage release experiment. The solid lines are fitted regression curves (Eq 3.2).

release cage of 223 parasitoids per m^2 versus a low of about 0.5 kg (911 kg per ha) in the no-releases cages (Fig.3.5). Translating these values into the more commonly used measure of bales of lint cotton per acre, gives yields of 2.5 and 0.6 bales per acre respectively.

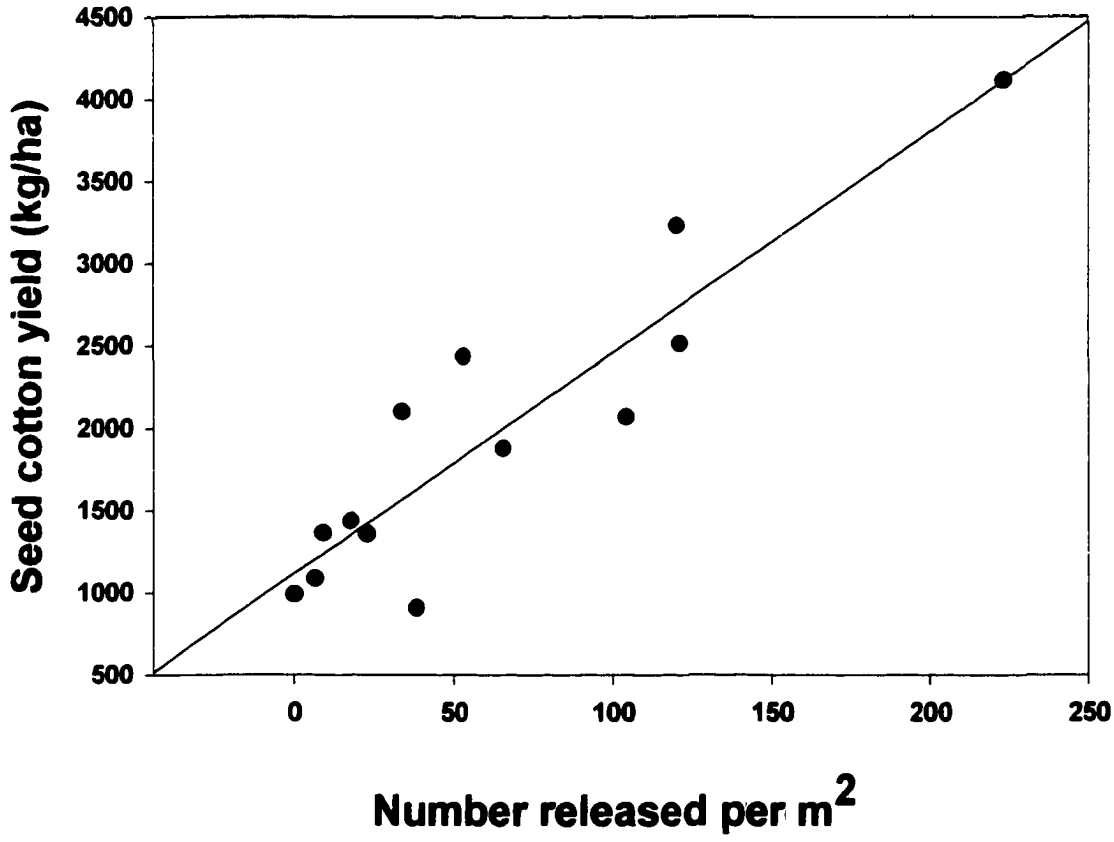


Fig. 3.5. Seed cotton yield (converted to kg/ha) versus number of *E. eremicus* released in 1993 cage release experiment. Solid line is a fitted least squares regression line.

3.4 Open Field Release Experiment in 1993

3.4.1 Methods

Test plots were planted with short staple cotton ('Deltapine 5461') on 4 April at the USDA-ARS Irrigated Desert Research Station in Brawley, CA. Plots measured 12 m wide by 9 m long with 1-m row spacing and were maintained using standard agronomic practice for cotton grown in the area. There were 12 plots of cotton in two fields with six plots in each field. Field "N" was 1.8 ha and the "river bottom" was 2. 8 ha. The plots were surrounded by bare ground and arranged to maximize the distance between plots, which ranged from 35 to 85 m.

There were six replications with two treatments deployed in a randomized complete block design. The treatments were parasitoid release (herein release) and no parasitoid release (herein no-release). Parasitoids of *E. eremicus* were released in the form of parasitized pupae of greenhouse whitefly, *T. vaporariorum*. These were shipped weekly as loose pupae from Koppert B.V. (Netherlands). A total of six releases of parasitoids were made beginning the week of 23 May and continuing until 25 July. The goal was to release the same number of parasitoids released in the high release treatment in the 1992 cage experiment, a range of 295 to 755 parasitoids per m^2 (Simmons and Minkenberg 1994. Appendix A), which successfully suppressed whitefly densities. On each release date, pupae were mixed thoroughly with 1 liter of sawdust that was then divided into 12 equal portions per plot. These were set out in 0.47 liter paper cups placed underneath cotton plants along every 3 m of row of cotton in alternate rows. The outsides of the cups were coated with a 3 cm wide band of Tanglefoot™ to prevent predators from

entering the cups and killing parasitoids. Before release, the number of wasp pupae per g and the percentage of emergence that had occurred prior to release (during shipping and handling) were determined by counting the number of pupae and emerged pupae in a 0.1 g sample from each shipment. This allowed the estimation of the number of parasitoids per gram so that the weight of pupae could be used to measure the number of wasps released on each date.

Because wasp emergence was always less than 100%, it was necessary to estimate the actual number of adult wasps released in each plot. To do this, 1 wk after release, all containers were collected and a total of 50 wasp pupae were counted per container to estimate the percentage of emergence. From this value, the value for the percentage of emergence, which had occurred before release, was subtracted to determine the percentage of emergence that had occurred during the release interval. This was multiplied times the number of pupae put out to obtain the number of wasps released in each interval. Most wasps that had emerged during shipping prior to release were dead, so it was assumed that the correct estimate of the total number of wasps released were only those that emerged during the release interval.

On each sampling occasion, a random sample of 40 mainstem leaves most infested (MIL) with *B. tabaci* pupae were selected; one leaf per plant. At the start of the experiment, all plots were sampled to determine initial densities of whiteflies and parasitism caused by naturally occurring parasitoids. Thereafter, plots were sampled about every two weeks until the end of the growing season. On 23 May, the entire leaf was censused, thereafter a 5.1 cm^2 leaf disk was censused (taken from the third distal

sector of the cotton leaf with the edges of the disk tangent to the leaf veins that mark the third sector). All whitefly counts were transformed into a per cm^2 basis. The percentage of parasitism was arcsine transformed; B. *tabaci* pupal counts were transformed by log(x+1). These transformed variables were analyzed with ANOVA repeated measures analysis of variance (SAS, PROC GLM, Littell et al. 1991) and if found significant, treatment means were separated using Tukey's HSD Test (Littell et al. 1991).

There were some potential problems in evaluating the effects of parasitoid release in small plots. Because of movement of released natural enemies or pests, experiments conducted on a small scale may become contaminated when the control treatment is too near the experimental treatments or if the plot size is too small (Stinner 1977, Sterling et al. 1992). In the case of this study, the parasitoids released may move out of release plots into the control plots obscuring differences between treatments. Because of this possibility, additional sampling was conducted in other fields of untreated cotton to serve as a comparison with the no-release plots in the event the movement of released parasitoids contaminated them. There were two fields sampled: an untreated research plot on the Brawley station of 'Deltapine 5461' of 2.7 ha (field I) and a 23 ha untreated commercial field also planted to 'Deltapine 5461'. The field on the Brawley station was about 800 m to the nearest release plot; the commercial field was 20 km from the release plots. Because of early defoliation, only one sample of 89 leaves was collected from the field on the Brawley station on 30 July. Two samples were collected from the 23 ha commercial field: 215 leaves on 10 July; and 229 leaves on 10 August. Whitefly

densities and the percentage of parasitism were estimated in the same manner as the samples from the release experiment.

Seed cotton yield was estimated by hand picking all the lint from all plants within four randomly selected 3 m sections of row, not sampling from the outside rows nor from within 2 m of the ends of the plot. Yield data were analyzed by ANOVA (PROC GLM, Littell et al. 1991) and treatment means were separated using Tukey's HSD test (Littell et al. 1991).

3.4.2 Results

There were a total of six releases of *E. eremicus* beginning on 23 May and continuing until 25 July (Table 3.4). The total number of parasitoid pupae put out over this time period in the six release plots was 1,448,000 (Table 3.4). Emergence ranged from 19 to 93% for the six releases, which resulted in a total release of 891,000 parasitoid adults (Table 3.4). This corresponds to a release rate of 786 wasps/m^2 (Table 3.4).

At the start of the experiment on 23 May, the mean (± SEM) number of whitefly nymphs per cm^2 was 0.027 ± 0.002 in the release plots versus 0.025 ± 0.003 in the no-release plots. An ANOVA indicated this was not a statistically significant difference (F = 0.03; df = 1, 10; P = 0.88). Therefore, there was no bias resulting from initial levels of whitefly that could affect the results of the experiment.

Repeated measures ANOVA indicated that there were no significant differences in the percentage of parasitism for any of the sample dates (F = 2.95; df = 1, 5; P = 0.15) The percentage of parasitism peaked on 12 August in both the release and control plots with a mean (± SEM) of 41 ± 2 % and 36 ± 2% respectively (Fig. 3.6).

Repeated measures ANOVA indicated that there were no significant differences in the number of whitefly pupae per cm^2 of leaf between the release and no-release treatments for any of the sample dates (F = 0.83; df = 1, 5; P = 0.40). The highest number of *B. tabaci* pupae per cm^2 occurred on 29 July in the control plots with a mean ± SEM of 2.6 ± 0.2 pupae /cm^2 (Fig. 3.7). The highest density of whitefly in the release plots was on 30 June with a mean (± SEM) of 1.8 ± 0.2 pupae /cm^2. Afterwards, whitefly densities in the release plots declined (Fig. 3.7).

Table. 3. 4. Number of parasitoids released in 1993 open field release. *(Cumulative number released based on a total plot size of 1134 m^2).

Release date	No. of pupae released (thousands)	Mean % emergence (n = 10)	No. of adult wasps released (thousands)	Cumulative No. of adult wasps released per m^2 *
23 May	186	82 ± 5 %	153	135
28 May	248	19 ± 5 %	47	176
12 June	310	58 ± 3 %	180	335
20 June	208	93 ± 2 %	193	505
28 June	310	69 ± 5 %	214	694
25 July	186	56 ± 1 %	104	786
Totals	1448	63 ± 10%	891	786

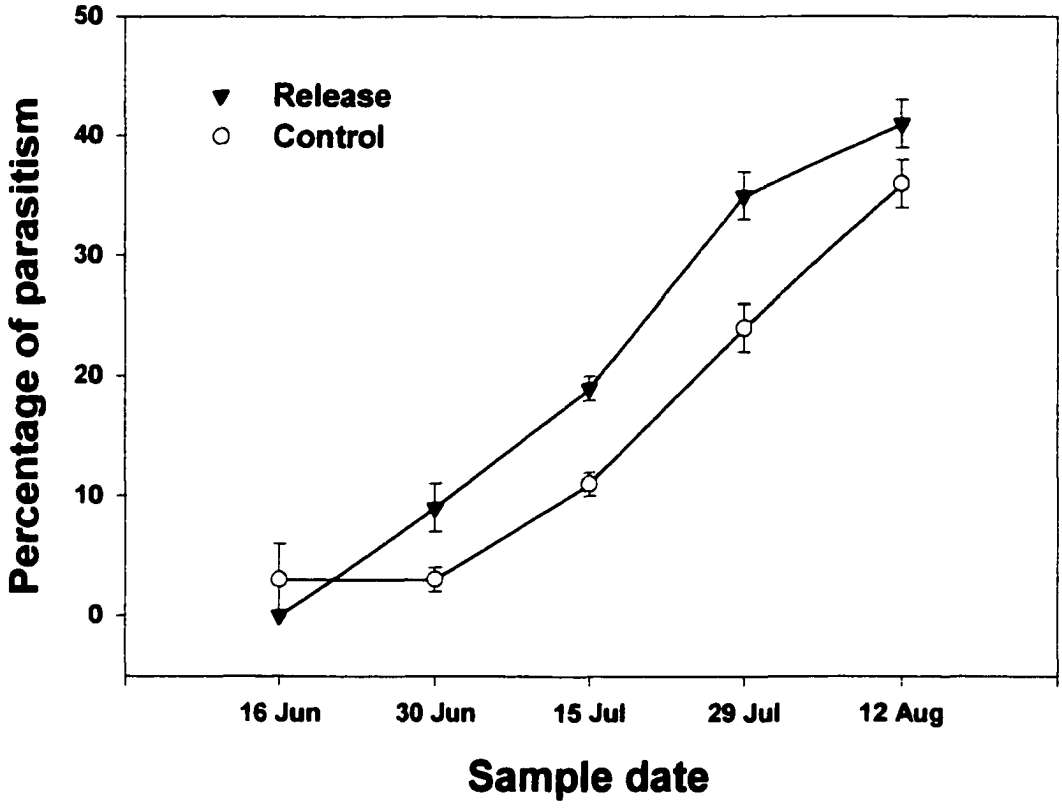


Fig. 3.6. Mean percentage of parasitism in release and control plots in the open field release experiment in 1993. Error bars are one standard error of the mean (n=6).

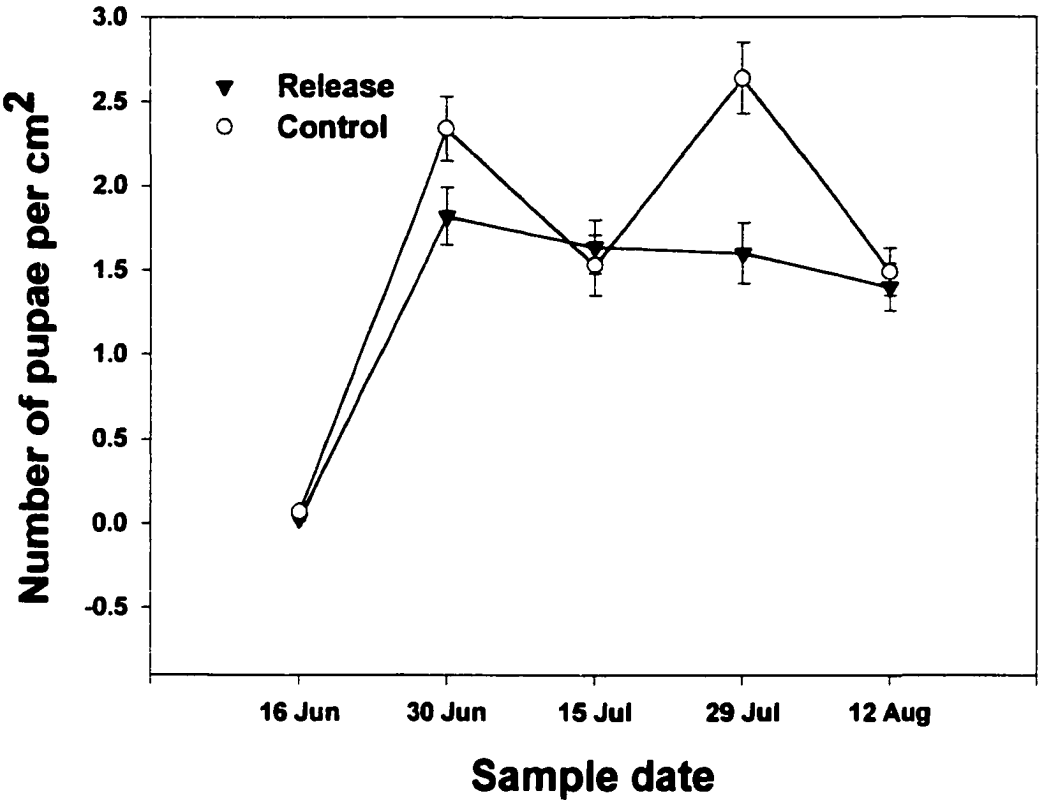


Fig.3.7. Mean number of *B. tabaci* per cm^2 in the open field release experiment in 1993. Error bars are one standard error of the mean ($n = 6$).

The cotton yield of the four row samples from the release treatment plots was numerically higher than the yield of control plots with a mean (± SEM) seed cotton yield of 0.69 ± 0.12 kg versus 0.55 ± 0.12 kg. ANOVA indicated that this difference was not significant (F = 0.71; df = 1, 5; P =0.44). Extrapolating these values to a per ha basis gives a yield of 200 and 153 kg/ha for the release and control plots respectively. Converting these values to the more commonly used measure of bales of lint cotton per acre, gives 0.12 and 0.10 bales/ac for the release and control plots respectively.

The percentage of parasitism in the nearby field on the research station, sampled on 30 July, was (mean ± SEM) 11 ± 2%. Mean (± SEM) rates of parasitism in the non-treated commercial field were 4 ± 1% and 35 ± 2% on 10 July and 10 August respectively.

3.5 Release Rate Experiment in 1995

3.5.1 Methods

A release rate study was conducted on 18 August on an organic cotton farm near Hyder, AZ. Parasitoid pupae were released in the center of small 5 x 5 m plots embedded in a larger field of 22 ha of cotton. Release rates were chosen to span the range of release rates tested in the 1992-93 cage studies. There were six plots with release rates of 0, 51, 129, 150, 430, and 641 parasitoids/m^2. Release plots were separated from other plots by about 100 m. A sentinel host technique was used to estimate daily parasitism rates. Sentinel hosts that were in the field for a short period of time are more likely to escape parasitism by naturally occurring parasitoids and may better reflect patterns of parasitism resulting from the release.

To produce the sentinel hosts, plants of two month old, greenhouse-grown short staple cotton ('Deltapine 5461') were placed in a cage and exposed to adult whitefly for 1 d after which all adult whitefly were removed with an insect vacuum (D-vac™, Dietrich Industries). The goal was to reach a target density of 10 whitefly eggs per leaf. These plants were kept in the greenhouse in a parasitoid-proof cage for 8 days to development whitefly to second instar at about 14:10 L:D, at 32 to 43^{o} C, and 30 to 60% rh. After the majority of the whitefly had developed to second instar, leaves were collected, the number whitefly nymphs per leaf were counted, and immediately placed in 40 ml florist tubes filled with water. The petioles of each leaf were re-cut under water to prevent an air bubble from entering the xylem of the leaf petiole that would block water transport. For each replicate, 540 sentinel leaves were prepared (6 treatments x 3 days x 30 leaves).

Parasitoids of *E. eremicus* reared on *T. vaporariorum* were provided by the University of Arizona insectary. To estimate the number of parasitoids per g of pupae and the amount of emergence that had occurred before release, emerged and unemerged wasps were counted in ten 0.1 g samples of wasp pupae. The number of parasitoid pupae needed for each release was estimated by weight and these were placed in a one-liter paper carton. Receptacles were protected from ants with Teflon™ tape and placed in the center of each plot under the shade of a cotton plant. Daily estimates of the number of parasitoids released were calculated by taking small samples of pupae (150 to 200 pupae) from each release container. The numbers of emerged and unemerged parasitoid pupae were counted and the percentage of daily emergence was estimated for each release area. These emergence estimates were multiplied times the total number of parasitoid pupae in each release area to calculate the number of wasps that emerged each day.

The sentinel host leaves were placed in each plot at 1 m intervals, six sentinel hosts per row on each of five rows within the release area for a total of 30 leaves per plot (Fig 3.8). The tubes containing the host leaves were attached to the mainstem of each plant with a 7.6 cm nylon quick-tie strap. Each tube was attached so that the level of the leaf was the same as naturally occurring node 3 leaves. This is the same position within a plant where leaves with many second instar whitefly are found (personal observation). These were changed daily over a 3 d period. The collected sentinel host leaves were taken to the laboratory and held for 2 wk. under natural light conditions ≈14:10 L:D, ≈ 22^{o} C. 30-45% rh to allow parasitoids to develop into pupae.

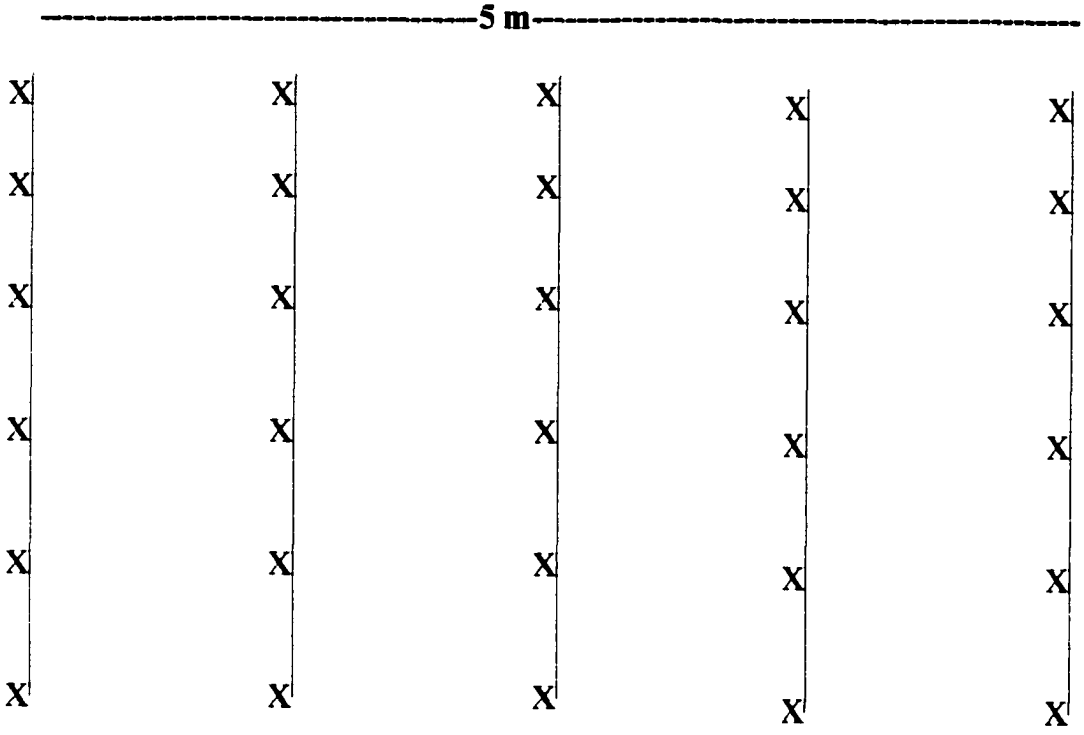


Fig. 3.8. Diagram of release plot with sentinel hosts in 1995 release rate experiment In August 1995. Each "**X**" represents the location of one sentinel host leaf within a row of cotton.

As a further estimate of the effects of the release, samples were collected from cotton plants within and surrounding the plots, three weeks after the release date when appropriate stages were present, to estimate rates of parasitism caused by the released parasitoids. Leaf samples were collected from four areas of concentric rings, 1,2, 3, and 4 m from the release point (Fig. 3.9). Leaves were collected from nodes 6-9 (one per plant), which contained the stages of whitefly that were exposed to searching parasitoids during the release period. The number of leaves collected were in approximate proportion to the area in each ring 25, 75, 100, 140 (ratio of areas: 1:3:5:7).

In addition to the calculation of the percentage of parasitism, another derived variable was calculated, the percentage of leaves that had one or more pupae of *E. eremicus* present ("percent discovered") as a further measure of the effects of each release rate. This variable is useful because it is more sensitive to the effects of a single release than the overall percentage of parasitism as a single release may not greatly increase parasitism. This depends a great deal on the behavior and the fecundity of the searching female wasps as well as the length of time after release. If a female wasp only oviposits once or a few times per leaf, a single release may not greatly change parasitism; particularly when parasitism by naturally occurring parasitoids may occur. However, a larger release should increase the number of leaves with hosts that become parasitized relative to a smaller release.

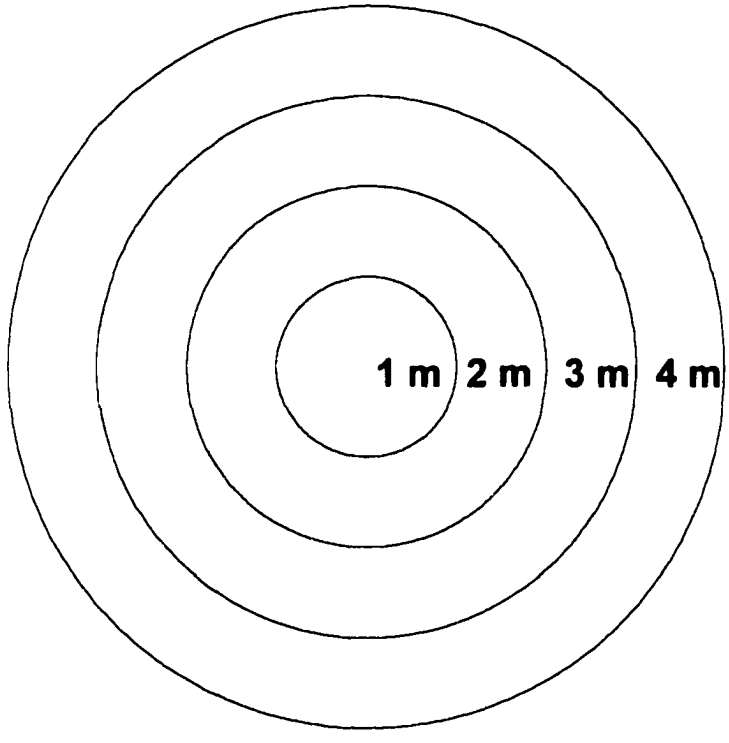


Fig 3.9. Diagram of sample area for leaf samples from cotton plants in each release plot for the release rate experiment in August 1995.

For the sentinel leaf data, the percentage of parasitism and the percentage of discovered leaves were arcsine transformed and analyzed with two-way ANOVA (PROC ANOVA, Sokal and Rohlf 1981, SAS Institute Inc. 1989) with the release rate and sample interval as the independent variables. The daily sentinel leaf data were also analyzed with least squares linear regression, regressing the percentage of parasitism or the percentage of discovered leaves against the number of released parasitoids (PROC REG, Freund and Littell 1991). If significant, the linear regressions were used to predict the level of parasitism possible at different release rates. Changes in the slope of the regression curves over time can be used to estimate the rate of wasp mortality or rates of emigration from the release area (Andow and Prokrym 1991).

For the cotton plant sample data, the percentage of parasitism, the percentage of discovered leaves and the number of whitefly pupae/cm^2 were analyzed with two-way ANOVA (PROC ANOVA, Sokal and Rohlf 1981, SAS Institute Inc. 1989) with the release rate and distance from the release point as the two independent variables. The percentage of parasitism and the percentage of discovered leaves were arcsine transformed and the number of whitefly pupae was transformed by log (x+1). Because the release rates were not replicated it was not possible to estimate the sums of squares of the interaction of the release rate by distance from the release. As it was hypothesized that increasing the distance from the release point would decrease the effect of the released parasitoids, sample data from each concentric ring at different distances were also analyzed separately. Least squares linear regression of the percentage of parasitism, the percentage of discovered leaves, and the number of whitefly pupae/cm^2 was

performed to predict the impact of released parasitoids at increasing distances from the release point. The percentage of parasitism and the percentage of discovered leaves were also analyzed separately for each sample distance with nonlinear regression fitting of the data to Eq.3.1 (PROC NLIN, Freund and Littell 1991) to determine if there was an asymptote in parasitism. For this analysis, the data were transformed by subtracting the 0-release value from all the data so that the 0-release values were set to 0.

3.5.2 Results

The percentage emergence of *E. eremicus* adults in each of the release plots varied from 57 to 99%. The number of parasitoids released in the five release plots ranged from 51 and 640 parasitoids per m^2 (Table 3.5).

For analyzing the daily levels of parasitism on the sentinel hosts, it was necessary to estimate the cumulative number of parasitoids released on each of the three days of the trial. The greatest emergence of parasitoids occurred on the second day by when the cumulative number of released parasitoids ranged between 45 to 79% of the total number released (Table 3.5). By the third day of the experiment, the cumulative number of released parasitoids ranged between 53 to 94% of the total number released (Table 3.5).

Table 3.5. Cumulative number of *E. eremicus* adults released at the end of each time interval in the release experiment in 1995.

Release plot	No. released (day 1)	No. released (day 2)	No. released (day 3)	Total No. released (± SEM)	Total No. per m^2 *
B	328	1154	1367	2541 ± 192	51
C	837	2947	3492	6426 ± 486	129
D	1691	5952	7053	7473 ± 565	150
E	3341	11759	13935	21497 ± 1625	430
F	6557	23077	27347	32023 ± 2421	641

* Number released per m^2 based on 50 m^2 plot size.

Sentinel Host Leaf Data. Sentinel host leaves were changed daily the first three days after the release. There were a total of 540 sentinel host leaves. Of these, 326 survived 2 weeks, long enough for development to occur so that the leaves could be censused for the presence of parasitoids (Table 3.6). At the start of the experiment, the mean number of *B. tabaci* nymphs on the sentinel leaves ranged between 0.35 to 0.76 nymphs/cm^2 of leaf (Table 3.6). ANOVA indicated that there were no significant differences between treatments in the number of nymphs on the sentinel host leaves. (F = 0.42; df = 5, 12; P = 0.83).

The percentage of parasitism on sentinel leaves ranged from 1 to 14% over all treatments and peaked at 14% in the plot receiving the highest release rate on the first day of the release (Table 3.6). Analysis with two-way ANOVA, indicated that there was a significant effect of both release rate and day after release on the percentage of parasitism (release rate, F = 7.2; df = 5, 10; P = 0.004, day since release, F = 5.9; df = 5, 10; P = 0.02). It was not possible to test for interaction terms since the release treatments were not replicated. Mean separation using Tukey's HSD test indicated that there was a significant effect of release rate compared with no-release only at the highest release rate of 641 parasitoids released per m^2 ($P < 0.05$).

The percentage of discovered leaves ranged from 12 to 78%. The highest level of 78% occurred in the highest release treatment on the first day sentinel hosts were put out (Table 3.6). On subsequent days, the levels of the percentage of discovered leaves declined. Analysis of the percentage of discovered leaves with two-way ANOVA

Table 3.6. Sentinel host leaf data in the 1995 release rate experiment. Estimates of the number of *E. eremicus* released; the number of *B. tabaci* pupae per cm^2 of leaf; the percentage of parasitism; and the percentage of discovered whitefly leaves.

Day	Release treatment*	No. of leaves	Mean No. of *B. tabaci* nymphs/leaf	Mean % parasitism	Mean No. of *E. eremicus*/leaf	% of discovered leaves
1	A(0)	25	0.51	7	1.08	48
1	B(328)	15	0.53	2	0.47	33
1	C(837)	15	0.62	4	1.47	47
1	D(1691)	14	0.46	2	0.71	43
1	E(3341)	19	0.40	3	1.68	26
1	F(6657)	18	0.43	14	3.72	78
2	A(0)	16	0.72	4	1.69	25
2	B(1154)	10	0.39	3	1.40	40
2	C(2947)	20	1.53	4	3.75	55
2	D(5972)	22	1.52	2	2.45	41
2	E(11759)	16	0.49	3	2.25	38
2	F(23077)	18	0.38	7	1.05	44
3	A(0)	16	0.17	3	0.53	25
3	B(1367)	15	0.78	1	0.38	20
3	C(3492)	17	0.12	1	0.18	12
3	D(7053)	17	0.08	2	0.15	12
3	E(13935)	17	0.19	1	0.21	12
3	F(27347)	27	0.24	8	0.89	37

*Numbers in parenthesis are the daily cumulative number of released parasitoids.

indicated that there was no significant effect of release treatment ($F = 2.3$; df = 5, 10; $P = 0.1187$). There was a significant effect of day after release ($F = 10.6$; df = 5, 10; $P = 0.003$).

Parasitism data were also analyzed by a series of linear regressions for each of the three days after release. These regressions were not significant for the first two days (day 1, $F = 4.2$; df = 1, 4; $P = 0.11$, day 2 , $F = 3.7$; df = 1, 4; $P = 0.13$). There was a significant regression on the third day with an R^2 of 0.65 ($F = 7.3$; df = 1, 4; $P = 0.05$, Fig. 3.10). While the regressions were not significant for two of three days, there was a trend of a decreasing slope for the regression lines that suggests the possibility that released parasitoids were either dying by the third day or leaving the release arena (Fig. 3.10).

The percentage of discovered sentinel leaves was also analyzed with linear regression. For these regressions there also was a trend of a decreasing slope with time although none of these regressions were significant (day 1, $F = 2.4$; df = 1, 4; $P = 0.20$, day 2 , $F = 0.22$; df = 1, 4; $P = 0.66$, day 3, $F = 1.8$; df = 1, 4; $P = 0.26$) (Fig. 3.11).

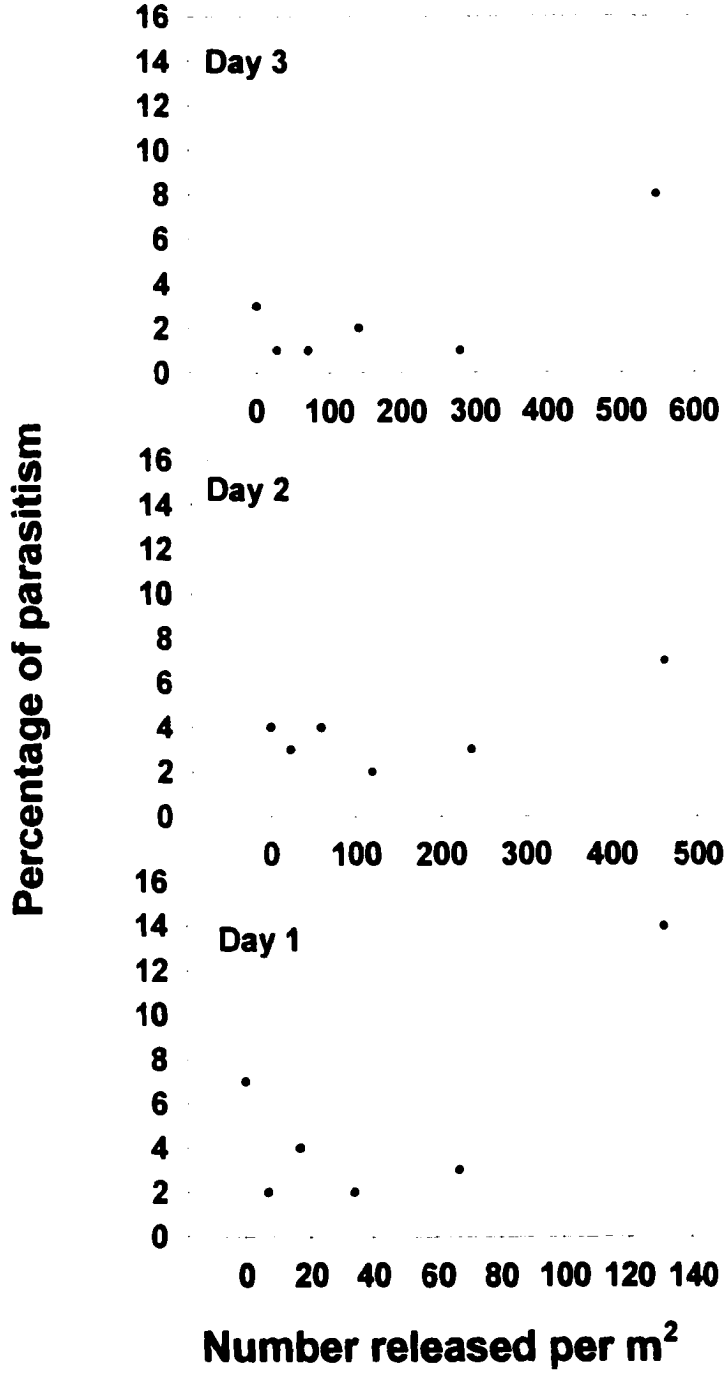


Fig. 3.10. Percentage of sentinel hosts parasitized on days 1-3 of 1995 release rate experiment plotted against the daily cumulative number of released wasps per plot. Solid line is a fitted least squares regression curve.

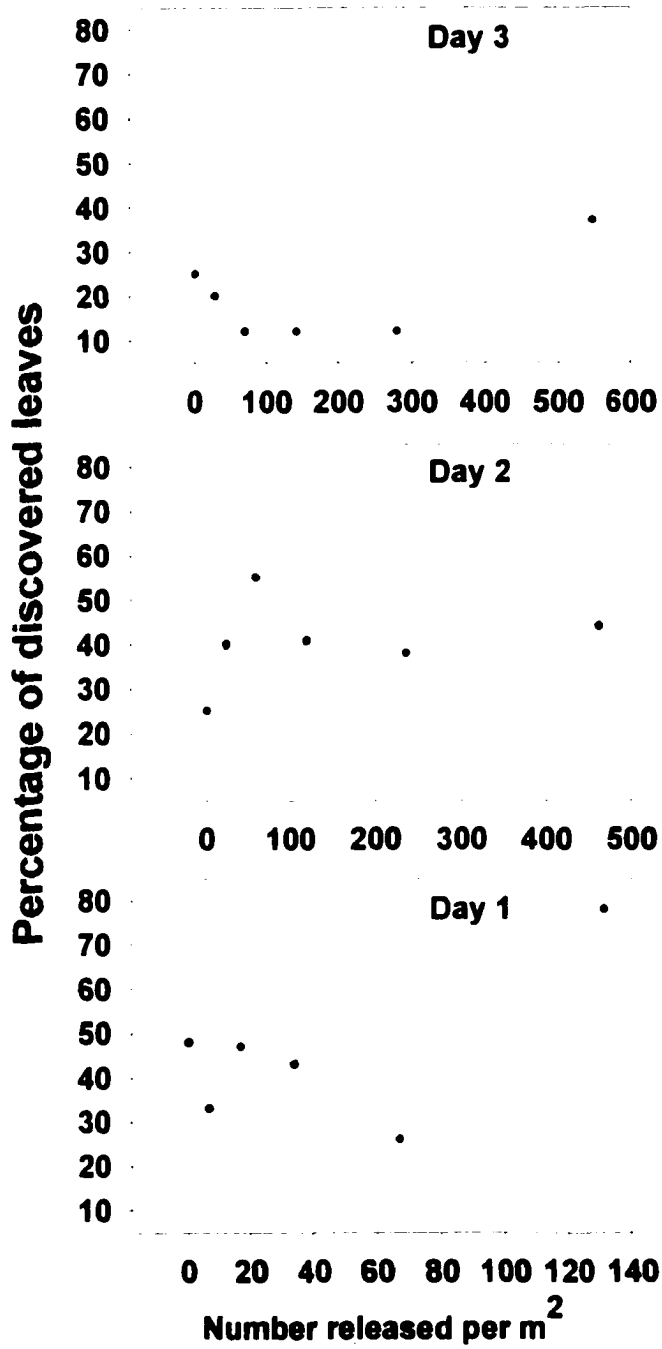


Fig. 3.11. Percentage of discovered sentinel leaves (with one or more parasitized whiteflies) plotted against the daily cumulative number of wasps released per plot on days 1-3 of the 1995 release experiment. The solid line is a fitted least-squares regression curve.

Cotton Plant Sample Data. The percentage of parasitism within the cotton plots ranged from 10 to 52 % (Fig. 3.12). The highest rates of parasitism occurred within the two highest release plots of 430 and 641 parasitoids per m^2 and within 1 m of the release point (Fig. 3.12). There was a general trend for parasitism to decrease with increasing distance from the release point (Fig. 3.12). Parasitism by naturally occurring *E. eremicus* in some cases exceeded that caused by parasitoid release (0 release plot; Fig. 3.12). The percentage of discovered leaves ranged from 42 to 99 % and declined with increasing distance. The decrease in the percentage of discovered leaves was not as great as the decline of parasitism with distance (Fig. 3.12).

Analysis with two-way ANOVA indicated there were no significant effects of release rate or distance from the release point on the percentage of parasitism (release rate, F = 1.1; df = 5, 15; P = 0.38, distance from release point, F = 2.8; df = 3, 15; P = 0.07). There was a significant effect of release rate on the proportion of discovered leaves (F = 10.0 ; df = 5, 15; P = 0.0002). Mean separation with Tukey's HSD test indicated that there were significantly higher percentages of discovered leaves in the high release plots of 430 and 641 parasitoids per m^2 than in the lowest release plot of 51 parasitoids per m^2 and the no-release plot (Table 3.7).

Analysis with two-way ANOVA indicated that there were no significant effects of either release rate or distance from release on the number of whitefly pupae/cm^2 of leaf (F = 1.6 ; df = 5, 15; P = 0.17). The density of *Bemisia* pupae ranged from (mean ± SEM) 0.11 ± 0.01 to .24 ± 0.02 pupae/cm^2 (Table 3.7). There was no pattern of whitefly density correlated to the number of parasitoids released (Table 3.7, Fig. 3.13).

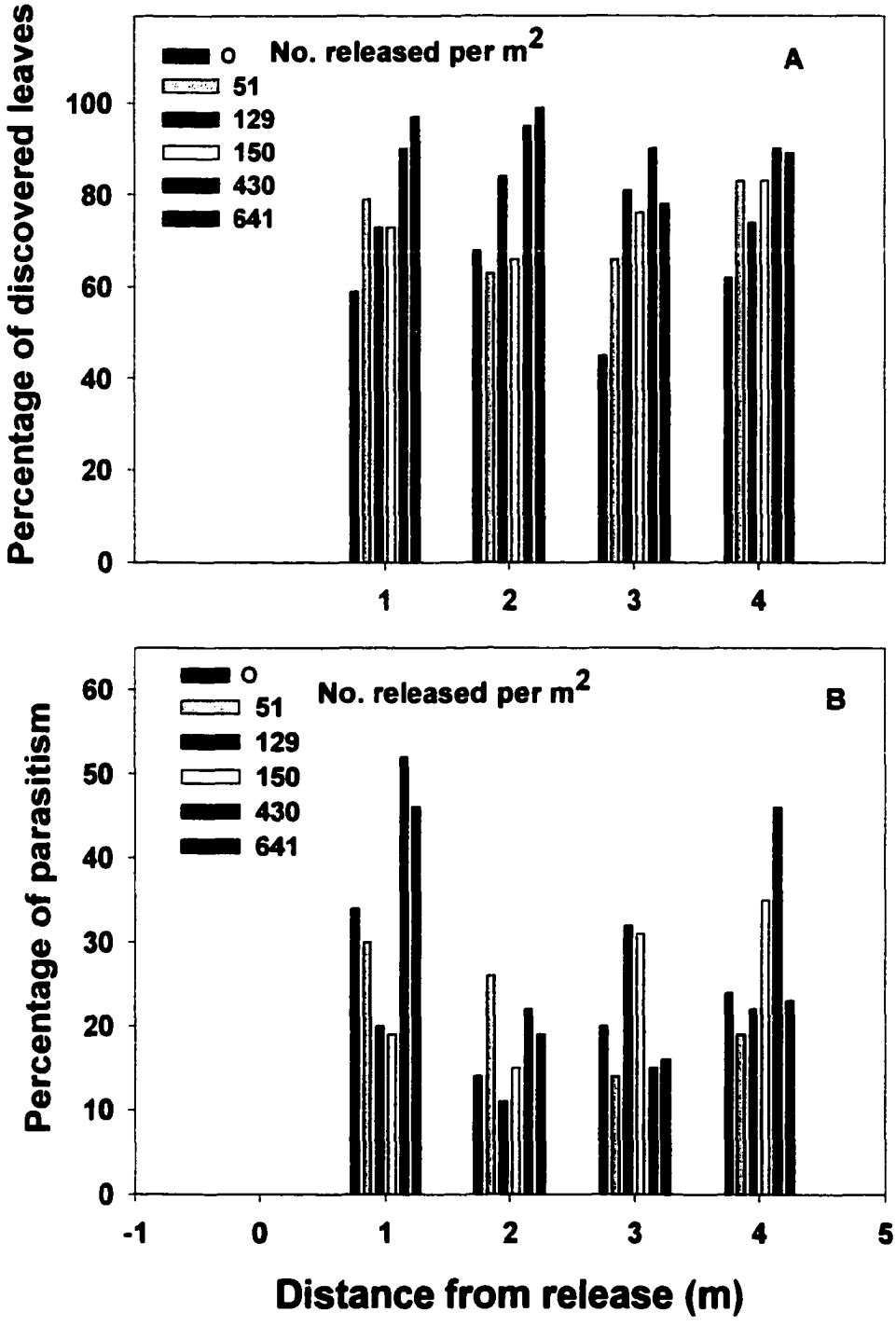


Fig. 3.12. Mean percentage: **A** of leaves discovered by *E. eremicus*; **B.** of parastism, on cotton plant samples at different release rates plotted against distance from release point in 1995 release rate experiment.

Table 3.7. Estimates of the number of *E. eremicus* released, and the means (± SEM) of the percentages of parasitism: the percentage of discovered whitefly infested leaves; and the number of *B. tabaci* pupae per cm^2 of leaf from the cotton plant samples in the 1995 release rate experiment.

Release treatment	No. of *E. eremicus* released	*n* of leaf samples	% parasitism	% discovered leaves	No. of *B. tabaci* pupae per cm^2
A (0)	0	321	22 ± 2a	59 ± 5c	0.12 ± 0.01a
B (2541)	2541 ± 192	332	21 ± 1a	73 ± 5bc	0.24 ± 0.02a
C (6426)	6426 ± 486	340	22 ± 1a	78 ± 3ab	0.14 ± 0.01a
D (7473)	7473 ± 565	339	28 ± 1a	75 ± 3abc	0.11 ± 0.01a
E (21497)	21497 ± 1625	342	32 ± 1a	91 ± 1a	0.21 ± 0.01a
F (32023)	32023 ± 2421	297	22 ± 1a	91 ± 5a	0.14 ± 0.01a

Means followed by the same letter do not significantly differ between treatments at $P < 0.05$ (Tukey's HSD, SAS 1989).

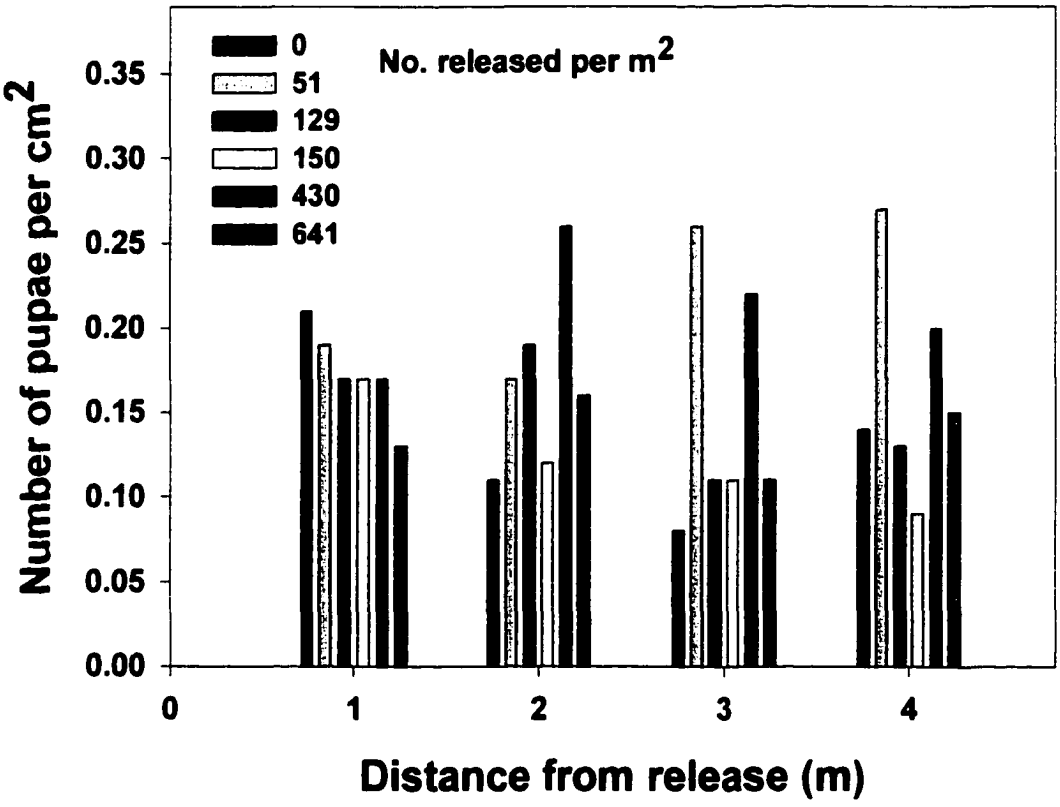


Fig. 3.13. Mean number of *B. tabaci* per cm^2 on cotton plant samples from the August 1995 release rate experiment plotted against distance from the release point.

As there were too few degrees of freedom to analyze the interaction between the number released and the distance from the release point, a further series of analyses were conducted analyzing the sample data at each distance with a separate least squares regression. Regressions of the percentage of parasitism versus the number released were not significant for any of the four sample distances from the release point (Table 3.8). Though none of these regressions were significant, there was a trend of a decreasing slope of the fitted regression line with increasing distance from the release point, suggesting that the parasitoids had a decreasing effect with increasing distance (Fig. 3.14).

Regressions of the percentage of discovered leaves versus the number released were significant for the 1 and 2 m sample distances with R^2 values of 0.82 and 0.79 respectively (Fig. 3.15, Table 3.9). Beyond 2 m, the regressions were not significant (Fig. 3.15, Table 3.9). There was a trend of a decreasing slope in the fitted regression lines beyond the 2 m distance (Fig. 3.15).

The data for each sample distance were also analyzed with nonlinear regression by fitting the data to Eq.3.1 Before analysis, the data were transformed by subtracting the

Table 3.8. Least squares regressions of the percentage of parasitism on the leaves of the cotton plant samples versus the number of released *E. eremicus* in the 1995 release experiment.

Distance from release (m)	R^2	F	df	P	Slope	Intercept
1	0.50	4.1	1,4	0.11	0.04 ± 0.02	24.6 ± 6.2
2	0.06	0.24	1,4	0.65	0.005 ± 0.011	16.6 ± 3.5
3	0.13	0.61	1,4	0.48	-0.012 ± 0.015	24.1 ± 5.0
4	0.10	0.46	1,4	0.53	0.01 ± 0.02	25.1 ± 6.4

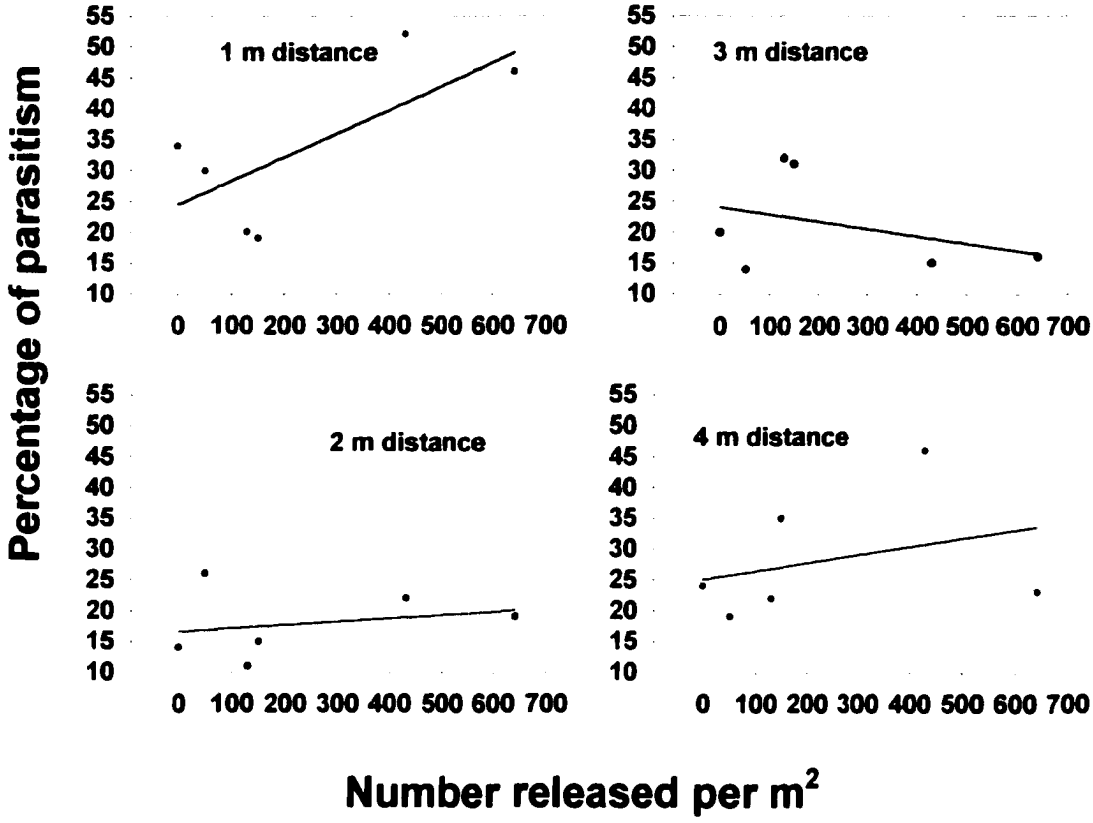


Fig. 3.14. Percentage of parasitism on cotton plant samples versus the number of released *E. eremicus* in the August 1995 release experiment. Each figure represents data from samples areas at increasing distance from the release point. Solid lines are fitted least squares regression curves.

Table 3.9. Least squares regressions of the percentage of discovered leaves for the cotton plant samples vs. the number of released *E. eremicus* in the 1995 release experiment.

Distance from release (m)	R^2	F	df	P	Slope ± SEM	Intercept ± SEM
1	0.82	18.6	1,4	0.013	0.05 ± 0.01	67.0 ± 3.7
2	0.79	15.1	1,4	0.018	0.06 ± 0.01	66.1 ± 4.7
3	0.39	2.5	1,4	0.19	0.04 ± 0.02	63.6 ± 8.0
4	0.54	4.7	1,4	0.097	0.03 ± 0.01	72.9 ± 4.7

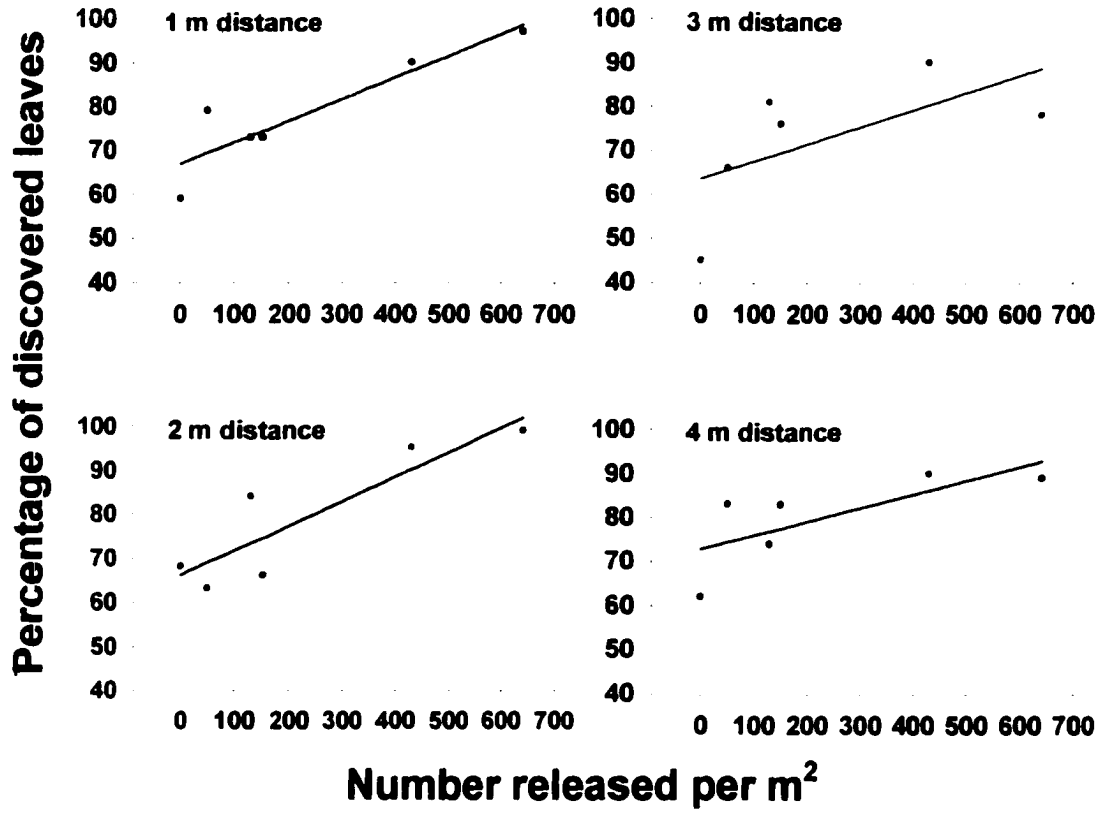


Fig. 3.15. Percentage of discovered leaves (with one or more whitefly parasitized) of the cotton plant samples versus the number of released *E. eremicus* in the 1995 release experiment. Each figure represents sample data from sample areas at increasing distance from the release point. Solid lines are fitted least-squares regression curves.

baseline value of parasitism (the value of parasitism in the 0 release plot) from all values for parasitism in each sample. All regressions had high R^2 values and were significant for all distances (Table 3.10). By inspection of the 3 and 4 m distance fitted curves it appears that an asymptote in the percentage of discovered leaves occurs between the release rates of 200 to 400 (Fig. 3.16) this was not the case for the 1 and 2 m distances where the regressions appear to be linear.

For the regressions of the number of whitefly pupae versus the number released only the 1 m distance was significant with a R^2 of 0.78 (Table 3.11, Fig. 3.17). Beyond the 1 m sample distance there was no apparent pattern of whitefly density in relation to the number of parasitoids released (Fig. 3.17).

Table 3.10. Nonlinear regressions of the percentage of discovered leaves for the cotton plant samples versus the number of released *E. eremicus* in the 1995 release experiment. Before fitting with Eq. 3.1, data were transformed by subtraction of the value for the percentage of discovered leaves from the no-release plot from all other values.

Distance from release (m)	R^2	F	df	P	$a \pm$ SE	$b \pm$ SE
1	0.80	15.9	1,4	0.02	39.0.0 ± 9.8	0.0041 ± 0.0024
2	0.82	18.8	1,4	0.01	138872 ± 796099280	0.0 ± 0.0022
3	0.92	47.7	1,4	0.002	38.6 ± 3.3	0.02 ± 0.005
4	0.71	9.9	1,4	0.03	25.9 ± 4.5	0.012 ± 0.007

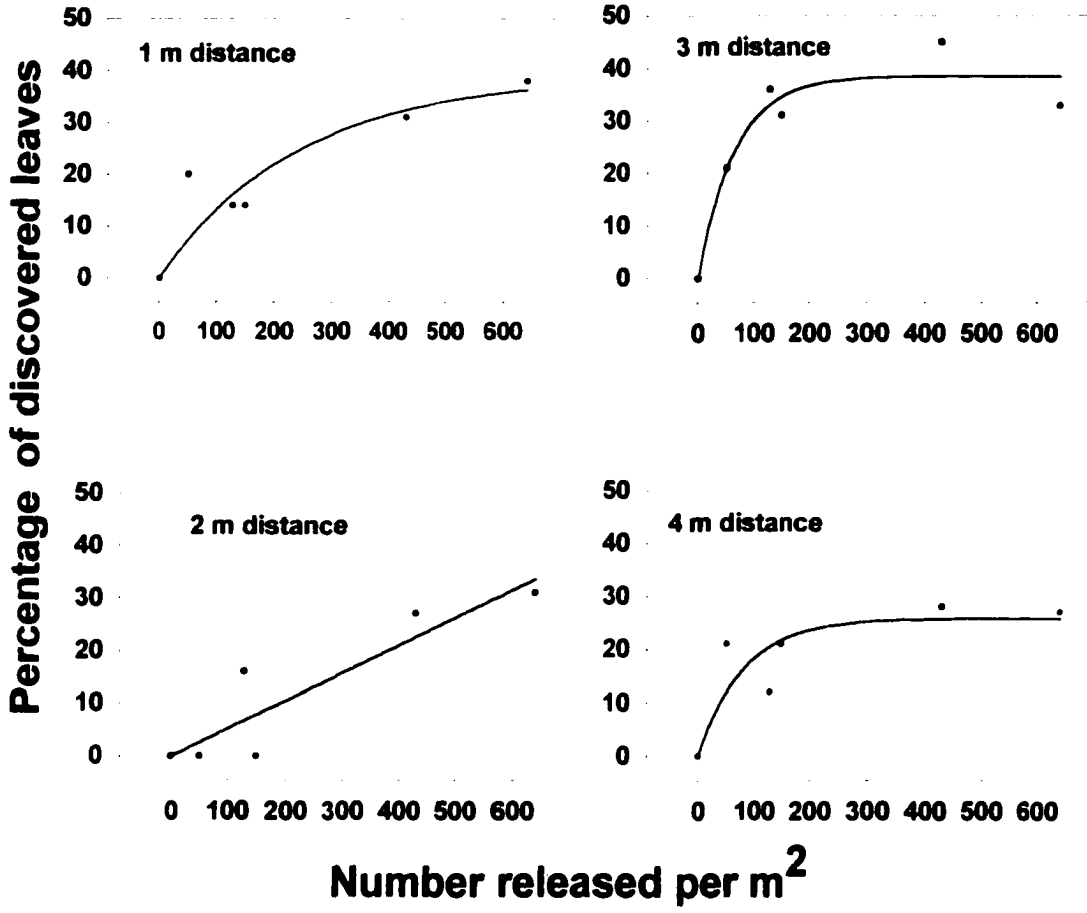


Fig. 3.16. Percentage of discovered leaves (with one or more whitefly parasitized) from the cotton plant samples versus the number of released *E. eremicus* in the 1995 release experiment. Each figure represents sample data from sample areas at increasing distance from the release point. Solid lines are fitted regressions to Eq. 3.1. Data were transformed by subtracting the value of the percent discovered leaves from the 0 release plot.

Table 3.11. Nonlinear regressions of the density of *B. tabaci* on the leaves of the cotton plant samples versus the number of released *E. eremicus* in the 1995 release experiment. Data were fit to Eq. 3.2.

Distance from release (m)	R^2	F	df	P	Slope ± SEM	Intercept ± SEM
1	0.78	14.3	1,4	0.019	-0.00004 ± 0.00001	0.20 ± 0.008
2	0.19	0.96	1,4	0.38	0.00004 ± 0.00001	0.15 ± 0.03
3	0.0	0.0	1,4	0.99	0.0 ± 0.0	0.15 ± 0.05
4	0.0	0.0	1,4	0.95	0.0 ± 0.0	0.17 ± 0.04

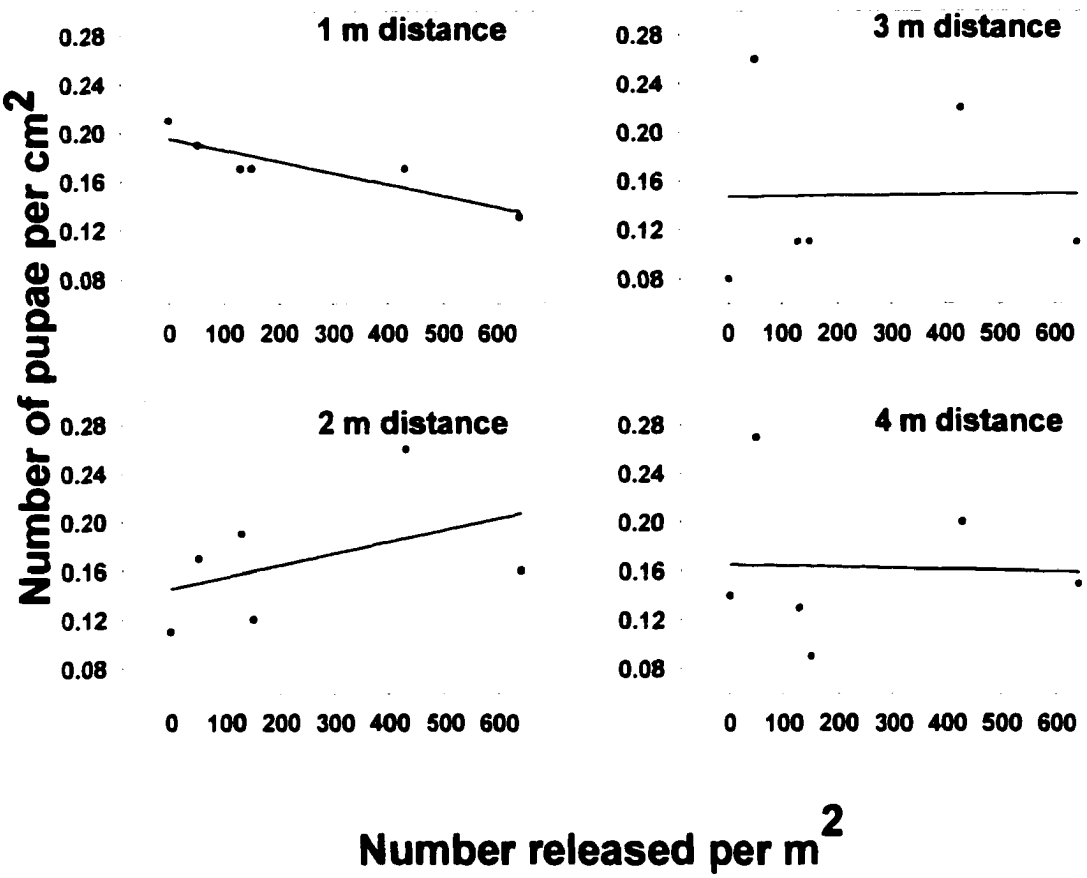


Fig. 3.17. Mean number of whitefly pupae versus the number of *E. eremicus* released in the August 1995 release experiment. Each figure represents sample data from sample areas at increasing distance from the release point. Solid lines are fitted linear regression curves.

3.6 Discussion

3.6.1 Estimating Release Rates From Inclusion Cage Experiments.

Results from the 1992 and 1993 parasitoid inclusion cage studies demonstrated that the release of *E. eremicus* can increase parasitism of *B. tabaci* infesting cotton. The highest release rates, increased parasitism, suppressed whitefly densities and increased cotton yields. The peak level of parasitism achieved in 1993 of 79% in the highest release cage was higher than the peak level of 61% achieved in the 1992 cage experiments (Simmons and Minkenberg, 1994) but is similar to the levels reported by other workers on *B. tabaci* (e.g. Gerling 1966, 1967, Natwick and Zalom 1984, Gerling 1986, Bellows and Arakawa 1988).

For the 1993 inclusion cage experiment, the three highest release rates (120 to 223 parasitoids/m^2) resulted in peak levels of parasitism earlier than the peak levels observed in the lower release treatments (<120 parasitoids/m^2). Estimates from nonlinear regressions suggest that releases above 128 to 146 parasitoids/m^2 produced an asymptote in parasitism of about 70%. By inspection of the nonlinear regressions of whitefly pupae density, releases above 109 parasitoids/m^2 resulted in the lowest whitefly (and about equal) densities suggesting that higher release rates would not result in further reductions in whitefly.

Taken together, the levels of parasitism and the degree of whitefly suppression achieved in the 1993 release rate experiment suggest that releases above 109 to 146 parasitoids/m^2 produced the greatest suppression of whitefly populations and the highest levels of parasitism. However, linear regression of the cotton yield data shows that there

was a increase in yield with increasing releases of parasitoids. So there appears to be a benefit of releasing parasitoids at levels higher than 109 to 146 parasitoids/m^2. This may be due to the fact that at the higher release rates, whitefly pressure on the cotton plants was reduced in the cages earlier than in cages receiving lower release rates. This result, in spite of high levels of parasitism achieved with the release rates lower than the top rate of 223 parasitoids/m^2, led to healthier plants and higher yields.

The last parasitoid release on 8 July, probably contributed little to the rates of parasitism and the suppression of whitefly achieved in this experiment because high rates of parasitism had already been reached by the 6 July sample date. Further increases in parasitism to the peak levels observed on 20 July, were likely caused by the reproduction of the existing population of parasitoids. Coupled with the observation that the peak emergence of the released parasitoid pupae takes place about 3 to 4 days after introduction (personal observation) and that parasitoid development may take between 12 to 18 days (Powell and Bellows 1992b), it seems likely that the first three releases contributed the most to increases in parasitism. This suggests a release rate of between 77 to 109 parasitoids/m^2 (the number released on 8 July subtracted from the range of totals of the top three release rates, see Table 1) would be effective. This rate of release is lower than the rate suggested by the 1992 cages studies of 113 to 367 parasitoids/m^2.

Release rates estimated from cage studies can, at best, provide only approximate estimates of the range of release rates that may be effective. Cages limit immigration of whitefly onto the cotton plants so that the whitefly populations that develop within a cage are the result only of the reproduction of the existing population and interactions with the

parasitoids. In an open environment, both whitefly and parasitoid populations are influenced by immigration and emigration. There is a significant source of additional whitefly that emigrates from the surrounding spring melon crop throughout June, after the time that the cages were set up over the cotton. Furthermore, the cages eliminated emigration of other natural enemies into the cotton (e.g. *Geocoris spp.*, *Orius tristicolor*) which have been found to be important predators of whitefly in Arizona cotton production systems (Hagler and Naranjo 1994).

Finally, cages alter the growing environment by shading the plants, causing a decrease in air temperatures, decreasing light levels, and reducing soil water evaporation rates. As a result, soil within the cages retains moisture longer than sun exposed soil. These factors cause the cage grown cotton plants to grow taller, and to produce lush green foliage with larger than normal leaves. These differences in the plant may influence the growth of whitefly and parasitoid populations as well as their interactions.

3.6.2 Estimating Release Rates from Open Field Releases

Open Field Release Experiment in 1993. Further limitations of using cage studies to estimate release rates were revealed by the results of the 1993 open field release experiment. A release rate of 786 parasitoids/m^2 (approximately 3.5 greater than the highest release rate in the 1993 cage experiment) resulted in a peak rates of parasitism of only 42%, about half that of the highest rate of parasitism achieved in the high release cage in 1993. Furthermore, there were no significant differences in rates of parasitism, whitefly density, nor yield between the release and control plots.

A major factor for the low levels of parasitism achieved in the open field release appears to be the large number of whitefly that migrated from the spring melon crop into the plots during a three week period from about 5 June until 25 June, a period of time after which the cages in the cage experiment had been closed. This resulted in a large nymphal population that diluted the effects of the parasitoid release. Near continuous development of whitefly populations caused by the cultivation of concurrent or overlapping whitefly-sensitive crops (as is the case for *B. tabaci* in the Imperial Valley) has been cited as a major factor for the failure of augmentative biological control efforts (van Lenteren et al. 1996). Unfortunately, the number of whitefly that migrated into the field plots during this time was not quantified. An indication of the magnitude of this migration can be shown by comparing the mean density of whitefly pupae in the two no-release cages on 22 June (these cages were closed before the increase in whitefly immigration) versus the mean number of whitefly pupae just one week later on 30 June in the control plots of the open release experiment, 0.9 versus 2.2 whitefly pupae/cm^2 respectively.

There also appears to have been a large degree of emigration of parasitoids from the release plots into the control plots, as the rates of parasitism in the control plots were higher than in the field on the research station and in the commercial field where no parasitoids were released. Parasitoids may have left the release plots because the size of the release plots was small relative to the scale of parasitoid dispersal (c.f. Sterling et al. 1992, Diehl et al. unpublished manuscript). In the movement experiments (Chapter 4), it was estimated that the median dispersal distance of *E. eremicus* was on the order of 2.4

to 4.4 m in 4 to 8 d. Parasitoid release containers on outer rows were within 2 m of the plot boundary and many of these parasitoids may have left the plot. Nothing is known about the behavior of dispersing wasps on the boundary of a release plot. Upon encountering a boundary edge, they may either turn back into the cotton plot (boundaries reflective, Turchin 1998) or they may emigrate from the plot and disperse at greater distance than they would otherwise move within a solid block of cotton. It would be of interest to study the dispersal behavior of *E. eremicus* along the edges of cotton fields and in the interface between crop and non-crop areas because knowledge of their behavior at this interface may be important for implementing augmentative biological control as well as for designing refuges for the conservation of natural enemies in *B. tabaci* infested agricultural areas.

Lastly, there is the possibility that the high release rate led to density dependent dispersal caused by mutual interference (Hassell 1978). If female parasitoids have a tendency to leave the leaf or plant it is searching upon encountering another searching female this may lead to a higher than normal rate of emigration (Hassell 1978, van Alphen and Vet 1986, Godfray 1994). It is not known whether mutual interference is an important phenomenon for *E. eremicus*.

Open Field Release Experiment in 1995

Sentinel Host Samples. Linear regression revealed only one significant relationship between rates of parasitism and release rate for the third and last sample period with sentinel hosts. Except for the highest release plot, parasitism was higher in the no-release

plot for each of the 3 days. This was due to the action of naturally occurring parasitoids, which made it difficult to determine if the trend of higher rates of parasitism with increasing release rates was significant for the sentinel hosts. Therefore, it was not possible to draw many conclusions from the sentinel host leaf data except there was a trend over the three days of the experiment of decreasing rates of parasitism and slopes of the parasitism versus number released regressions. This suggests the possibility that parasitoids were leaving the release arena or were dying. Andow and Prokym (1991) found a similar result with *Trichogramma nubiale* Ertle and Davis released against European corn borer (*Ostrinia nubilalis* Hubner) and suggested that the rate of disappearance (as a result of mortality or leaving the release arena) needs to be reduced in order to increase the efficacy of releasing parasitoids in that system. This may be an important consideration that should be addressed for the effective use of augmentative releases of *E. eremicus* for control of whitefly infesting cotton.

Cotton Plant Samples. Increasing the number of parasitoids released did not cause any significant increases in the rates of parasitism for any of the four sample distances. This may be due to the fact that there were already parasitoids present in the field at the start of the experiment that partly masked the effects of the released parasitoids. The successful use of inoculative augmentative biological control is dependent on the population growth of the released natural enemies in order to exert control on the target pest. For these same reasons, it was expected that a single release would not greatly change the density of whitefly immediately, as was the case in this experiment.

Increasing the number of released parasitoids increased the percentage of discovered leaves at all sample distances. The greatest effect was seen at the highest release rates of 430 and 640 parasitoids per m^2. The nonlinear regressions (with Eq. 3.1) of these data, revealed that an asymptote in the percentage of discovered leaves occurred at between 200 to 400 parasitoids per m^2. Releases at these levels resulted in levels of the percentage of discovered leaves of greater than 80%.

3.6.3 Summary of Effective Release Rates and Economic Analysis

The results from 1995 release experiment suggests that a release rate in an open field of between 200 to 300 parasitoids per m^2 should result in very high levels of discovery of whitefly infested leaves by *E. eremicus*. Whether these release rates would result in high rates of parasitism and suppression of whitefly densities remains to be determined. The density of whitefly at the start of this experiment in 1995 was about one fortieth less than the density at the start of the 1992 cage experiment (0.006 versus 0.24 pupae/cm^2 respectively) and about one fifth less than the density of whitefly in the 1993 open field release (0.027 pupae/cm^2). Furthermore, the field used in the 1995 experiment was not subject to the same degree of whitefly immigration that occurred in the 1993 open field experiment. So release rates at these levels may not be appropriate when whitefly populations are higher. On the other hand, the cage studies in 1993 were initiated with an initial whitefly density about equal to the 1995 study (0.005 versus 0.006 pupae/cm^2 respectively). Very high levels of parasitism were achieved and whitefly populations were greatly reduced with the higher release rates. Though I have speculated that cage effects may have been important in this experiment; I did not attempt to estimate what these effects might be, the only suggestion of such an effect was the much higher number of parasitoids needed for the open field release relative to the cage experiments in 1993.

Finally, all of these releases were conducted in small plots and cages and a true test of what is possible has not been conducted in an entire field. If an entire field was treated, the effects of whitefly immigration into treated areas from untreated parts of the field would presumably be mitigated as would within field parasitoid movement out of the release areas.

For the purpose of making a tentative economic assessment to gauge the current feasibility of using *E. eremicus* in a release program against *B. tabaci*, I will assume that the range of effective release rates are between the lowest release rate in the cage study and the highest release rate estimate from the 1995 release rate study i.e., between 77 to 300 parasitoids/m^2 respectively. On a per ha basis, these translate to a range of release rates of 770,000 to 3,000,000 parasitoids/ha. The lowest price for commercially produced parasitoids is $6.00 per 1,000 for volume purchases (D. Cahn, Oxnard, CA, Novartis BCM, personal communication). Cost estimates for treating one hectare of cotton with parasitoids range from a low of $4620 (770,000 @ $6.00/1000) to a high of $18,000 (3,000,000 @ $6.00/1000). An adjustment in costs must be made to account for rates of emergence less than 100%, as the above figures are based on the actual number of adults that were released. Emergence rates in the 1993 and 1995 experiments ranged between 56 to 99% (discarding an outlier of 19%) with a mean of 72%. Therefore the previous figures should be increased by 28%, which results in an estimate of $5,914 to 23,040 to treat one hectare with parasitoid release. In the Imperial Valley of California, where whitefly population densities on cotton have been among the highest as anywhere in the cotton belt (D. Weddle, Imperial County, CA, Office of the Agricultural Commissioner, personal communication), the highest price growers have been willing to spend on pesticidal control of whitefly and still achieve economic returns has been about $741 per hectare (J. Benson, Benson Farms, Brawley, CA, personal communication).

Therefore, the lowest estimate of the cost of an effective parasitoid release based on the current study is about 8 times higher than the highest amount growers are willing to spend and still achieve some economic returns. Clearly, even if it was certain that these release rates would result in effective control and yields comparable to those achieved with pesticides, the current costs of using parasitoid release against whitefly infesting cotton are far too high to be considered a viable method of pest control.

3.6.5 Future Prospects for the Use of Parasitoid Release Against Whiteflies in Cotton

In the years since 1992 to 1995, regional whitefly populations in cotton and vegetable production areas have been declining in the most severely affected areas in Arizona and California (Ellsworth 1999, Palumbo 1999, D. Weddle, Imperial County, CA, Office of the Agricultural Commissioner, personal communication). Lower overall regional whitefly densities should result in fewer whiteflies migrating from surrounding crops, improving the odds for the successful use of parasitoid release to control within field populations of whitefly. Probably the most significant factor responsible for this decline is the introduction of more effective pesticides that are used in the spring melon crop and in cotton. Three of the most important of these chemicals, buprofezin, imidacloprid and pyriproxyfen, have the advantage of having few to no direct effects on natural enemies (Jones et al. 1995, Naranjo and Hagler 1997, Simmons et al. 1997b, Naranjo et al. 1998b). The combination of the use of the new selective materials and reduced regional whitefly populations, raises the possibility of using lower economical release rates of parasitoids combined with pesticide applications as part of an integrated control program. Combining parasitoid release with the use of these selective materials may be needed because whitefly survivorship after treatment can range as high as 18 to 20% four to five

weeks after treatment (Naranjo and Ellsworth 1999). Thus, the remaining whitefly may still pose a significant threat to the cotton requiring further sprays.

The additional materials that can be used after buprofezin and pyriproxyfen, the two most effective selective materials for use in cotton (label restrictions allow only one use of each material), are mostly highly toxic (e.g. pyrethroids and organophosphates) to both whitefly parasitoids and other natural enemies (Kapadia and Puri 1991, Jones et al. 1995). Besides the need to treat whitefly populations surviving pesticide treatment, it is desirable to try and conserve the natural enemies of other key pests in the cotton system (Hagler and Naranjo 1994). If parasitoid release could be exchanged for further pesticide treatments, this would be advantageous for the conservation of the natural enemy complex of the other pest species. Finally, relying on augmentative biological control for pest control is desirable due to increasing concerns about the development of insecticide resistance and rising costs associated with pesticide regulatory issues (Heinz et al. 1993). All of these factors provide financial incentives for using augmentative biological control that goes beyond only the consideration of the costs of parasitoid release versus pesticide use.

An additional factor that has improved the economic prospects for using parasitoid release is the collection of new more effective *Eretmocerus* spp. from other regions in the world (Goolsby et al. 1998). These have been introduced into cotton and vegetable growing areas of California and Arizona and numerous over-wintering recoveries have been made in the last several years (Roltsch and Simmons 1997, Hoelmer et al. 1998b, Pickett et al. 1998a). The most effective of these species (*E. emiratus* Zolnerowich and Rose, *E. mundus* Mercet, and *E. hayati* Zolnerowich and Rose) have field measured fecundities more than two times greater than *E. eremicus* (Hoelmer 1998). Using *Eretmocerus* species with greater fecundity should allow the use of lower effective

release rates than those that were estimated in the current study. Several of these species have recently been introduced into commercial trade, which will provide access to growers and researchers interested in conducting further testing.

3.6.6 Considerations for Further Research

New research on efficacious release methods is needed to increase the survival, emergence rates, and the distribution of released *Eretmocerus* spp (c.f. Giles et al. 1995, Obrycki et al. 1997). In these experiments the survival of the released parasitoids is not known, but survival and emergence of parasitoids are often correlated (Obrycki et al. 1997). The emergence rates in these experiments generally ranged between 56 to 99% with a mean of 72% (though it was as low as 19% for one of the release dates). Given the high numbers of parasitoids needed for release, and the high cost of parasitoids, it is clear that emergence rates will need to be improved in order to make augmentative releases economically possible.

Quality control in the processes of rearing and shipment of biological control agents also has important consequences for their survival and performance (Stinner 1977, Obrycki et al. 1997). To improve the prospects for the successful use of parasitoids of *Eretmocerus* spp. there is an urgent need for research on the both the temperature and length of time at which they can be stored before use in the field. Frequently, *Eretmocerus* spp. purchased from commercial suppliers are of poor quality and have low emergence rates (personal observation). Currently, there are no industry standards for the storage or shipment of these parasitoids (D. Cahn, Oxnard, CA, Novartis BCM, personal communication).

It is also important to learn how much natural enemy induced mortality is needed in order to manage whitefly populations. Information on whitefly mortality within the cotton system is becoming available though the efforts of researchers making detailed life-table studies on the sources and intensity of whitefly mortality in the cotton system (Naranjo et al. 1997, Naranjo et al. 1998a). Naranjo et al. (1997, 1998a) have quantified how much mortality can be attributed to pesticides, natural enemies, and unknown causes. If this information were coupled with important details of *E. eremicus* biology such as fecundity, survival, search rate, and dispersal; a population model could be developed to estimate the effects of varying release rates, frequency, and timing. A population model that modeled key features of the *B. tabaci-E. eremicus*-cotton system could then be used to predict outcomes of different augmentative release strategies under varying conditions. For example, what is the effect of parasitoid release with and without whitefly migration from surrounding fields? A modeling approach could answer this and other key questions and help to design future field studies. An important result of some augmentative parasitoid release models has found that the timing of release can be more important than the absolute number of parasitoids released (Flinn and Hagstrum 1995, van Roermund et al. 1997). Perhaps in this study a lower rate of parasitism would have been effective if releases were made very early. An effective experiment to test this idea would be difficult to conduct in the field, a model could help determine if such an effort was worthwhile.

Lastly, some effective augmentative strategies from biological control efforts of whitefly and other pests in greenhouses are beginning to be adopted in augmentative biological control in field crops (Obrycki et al. 1997). One particularly promising strategy, which has been described as the "pest in first" or the "banker plant" (c.f. Bennison and Corless 1993) strategy has been experimentally tested against whitefly

infesting melons (Goolsby and Ciomperlik 1997, Goolsby and Ciomperlik 1998, Pickett et al. 1998b). In this technique, parasitoids are put in a field along with small quantities of whitefly very early in the season on melon seedlings bearing both host and parasitoid. After sufficient degree days have accumulated for whitefly populations to build up and migrate from the surrounding winter vegetable crop into the melons, there is already a resident population of parasitoids present in the field that will attack the new migrants as soon as nymphs develop. Use of the banker plant technique significantly reduced whitefly relative to the release of a larger number of loose parasitoid pupae suggesting that it should be possible to achieve better results with lower release rates using this technique (Pickett et al. 1998). It is conceivable that this same technique could be adopted for use in the cotton system, and greatly reduce the number of parasitoids needed for release.

CHAPTER 4

DISPERSAL OF *E. EREMICUS* IN COTTON AFTER A POINT RELEASE

4.1 Introduction

The degree of mobility of natural enemies is an important characteristic in determining their effectiveness in controlling their prey (Kareiva and Odell 1987, Andersen and Kareiva 1993, Corbett and Rosenheim 1996b, Rudd and McEvoy 1996, Weisser 1997). For example, in classical biological control mobility is a critical factor in determining the success in controlling the pest, or the rate of establishment of the new agent (Huffaker et al. 1976, Strong et al. 1984, Greathead 1986, Stiling 1993, Corbett and Rosenheim 1996b, Rudd and McEvoy 1996). However, the rate of movement of introduced natural enemies is rarely documented in studies concerning the success of classical biological control programs (c.f. Hall and Ehler 1979, Greathead 1986, Waage and Greathead 1988, Kareiva 1990b, Stiling 1993). There is also a paucity of information concerning the mobility of natural enemies used in augmentative control (Keller et al. 1985, Cronin and Strong 1990, Corbett and Plant 1993, Pedgley 1993, Corbett and Rosenheim 1996b, Quicke 1997, Weisser 1997). Knowledge of how far an agent is likely to move can be used to devise release patterns, e.g. is it sufficient to make a large release from a central release point or are several small releases with greater dispersion necessary? Also important is that limited dispersal of parasitoids may lead to poor establishment or extinction of the released parasitoids as many female parasitoids will go unmated (Hopper and Roush 1993). This factor can impact both attempts to establish new species and the efficacy of augmentative control, and should be an

important consideration when designing release strategies. Another consideration is whether the released agent will stay in the release area (Keller and Lewis 1985, Keller et al. 1985, Andow and Prokrym 1991, Corbett and Plant 1993, Pedgley 1993, Corbett and Rosenheim 1996b). Indeed, movement of the released natural enemies out of the target area has often been cited as an important factor in the failure of biological control efforts (Keller and Lewis 1985, Andow and Prokrym 1991, Sterling et al. 1992, Stiling 1993). Although numerous studies have documented the longest moves observed after release (c.f. several studies on Trichogramma against false codling moth: Stern et al. 1965, Stern and Mueller 1968, Yu et al. 1984, van den Berg et al. 1987, Newton 1988), what is most needed is to know what proportion of the released population will move distance x (Turchin and Thoeny 1993, Turchin 1998)?

The main reasons for the lack of quantitative dispersal studies in biological control are: that small highly mobile insects are difficult to observe directly in the field (Weisser and Volkl 1997, Turchin 1998); the relatively recent appreciation of the role that mobility plays in controlling pests that have patchy distributions (Kareiva and Odell 1987, Anderson and Kareiva 1993); the lack of analytical tools have made it difficult to interpret data from dispersal experiments (Turchin and Thoeny 1993, Turchin 1998). Recently, new methods of analysis have been developed with the adaptation of the diffusion model (Okubo 1980) and much progress has been made that allows these tools to be used by entomologists wishing to address questions about pest management issues (c.f. Kareiva 1983, Rudd and Gandour 1985, Kareiva and Odell 1987, Plant and Cunningham 1991, Turchin and Thoeny 1993, Corbett and Rosenheim 1996b, Turchin 1998). The diffusion model has been found

to accurately describe insect movement for a variety of species (e.g. Kareiva 1983, Rudd and Gandour 1985, Plant and Cunningham 1991, Turchin et al. 1991, Corbett and Plant 1993, Holmes 1993, Turchin and Thoeny 1993, Corbett and Rosenheim 1996b). The use of the diffusion model is an improvement over purely empirical models of dispersal such as various exponential decline with distance models (e.g. Hawkes 1972, Taylor 1978, Taylor 1980) because its parameters have a direct biological connection to the data and useful statistics such as the median distance of dispersal of the marked population can be calculated (Turchin and Thoeny 1993, Turchin 1998).

As discussed in chapter three, previous open field releases of parasitoids suggested a high degree of dispersal away from the release site. Obtaining more information about the pattern of dispersal within a field after a point release would provide information useful for implementing release strategies. Questions such as what pattern of dispersion of parasitoids is needed for release, how many parasitoids are needed per unit release area, and how many parasitoids are likely to leave the release area are important considerations. In addition, there is theoretical interest in differences in movement patterns between insect species and the kinds of models that can accurately characterize movement (Levin 1981, Kareiva 1983, Rudd and Gandour 1985, Turchin 1998). Also, if parameters of insect movement such as the diffusion coefficient (diffusion rate) are estimated for a particular biocontrol agent under one set of conditions (e.g. low host density) and contrasted with other sets of conditions (e.g. high host density) predictions can be made about the performance of the agent under different conditions. The diffusion coefficient could also be used to predict which agent from of a group of

potential natural enemies would be most efficient at searching out prey within a field after release. Finally, estimates of the diffusion coefficient could be used to determine which kind of biological control strategy might be most suited for a particular agent (Corbett and Rosenheim 1996b). For example, a parasitoid that had a relatively low rate of dispersal within a field may stay longer in the release area providing more effective long-term control and would be suitable for augmentative biological control, while another agent with higher dispersal rates might be more suited for use as a classical biological control agent because it could become quickly established within the pest habitat. The main question I wished to address was: How far and at what rate do *E. eremicus* disperse within a cotton field after a point release?

The strategy chosen to pursue this question was to conduct a series of mark-release-recapture experiments of *E. eremicus*. With traps deployed in a grid around a central release point, recaptures were made over several days. These data were fit to a diffusion model to estimate diffusion coefficients and dispersal distances. With the results from this study in mind, I suggest how estimates of diffusion coefficients could be used to evaluate potential biological control candidates and for devising biological control strategies.

4.2 Methods

4.2.1 General Design and Techniques of Release Experiments

Release grids were set up within larger cotton fields of approximately 20 to 28 ha. Each release grid consisted of a central release point surrounded by an array of traps. The release grid measured 16 by 16 m and was divided into 64 cells measuring 2 x 2 m (Fig. 4.1). Within the center of each cell an 18.7 x 33 cm yellow sticky trap (Olson Co., Medina, OH) was placed on a stake at canopy height so that the top of the trap was even with the canopy. Yellow sticky traps have been shown to be attractive to *E. eremicus* (Hoelmer et al. 1998a). For this reason, to avoid bias in recapture patterns of wasps, traps were placed within the cotton canopy so they would not be visible unless parasitoids were within a short radius from the trap and could only be attracted from a short distance away. Traps were stapled to the stake in the form of a cylinder so that the trapping surface was exposed in all directions. In some of the releases, traps were also placed outside of the central trapping grid along transect lines in each cardinal direction at 5 m intervals. Four mark-release experiments were conducted in untreated cotton during the summers of 1994 (three releases) and 1995 (one release).

Parasitoids were released as pupae within the center of each grid. Each experiment began at first daylight with the placement of the initial set of traps and the parasitoid pupae. Parasitoid pupae were lightly dusted with fluorescent dust (Day-Glo Color Co., Cleveland, Ohio) by gently shaking them in a 0.47 liter paper ice cream carton with a small quantity of dust so that an adult wasp would become marked upon leaving the whitefly exuvia. Pupae were then transferred to clean 0.47 liter paper ice cream

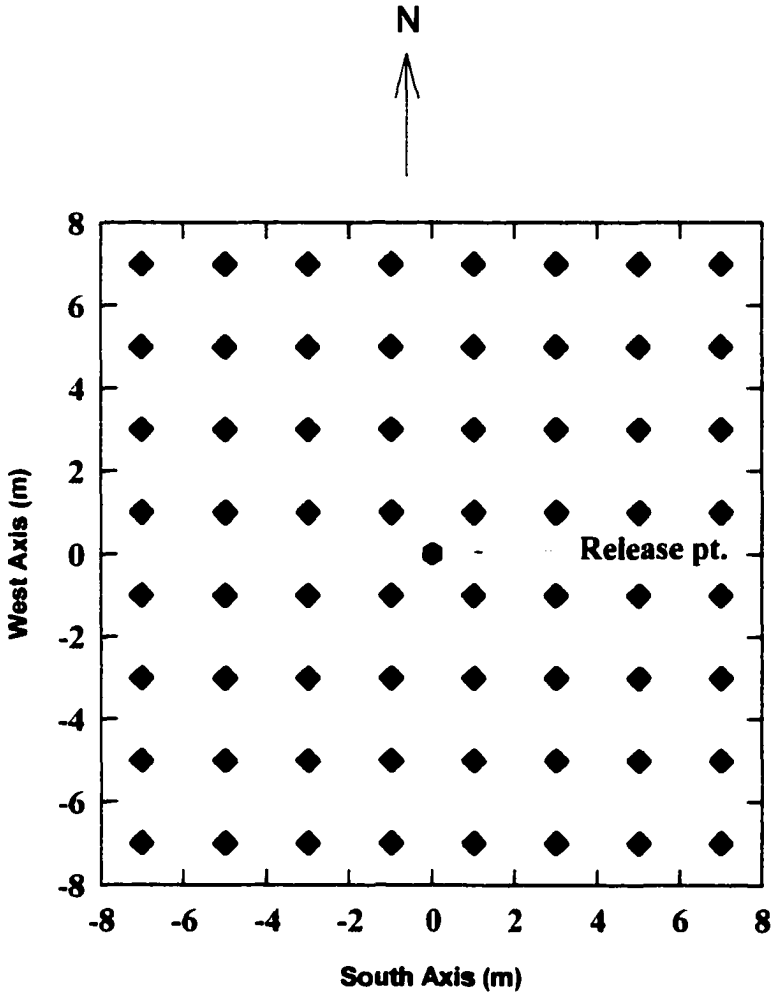


Fig. 4.1. Recapture grid for dispersal experiments. Each ◆ represents one trap. Transect traps not shown see text.

containers that were wrapped with Teflon™ coated tape (SureFire Insect Barrier Tape, Consep, Inc, Bend, Oregon) to protect wasp pupae from ants and other predators. So parasitoids would not desiccate in the sun, each carton was placed under the shade of a cotton plant. Leaves or branches touching the container were removed so they could not form a bridge ants could use to cross into the container.

To estimate the number of parasitoids released, a sample of 500 to 1000 pupae was removed at the start of each experiment and counted to estimate the rate of emergence at the start of the experiment. This was necessary because parasitoids may have emerged during shipment or storage before the start of the experiment. At the end of the experiment, the total number of emerged parasitoids, unemerged parasitoids, and dead adults were counted in each release container. The proportion of parasitoid emergence that occurred during the release was determined by subtraction of the initial proportion of emerged pupae from the final. To calculate the number of wasps that flew out of the container, the sum of the dead adult wasps was subtracted from the sum of emerged parasitoids. This figure represents the number of wasps that survived and flew from the container.

Traps were collected and replaced every 24 hr period for the duration of each release experiment. In a few instances, due to logistical problems, this interval was longer though this would not affect the analysis of recapture data as the analyses used were time integrated techniques independent of the time interval between trap collection periods (see section 4.2.4, Statistical Analyses and Turchin, 1998). Traps were covered in plastic wrap and taken to the laboratory for examination. Traps were examined and all

E. eremicus were counted, sexed, and inspected for the presence of the fluorescent powder with an ultra-violet fluorescent light (General Electric, F8T5-BLB) under a dissecting microscope at 50 x magnification.

To measure marking efficiency, a sample of 100 to 200 marked parasitoid pupae were collected at the start of each experiment. Adults were emerged and examined in the laboratory for the presence of dust. For each release, an estimate of the percentage of marked wasps was calculated and this figure was used to estimate the number of marked wasps released in each experiment.

4.2.2 Effects of Dust on Flight and Longevity

In mark-recapture experiments, precautions must be taken to insure that the marking technique does not differentially affect behavior. Marking sometimes disturbs insects causing them to move greater distances than they ordinarily would (Southwood 1978, Turchin and Thoeny 1993, Turchin 1998). Conversely, the marking may affect their flight ability so they make shorter flights than unmarked individuals (Turchin 1998). To determine if the mark had any affect on wasp flight, 30 marked and unmarked wasps were flown in a vertical flight chamber to measure length of flight and propensity to fly. Flight chamber details are described in Blackmer and Phelan (1991). Five hundred parasitoid pupae were dusted with 1 mg green fluorescent powder (Day-Glo Corp., Cleveland, Ohio) using the dusting technique previously described. These were placed in emergence containers in the laboratory and kept at 22 to 26° C. A control group of 500 unmarked parasitoid pupae was treated in the same manner. Wasps from each group

were collected, sexed, examined for dust, and placed individually into 33 ml plastic vials. Wasps from each group were flown in the flight chamber in alternating order. A vial with a wasp was set on a 16.5 cm high platform in the center of the flight chamber. A 400 W mercury vapor lamp illuminated the chamber from above. Once a wasp initiated flight, the downward airspeed was controlled by opening and closing a damper that maintained an air speed (range 0.5 to 1 m/s) that would keep the wasp at a height of about 0.5 m in the chamber. Flight duration was recorded in seconds until each flight was terminated, when a wasp landed on the wall or the base of the chamber. Flight duration was compared for the two groups using a paired *t*-test for comparison of means (Sokal and Rohlf 1981).

If the marking technique had any significant effects on mortality, then the estimate of the distance marked wasps flew would underestimate the actual dispersal distances compared to an unmarked population of wasps. To determine if there were any mortality effects of dust, 1,000 pupae of *E. eremicus* were split into two groups of 500 each and were put into a 0.95 liter paper ice cream carton with a lid. One group was dusted with 1 mg of green fluorescent dust using the same technique previously described. Both containers were placed into an emergence chamber where the humidity was maintained above 50% rh and temperature was maintained at 27° C. These containers were monitored daily until emergence began. One hundred wasps from each group were aspirated from the lid of the containers and put into 30 dram plastic vials in groups of ten. Each vial had a 15 mm x 30 mm strip of a Kim-wipe™ paper strip (Kimberly-Clark Corp.) that had been coated with a 1:1 honey/distilled water solution.

These honey strips were held in place by the vial cap and pressed to the side of the vial. Once wasps were in the vials, the number and sex of the wasps in each vial were recorded. Vials were placed on a laboratory bench and kept under natural light conditions ≈12L:12D, 25 to 45% RH at 20 to 23° C. Vials were observed each day and the number and sex of living wasps per vial were recorded until all wasps were dead. After all the wasps had died, the dusted group was examined to determine if they had been marked with the dust. Data were examined as the proportion of average daily mortality per group and differences in the resulting mortality curves were compared with a Kolmogorov-Smirnov two sample test (Sokal and Rohlf 1981).

4.2.3 Release Experiments

Release Experiment 1. This release experiment took place between 30 June and 4 July, 1994. The release plot was located near Hyder, Arizona embedded within a 14 ha organically grown field of short staple cotton cultivar ('Deltapine 5415') planted in mid-April and grown according to standard practices for this region. At the time of this experiment the cotton canopy was about 1 m high. Two releases of parasitoids were made three days apart. This was done because the first group of parasitoids had very low rates of emergence. The first group of 353,000 parasitoids was put in the release field on 30 June and was left until 2 July. These were dusted with 15 mg of green fluorescent powder. A second group of 325,000 parasitoids were put in the field on 2 July and were left until the end of the experiment. These parasitoids were dusted with 12 mg of orange

fluorescent powder. Trapping for recapture began with the first release and continued until 4 July for a total of 4 trapping intervals.

In addition to the 64 traps deployed in the release area, traps were also deployed along each of the cardinal directions spaced at 5 m intervals. There were 17 traps on transect lines along each of the four cardinal directions (for a total of 68 transect traps) which extended to a distance of 92 m from the central release point. The purpose of these additional traps was to provide information about longer-range movements outside of the main trapping grid.

Release Experiment 2. This release was conducted between 26 July and 1 August, 1994. The release field was located near Aztec, Arizona within a 16.2 ha block of untreated short stable cotton ("Deltapine 5415") which was grown according to standard practice for the area. At the time of this release the cotton canopy was about 1 m tall. On 26 July, 303,000 parasitoid pupae marked with 11 mg of orange fluorescent dust were put out at daybreak. Sixty-four traps were set out and collected on daily intervals except for the last set which were left out for a three day interval. There were a total of four trapping intervals. In addition to the 64 traps set up in the standard design of the trapping grid, 36 traps set at a 2 m height were placed on the stakes of the interior 12 x 12 m section of the grid. The purpose of these additional traps was to determine if a higher trapping height could be more efficient than the 1 m height (canopy height). Outside of the central trapping area, 5 traps were placed on transects along each of the cardinal

directions (for a total of 20 transect traps) at 5 m intervals which extended to a distance of 32 m from the central release point.

Release Experiment 3. This release was conducted between 22 August and 24 August, 1994. This release field was located near Brawley, California in about 2 ha of untreated short staple cotton ("Deltapine 5461"). At the time this experiment was conducted, irrigation had been terminated and the cotton bolls were ripening. The canopy height was about 0.8 to 1 m. On 22 August, 207,000 parasitoid pupae marked with 7 mg of orange fluorescent dust were put out at daybreak. The grid consisted of 64 traps, which were set out daily for two days. Outside of the central trapping area, 6 traps were placed on transects along each of the cardinal directions (for a total of 24 transect traps) at 5 m intervals which extended to a distance of 37 m from the central release point.

Release Experiment 4. This release was conducted between 28 August and 5 September, 1995 near Hyder, Arizona in within a 20.2 ha block of untreated short staple cotton ("Deltapine 5461"). The canopy height was about 1.1 m. At 10:30 AM, 81,000 parasitoid pupae marked with 5 mg of red fluorescent dust were placed in the center of the release grid. A central release grid of 60 traps was set up in each of the 2 x 2 m cells as in the previous replicate, however in the middle 4 cells of the grid no traps were placed. This change was implemented because of concern that traps too close to the

central release point might catch too many parasitoids depleting the population of dispersing wasps and biasing the recapture results.

Outside of the central trapping area, an additional 48 traps were placed, 12 in each of four arms placed along each of the cardinal directions. These arms were four traps wide by three traps long with traps placed in the center of 2 X 2 m cells with the farthest trap set at 13 m from the release point.

Because parasitoid emergence started slowly, there were only three trapping intervals. The first set of traps was in the field for two days (28-30 August). The second set of traps was in the field for one day (30-31 August). The third set of traps was in the field for five days, from 31 August-5 September.

For all releases, temperature, wind direction and speed were monitored using an on-site weather recording system (Weather Monitor II, Davis Instruments, Hayward Calif.). The wind paddle was mounted on a stake at canopy height in a center row (near the halfway point of one field edge) at a distance of 10 m from the end of the row. Wind speed and direction were monitored at approximately 30 min intervals from 6:00 AM until 3:00 PM. Wind monitoring terminated at 3:00 PM because wasp flight activity appeared to cease at 11:00 AM – 1:00 PM each day (this may have been related to the increase in wind speed which generally occurred after 11:00 AM). At each reading, the highest wind speed reading from the previous monitoring interval was recorded from the system memory. High and low temperature readings were collected from memory each morning at 6:00 AM.

4.2.4 Statistical Analyses

Directional Bias in Recapture Patterns. If there was a directional bias that affected the recapture distribution (effects of wind, orientation towards the sun etc.) a term for directional bias would need to be included in the model of diffusive movement. To determine if there were any directional effects, the average displacement of recaptured wasps was calculated for each of the releases with the following formula (from Turchin and Thoeny 1993):

$$X_j = \frac{\sum_{i=1}^{n} x_i C_{ij}}{\sum_{i=1}^{n} C_{ij}} \qquad (4.1)$$

Where C_{ij} is the cumulative recaptures in trap i over the course of recapture day j, x_i is the x coordinate of the location of trap i and n is the number of traps. The quantity $x_i C_{ij}$ is the sum of the recapture displacements along the x axis of all *E. eremicus* that flew to trap i. This equation gives the average displacement X_j, along the x coordinate. The average displacements along the y coordinate are calculated in the same manner and together give the mean displacement of recaptures within the grid. If there were no directional bias effects on the recapture distribution, there should be no statistically significant differences in average displacement from 0 along the x or y axes. This was tested with a t-test (Sokal and Rohlf, 1981).

Fitting Models of Dispersal. Because males and female wasps may have different dispersal behaviors, an analysis of dispersal by sex was conducted before proceeding to fit the recapture data. The sex ratio (proportion of males) of recaptures per trap per release was calculated. These data were arcsine transformed and fit with linear regression to the distance from release data to determine if there was a significant relationship between sex ratio and dispersal distance. If there are significant effects of sex ratio on dispersal distance then, the male and female recapture data should be fit separately to the dispersal models (Turchin and Thoeny 1993).

The recapture data were fit to three statistical models: two empirical models and a theoretical model based on an assumption of diffusive movement. An exponential model is commonly used to fit dispersal data (Hawkes 1972, Freeman 1977, Taylor 1978, Taylor 1980):

$$N = a \exp [-br] \tag{4.2}$$

Where N is the total number of recaptured wasps dispersing to distance r, and a and b are constants. Eq. 4.2 was linearized with log transformation and fit to the data with linear regression.

Another empirical equation based on an inverse linear relationship between the natural logarithm of the number of wasps recaptured and the square root of the recapture distance was also used to fit the dispersal data (from Hawkes 1972):

$$\text{Log } N = a - b\sqrt{r} \qquad (4.3)$$

Where N is the total number of recaptured wasps dispersing to distance r, and a and b are constants. Empirical models such as Eq. 4.2 and 4.3 fit the typical patterns of dispersal data of declining density with increasing distance from the release point. Both of these equations have been used to model numerous insect dispersal data sets (c.f. Hawkes 1972, Freeman 1977, Taylor 1978, Taylor 1980). These kinds of models, while often providing good empirical fits to dispersal data, have no particular theoretical justification for their use as the parameters have no biological meaning (Kareiva 1981, Rudd and Gandour 1985, Turchin and Thoeny 1993, Turchin 1998).

A diffusion based model was also used to fit recapture data. Diffusion models have been shown to accurately describe the movements of numerous species of insects (Kareiva 1983, Plant and Cunningham 1991, Turchin and Thoeny 1993, Corbett and Rosenheim 1996a, Rudd and McEvoy 1996, Turchin 1998) or they can serve as a null model for more complex patterns of movement (Kareiva 1981, Kareiva 1983, Turchin and Thoeny 1993). Furthermore, the parameters of these models have biological meaning and can be estimated independently from the model by experiments

(Turchin 1998). Following diffusion based model developed by Turchin and Thoeny (1993):

$$N = Ar^{-1/2} \exp[-r/B] \tag{4.4}$$

Where N is the total number of recaptured wasps dispersing to distance r, $A = \alpha N_0(8\pi)^{-1/2}(D^3\delta)^{-1/4}$, is a scale parameter which is proportional to the total number of wasps released times the recapture efficiency (α is a constant of proportionality equal to the recapture efficiency of the trap). Parameter $B = (D/\delta)^{1/2}$ measures the spatial scale of dispersal and is proportional to the square root of the diffusion rate (D = diffusion coefficient) divided by the loss rate (δ). The loss rate measures the rate at which insects are lost from the marked population (Turchin and Thoeny 1993). If the loss rate is constant, a large diffusion rate (D) will result in a population of insects that disperse farther than a population with a lower diffusion rate, a larger loss rate means the average lifetime of dispersing individuals is shorter, resulting in lower rates of dispersal and population spread (Turchin and Thoeny 1993). The diffusion rate (D) also has the advantage in that it is a parameter that can be used to compare to different rates of movement between species (Kareiva 1981, Kareiva 1983, Corbett and Rosenheim 1996a, Rudd and McEvoy 1996).

The diffusion coefficient can be estimated as:

$$D = B^2\delta \tag{4.5}$$

The value for B, the spatial scale parameter, will be estimated from fitting Eq. 4.4 to the recapture data from the different releases. To use Eq. 4.5 to estimate the diffusion rate (D) it will also be necessary to have an estimate for the loss rate (δ) of individual wasps from the dispersing population. The *E. eremicus* loss rate should be dependent on three processes (see Turchin and Thoeny 1993 and Turchin 1998 for examples from other systems): 1) wasps leaving the cotton field by flying up and away from the canopy, perhaps aided by wind; 2) wasps stopping dispersal to oviposit in hosts and not returning to the dispersing population; 3) they may suffer mortality by predation, running out of energy reserves, or by other mortality factors. It is not known how important factor 1 is for the loss of dispersing individuals from a cotton field. However, traps placed at different heights indicated that fewer wasps were captured at 2 m heights than at 1 m heights. For the purposes of this study it will be assumed that the number of wasps lost in this manner is relatively small (a point we shall return to in the Discussion). The importance of factors 2 and 3 are also unknown. The extent to which wasps remove themselves from the dispersing population to search for hosts and do not resume flight, should depend on the host density (and other factors such as egg load) (Kareiva and Odell 1987, Godfray 1994). The extent of predation (and other mortality sources) is also unknown and may vary depending on the field situation. Variation in these factors and how they might effect the outcome of fitting the diffusion model will be considered in the Discussion. For the purposes of these analyses, it will be assumed that the loss rate is constant which summarizes the effects of all of the above processes (Turchin 1998). In this experiment it was not possible to estimate the loss rate (δ) in the field. However, it

is possible to use laboratory flight data to estimate δ. The loss rate (δ) is equal to the inverse of the mean flight time (assuming all other factors that contribute to the loss rate are either constant or negligible; see above) such that $1/\delta$ = the mean flight duration, the time a wasp will stay in the dispersing population until it runs out of energy reserves and δ = 1/mean flight duration (Turchin 1998). Two different estimates of *E. eremicus* mean flight duration within a flight chamber were available and these were used in Eq. 4.5 to estimate the diffusion coefficient. The use of the mean flight duration data to estimate δ was based on the assumption that *E. eremicus* engage in only a single dispersal flight and that once they have flown, they do fly again (an assumption that appears to be correct, D. Bellamy, University of Arizona, personal communication).

Fitting the Diffusion Model. Eq. 4.4 was linearized by taking the natural logarithm of both sides of the equation which resulted in the following:

$$\text{Log}(N) + \text{Log}(r^{1/2}) = \text{Log}(A) - r/(B) \qquad (4.6)$$

The data were fit with linear regression to Eq. 4.6 to estimate parameters B and A. To eliminate the problem of log-transforming zero values, recaptures at equivalent distances were averaged and these average trap recaptures were then log transformed. This technique would only be appropriate if there were no directional component to the recapture distribution (see above, Directional Bias in Recapture Patterns). The normal procedure of adding a small number before log transformation is inappropriate as it

would change the form of equation 4.6 resulting in the loss of biological meaning of parameters A and B in terms of the diffusion rate (Turchin and Thoeny 1993).

To determine which of the three dispersal models provided the best fit to the data, estimated R^2 for each of the releases for each model were compared and analyzed with ANOVA (SAS Institute Inc. 1989). Since each of the models was fitted to Log(N) the R^2 of the models could be directly compared.

Estimation of Dispersal Distances. Using a formula derived by Turchin and Thoeny (1993), and the values of parameter B (estimated by regression of Eq. 4.6 with the data from each release), the following equation was numerically solved using Mathematica (Wolfram 1988) to estimate the median distance that $r_{0.5}$, the radius of a circle that encloses 50% of the dispersers. In a similar manner the radii that enclose 67, 95 and 99% of the dispersal distances were estimated.

$$\frac{\int_0^{r0.5} r^{1/2}\exp[-r/B]dr}{\int_0^{\infty} r^{1/2}\exp[-r/B]dr} = 0.5 \qquad (4.7)$$

4.3 Results

4.3.1 Effects of Fluorescent Dust on Survival and Flight

Because some wasps stuck to the honey-strip and died in the dust survival experiment, there were fewer than 10 healthy wasps per vial at the start of the experiment. There were a total of 86 wasps in the dusted treatment versus 57 wasps in the control treatment. The mean (± SEM) of each group was 6.1 ± 0.7 dusted wasps per vial versus 5.2 ± 0.8 not-dusted wasps per vial. Because I wanted to handle the wasps as little as possible while transferring them to vials, I was unable to sex them beforehand which resulted in a difference in sex ratios between the two treatment groups: 31% male in the dusted treatment versus 54% male in the not-dusted group. Because of this difference in sex ratios, I looked for differential mortality between sexes within each treatment group before proceeding with the analysis of the effects of dust. A Kolmogorov-Smirnov two-sample test revealed that there was no effect of sex on mortality for the dusted group ($D = 45.4$; n_1& $n_2 = 9$; $P > 0.1$); or for the control group ($D = 6.1$; n_1& $n_2 = 6$; $P > 0.1$).

Analysis of the effects of dust with the Kolmogorov-Smirnov two-sample test revealed that there was no significant difference between mortality of the dust treated and control groups of wasps ($D = 16.2$; $n_1 = 7$, $n_2 = 8$; $P > 0.1$, Fig. 4.2). Examination of wasps in the dust treated group revealed all were marked, with dust most often found on the wing fringe hairs. The most long-lived wasps were 2 females and 1 male in the dust-treated group that survived 13 days (Fig. 4.2).

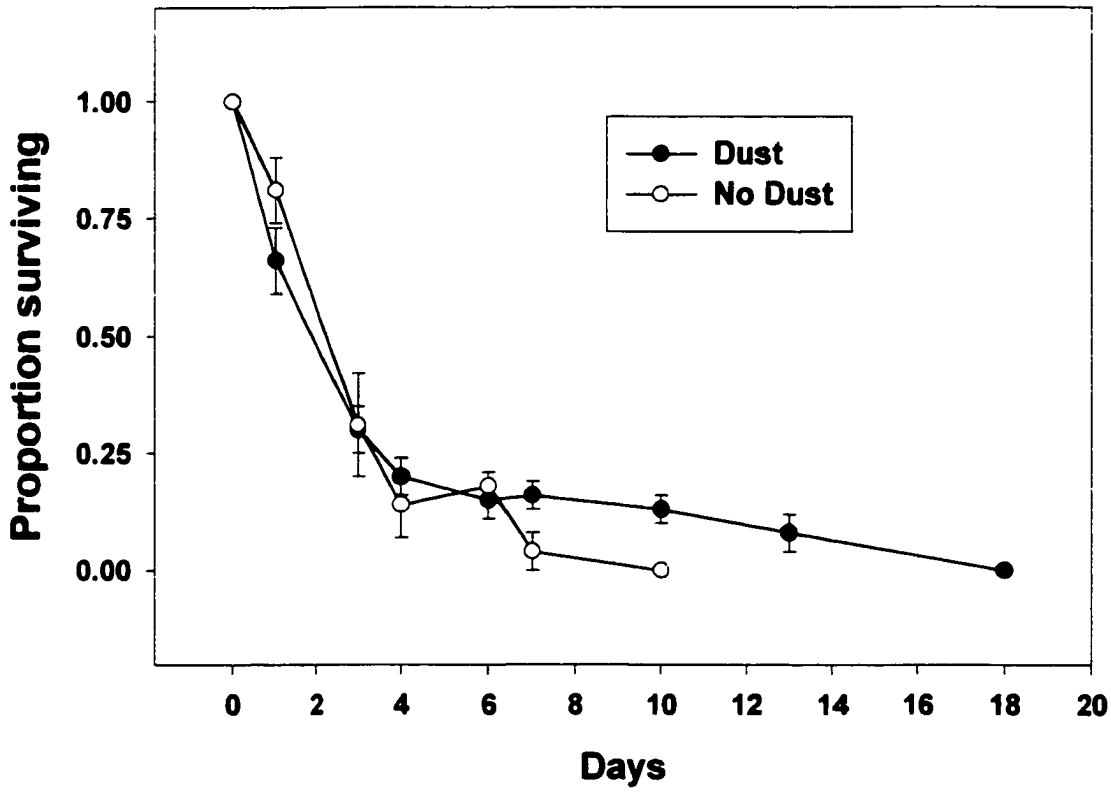


Fig.4.2. Effect of dust on *E. eremcius* survival. Error bars are one standard error of the mean.

The mean (± SEM) flight time of dusted wasps was 139.5 ± 28 s versus 134.2 ± 31 s for the non-dusted group. There were no significant differences in the mean flight time between these groups ($t = 0.02$, df. = 28 , $P > 0.9$).

4.3.2 Mark Recapture Experiments

The mean (± SEM) wind direction for release 1 was 209 ± 8 degrees (SSW). The mean (± SEM) wind speed was 5.0 ± 0.7 km/h, with a range of 0 to 13 km/h. Wind speeds were low in the mornings (readings before 8:00 AM were generally 0 to 1 km/hr) and tended to increase after 11 AM. For each 24 hr period, the maximum wind speeds occurred between 1:30 to 6:30 PM. Temperatures ranged from 19 to 41° C. For release 2, the mean (± SEM) wind direction during the course of the experiment was 221 ± 21 degrees (SW). The mean (± SEM) wind speed was 4.2 ± 0.4 km/h, with a range of 0 to 19 km/h. As in release 1, wind speeds were lower in the morning (0 to 2 km/hr, before 8:00 AM). The maximum high winds for each 24 hr period occurred between 11:50 AM to 10:00 PM. Temperatures ranged from 22 to 45° C. For release 3, the mean (± SEM) wind direction during the course of the experiment was 286 ± 12 (WNW) degrees. The mean (± SEM) wind speed was 3.8 ± 1.7 km/h, with a range of 0 to 8 km/h. Wind speeds were between 0 to 3.2 km/h before 7:30 AM and increased to speeds up to 8 km/hr after 7:30 AM. On each day, the maximum wind speeds occurred between 10:30 AM to 9:30 PM. Temperatures ranged from 24 to 46° C. For release 4, wind and temperature measurements were only collected for the first three days of the experiment as the final trapping interval was for five days and traps were left unattended in the field

during this time. Therefore, no weather monitoring was conducted during this last period and the following data apply only to the first three days of the trapping internals. The mean (± SEM) wind direction during this time period was 158 ± 23 degrees (ESE). The mean (± SEM) wind speed was 1.1 ± 0.7 km/h, with a range of 0 to 13 km/h. Wind speed before 8 AM were between 0 to 2 km/h, increasing afterwards. The peak wind speeds occurred between 9:30 AM to 7:00 PM. Temperatures ranged from 22 to 42° C.

Examination of laboratory emerged wasps from the samples of marked pupae collected from each release indicated a marking efficiency with a mean (± SEM) of 98 ± 1% marked wasps (n=5).

4.3.3 Wasp Emergence, Mortality and Recapture Patterns.

Although efforts were made to ensure that wasps were not coated with too much dust, some of the adults (especially those that emerged from the bottom of a pile of pupae) were too thickly coated with dust to fly and were unable to leave the container. Wasps that are able to walk up the sides of the container are capable of flight and all of these left the container after a period of grooming.

For release 1, the percentage of emergence during the trapping interval was 12 and 34% for the orange and green marked wasps respectively. Note that the emergence rates reported here (and for all releases) are only for the interval of time that parasitoids were left in the field and therefore are lower than if the parasitoids had been left in the field until all emergence was complete. The survival rates (the percentage of emerged wasps that were able to leave the container) of emerged orange and green dust treated wasps

were 8.4 and 30% respectively which resulted in a release of 1,904 orange and 8,775 green marked wasps (for a total release of 10,679 wasps, Table 4.1). Of these, a total of 349 marked wasps and 154 unmarked wasps were captured over the four days of trapping (Table 4.1, Fig. 4.3). Because the recapture pattern of unmarked wasps closely paralleled the recapture pattern of marked wasps (fig. 4.4), I hypothesized that the high number of unmarked wasp captures were released wasps that had lost the mark in the field. This hypothesis was tested by comparing the distribution of the percentage of recaptures of marked and unmarked wasps per trap with a Wilcoxon two-sample test (PROC NPAR1WAY, SAS Institute Inc. 1989). The Wilcoxon test is an appropriate test to use for these data because it is sensitive to differences in location between distributions (Sokal and Rohlf 1981). The two distributions were not statistically different (Z = -1.1799, df = 64, P > 0.238) therefore, both marked and unmarked wasps were included in the recapture totals. The overall recapture percentage of released wasps was 4.7%. Three female wasps were recaptured on the transect traps outside of the main trapping grid, at 12, 32 and 82 m from the central release point.

For release 2, the percentage of emergence over the trapping interval was 28%. The survival rate of emerged wasps was 27%, which resulted in a release of 23,000 green marked wasps. A total of 291 marked wasps and 136 unmarked wasps were recaptured over six days of trapping (Table 4.1, Fig. 4.5). As in release 1, the pattern of recaptures of unmarked wasps closely coincided with the pattern of marked wasp recaptures (Fig. 4.4). Testing for differences between recapture distributions of marked and unmarked

Table 4.1. Summary of *E. eremicus* releases : number of adults released and recaptured; and fits to various movement models.

		Recaptures				Estimates‡			R^2 of models‡‡		
Release	Date*	Released	Marked	Not marked	Used†	*A*	*B*	*P*	Dif-fusion	Expo-nential	Neg. Sqr-root
1	Jun 1994 (4)	10,679	348	155	503	55.3	2.00	0.0003	0.850	0.780	0.940
2	Jul 1994 (4)	23,000	273	145	418	43.4	3.70	0.0001	0.934	0.930	0.981
3	Aug 1994 (2)	3,774	22	97	22	3.64§	2.71§	0.16	0.714	0.792	0.890
4	Aug 1995 (3)	7,564	34	113	34	9.68	2.65	0.0031	0.851	0.870	0.898

* Numbers in parentheses are the number of trap collection intervals.

† In releases 1 and 2, both marked and not marked wasp recaptures were used in model fitting, in releases 3 and 4 only marked recaptures were used (see 4.3.3 Wasp Emergence, Mortality and Recapture Patterns).

‡ Parameter values and *P* values are from fitting the linearized form of the diffusion model, Eq. 4.6.

‡‡ R^2 of models are for: linearized form of the diffusion model, Eq. 4.6; exponential model, Eq. 4.2; negative square-root model, Eq. 4.3.

§ Not significant, parameter values not used in estimating median dispersal distance or *D*.

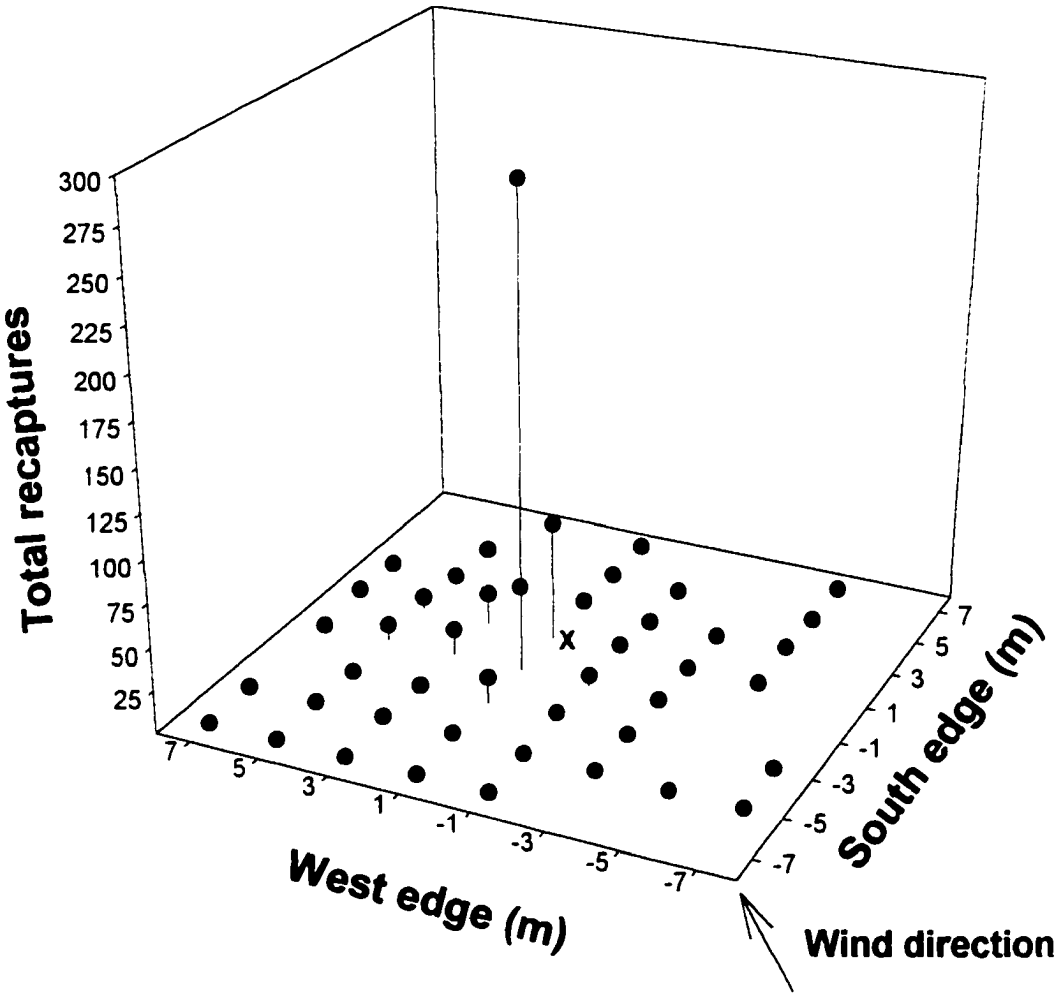


Fig. 4.3. Cumulative *E. eremicus* recaptures for release 1 over four trapping intervals (30 June – 4 July, 1994) of the dispersal experiment. Release point indicated by **X** (at 0,0); a trap with recaptures by ●.

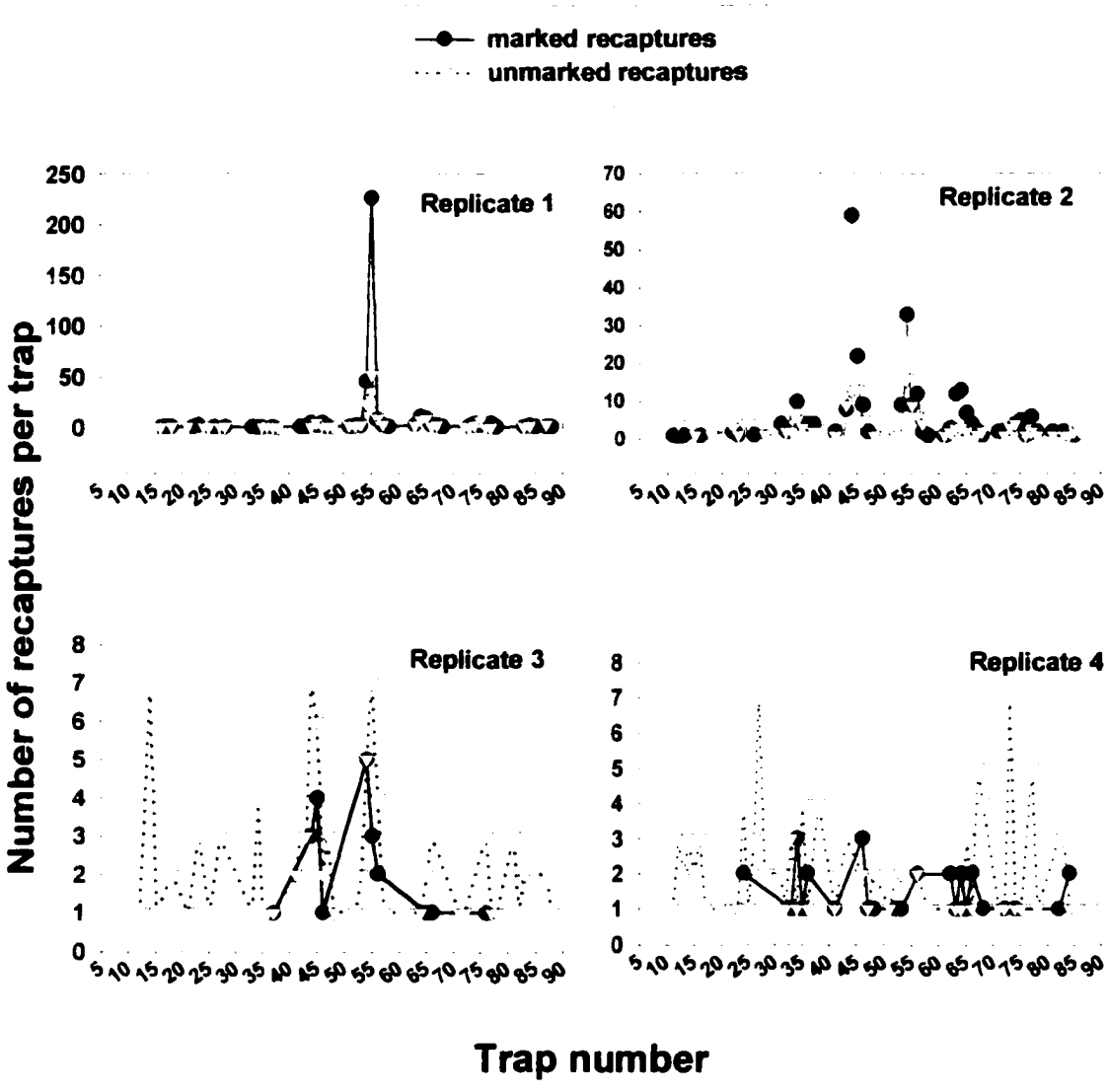


Fig. 4.4. Recapture distributions of marked and unmarked *E. eremicus* within the central trapping grid for the four releases of the dispersal experiments in 1994-95. Trap counts represent the cumulative number of recaptures over the entire trapping interval of each experiment. As a point of reference, traps numbered 45, 46, 55, and 56 are those closest (1 m) to the central release point.

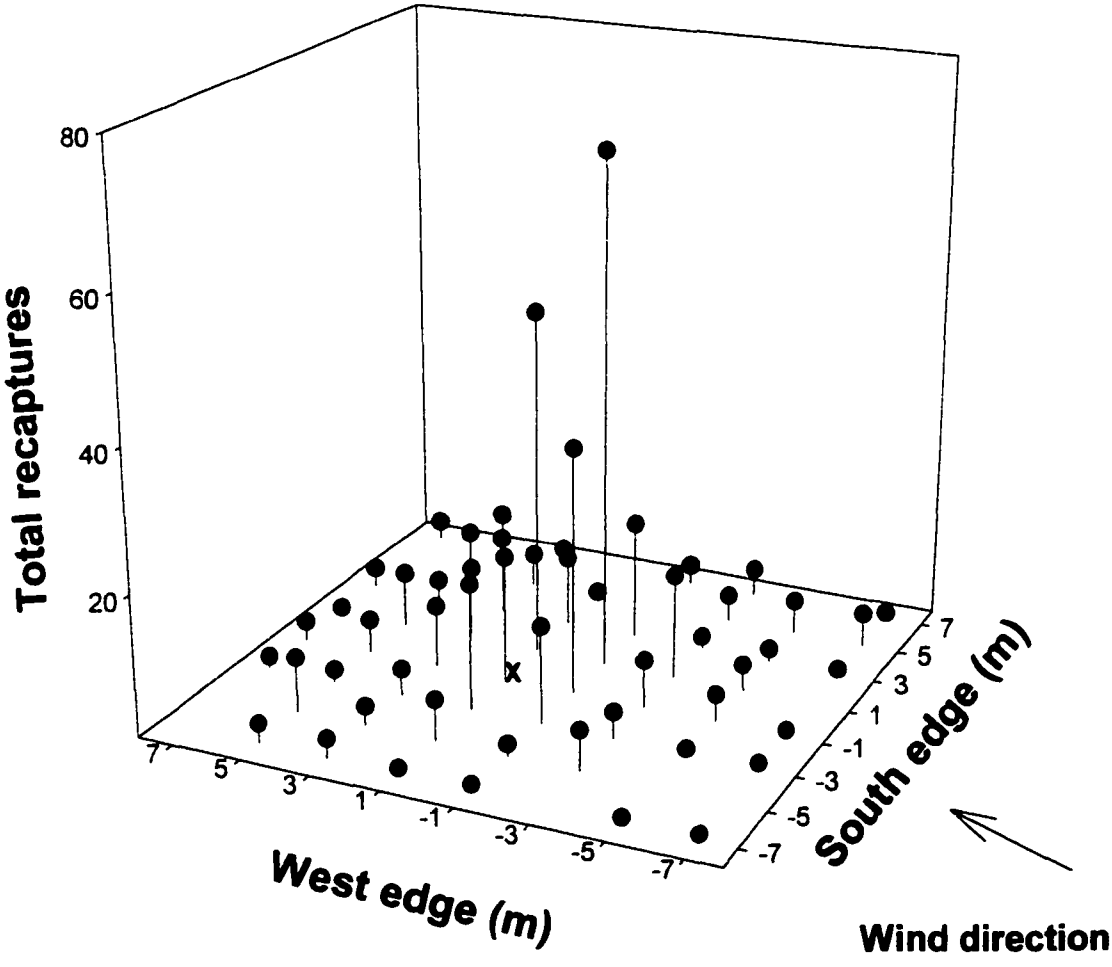


Fig. 4.5. Cumulative *E. eremicus* recaptures for release 2 over four trapping intervals (26 July – 1 August, 1994) of the dispersal experiment. Release point indicated by **X** (at 0,0); a trap with recaptures by ●.

wasps with the Wilcoxon two-sample test indicated that there were no statistically significant differences in recapture patterns between the two groups (Z = -0.4183, df = 64, P > 0.676). So both marked and unmarked wasps were included in the recapture totals, which resulted in a recapture percentage of 1.9%. The 1 m trap height caught significantly more wasps than the 2 m high trap: 6.4 wasps per trap versus 2.0 wasps per trap (t= 2.69; df = 94; P = 0.0085). Two wasps were recaptured on the transect traps outside of the main trapping grid, a male at 12 m and a female at 27 m from the central release point.

For release 3, the percentage of emergence over the two days of the trial was 14%. The survival rate of emerged wasps was 15%, which resulted in a total release of 3,774 wasps. A total of 22 marked wasps and 97 unmarked wasps were recaptured over two days of trapping (Table 4.1, Fig. 4.6). There was no pattern of recaptures of unmarked wasps that coincided to the recapture pattern of marked wasps (Fig 4.4). Distributions of marked and unmarked wasp recaptures were compared with a Wilcoxon two-sample test and the two distributions were statistically different (Z = -4.3828, df = 64, P > 0.0001). Therefore, only the marked wasps were included in the recapture totals, which resulted in an estimated recapture percentage of 0.6%. Two female wasps were recaptured on the transect traps outside of the main trapping grid at 32 and 37 m from the central release point.

For release 4, the percentage of emergence over the trapping interval was 20%. The survival rate of emerged wasps was 46%, which resulted in a release of 7,564 wasps. Of these, a total of 34 marked and 113 unmarked wasps were recaptured (Table 4.1, Fig.

4.7). There was no pattern of unmarked recaptures that coincided with the marked wasp recapture pattern (Fig. 4.4) and a Wilcoxon two-sample test indicated that the two recapture distributions were statistically different (Z = -2.5178, df = 64, P > 0.0118). Therefore, these captures were not included in the recapture analysis and the overall recapture percentage of marked wasps was 0.4%. There were no marked wasps recaptured on the transect traps outside of the main trapping grid.

Most marks were easily detected by viewing the wasps at 50X. For some specimens it was necessary to darken the room and view the wasps under the microscope holding an ultra-violet fluorescent tube close to the specimen in order to detect the mark. The number of dust particles found on a marked wasp ranged from 1 to 10 though most wasps were marked with 1 to 2 particles, which were most often found near a leg or wing coxa, attached to the fringe hairs of a wing, or dorsally on the pronotum or mesoscutum. In some instances, the mark was not found on the wasp but was detected nearby on the trap (within 1 to 2 mm). These individuals were assumed to be marked if no other wasps were nearby as the dust particle can migrate a small distance within the glue of the trap during handling.

For each release, there was a lot of variation in cumulative recaptures with a very few traps capturing many wasps (traps closest to the release point) and many traps with 1 or 0 recaptures (figs. 4.3-4.7). The recapture farthest from the release point was a female at 82 m after 1 day (release 1), so female wasps are capable of flying at least that far in a day.

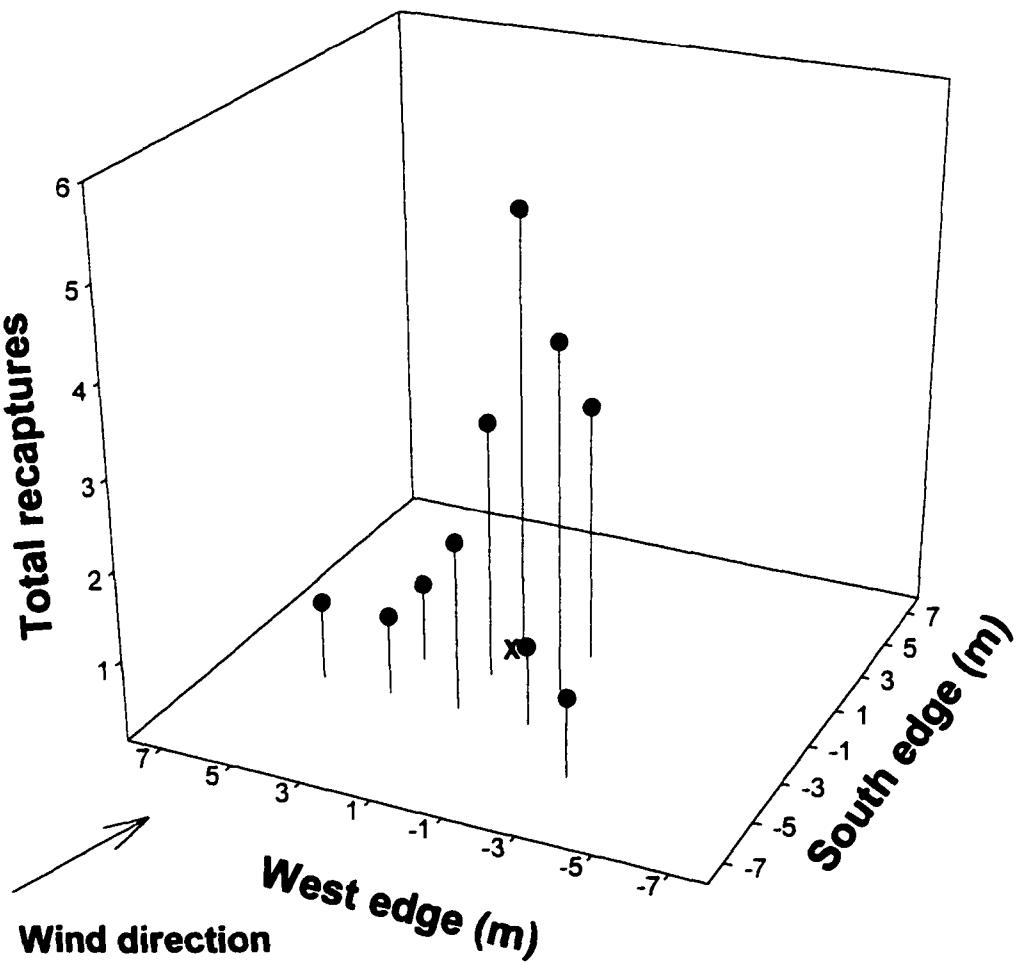


Fig. 4.6. Cumulative *E. eremicus* recaptures for release 3 over two trapping intervals (22–24 August, 1994) of the dispersal experiment. Release point indicated by **X** (at 0,0); a trap with recaptures by ●.

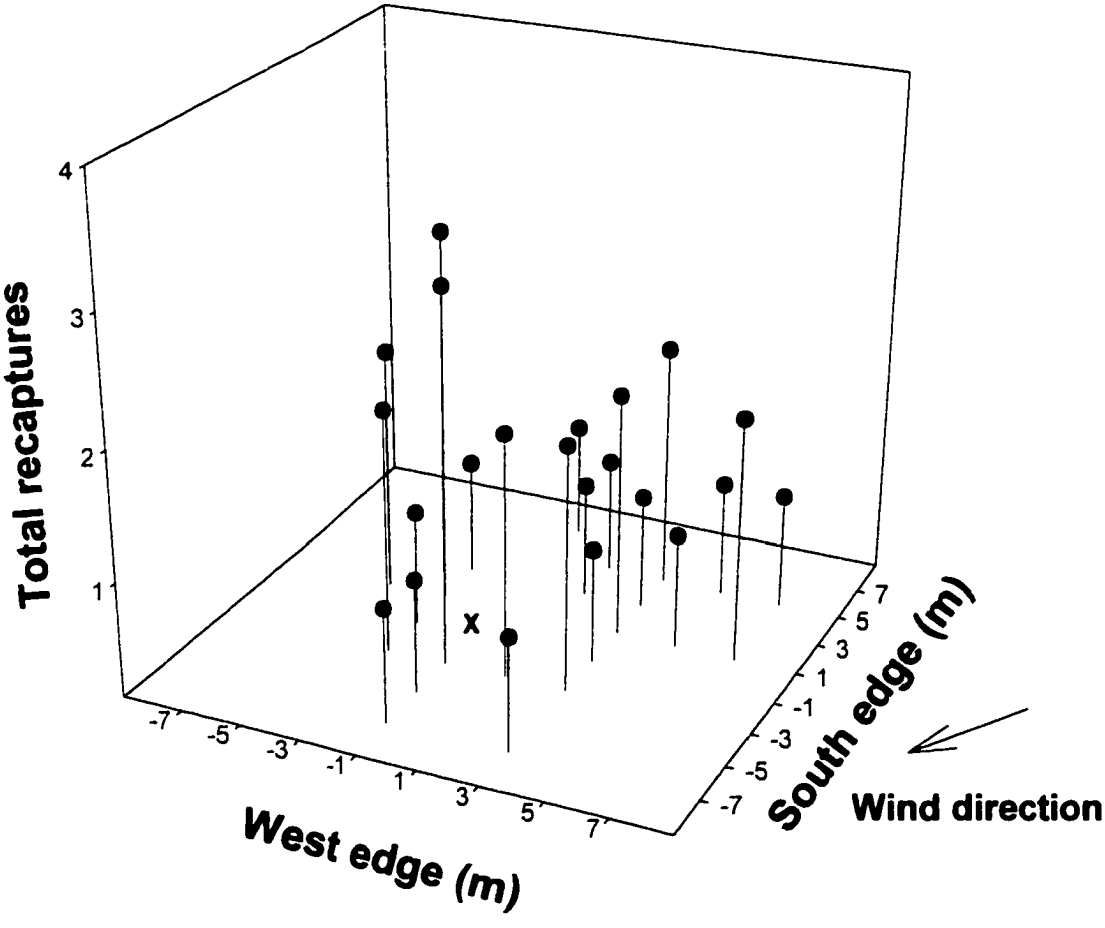


Fig. 4.7. Cumulative *E. eremicus* recaptures for release 4 over three trapping intervals (28–August – 5 September, 1995) of the dispersal experiment. Release point indicated by **X** (at 0,0); a trap with recaptures by ●.

Analysis of mean recapture displacements (with Eq. 4.1) shows there were no statistically significant differences from 0,0 (Fig. 4.8)(release 1, x displacement, $t = 0.18$, df = 3, $P > 0.8$, y displacement, $t = 0.12$, df = 3, $P > 0.8$; release 2, x displacement, $t = 0.59$, df = 3, $P > 0.5$, y displacement, $t = 0.39$, df = 3, $P > 0.5$; release 3, x displacement, $t = 3.34$, df = 1, $P > 0.10$, y displacement, $t = 12.2$, df = 1, $P > 0.05$; release 4, x displacement, $t = 0.58$, df = 1, $P > 0.50$, y displacement, $t = 0.78$, df = 1, $P > 0.50$). These results show that there was no directional bias in recapture patterns and allowed the fitting of the data to the diffusion model without including a term for drift.

The mean (± SEM) sex ratio of recaptures for all four releases was 31 ± 5% male. Linear regression of sex ratio on distance from release revealed no significant effect ($F = 0.15$, df = 1, 29, $P > 0.7$) suggesting that there were no differences in dispersal between males and females. Therefore, for fitting the recapture data, the sexes were not analyzed separately. To determine if release sex ratios differed from recaptured sex ratios, 100 to 300 adult pupae per release were set up in the laboratory for adult emergence to estimate sex ratios. This sex ratio was 48 ± 8% male (n = 6). A Chi-square test (Sokal and Rohlf 1981) indicated that there was no significant difference between released and recaptured wasp ratios ($\chi^2 = 10.0$, df = 9, P = 0.350).

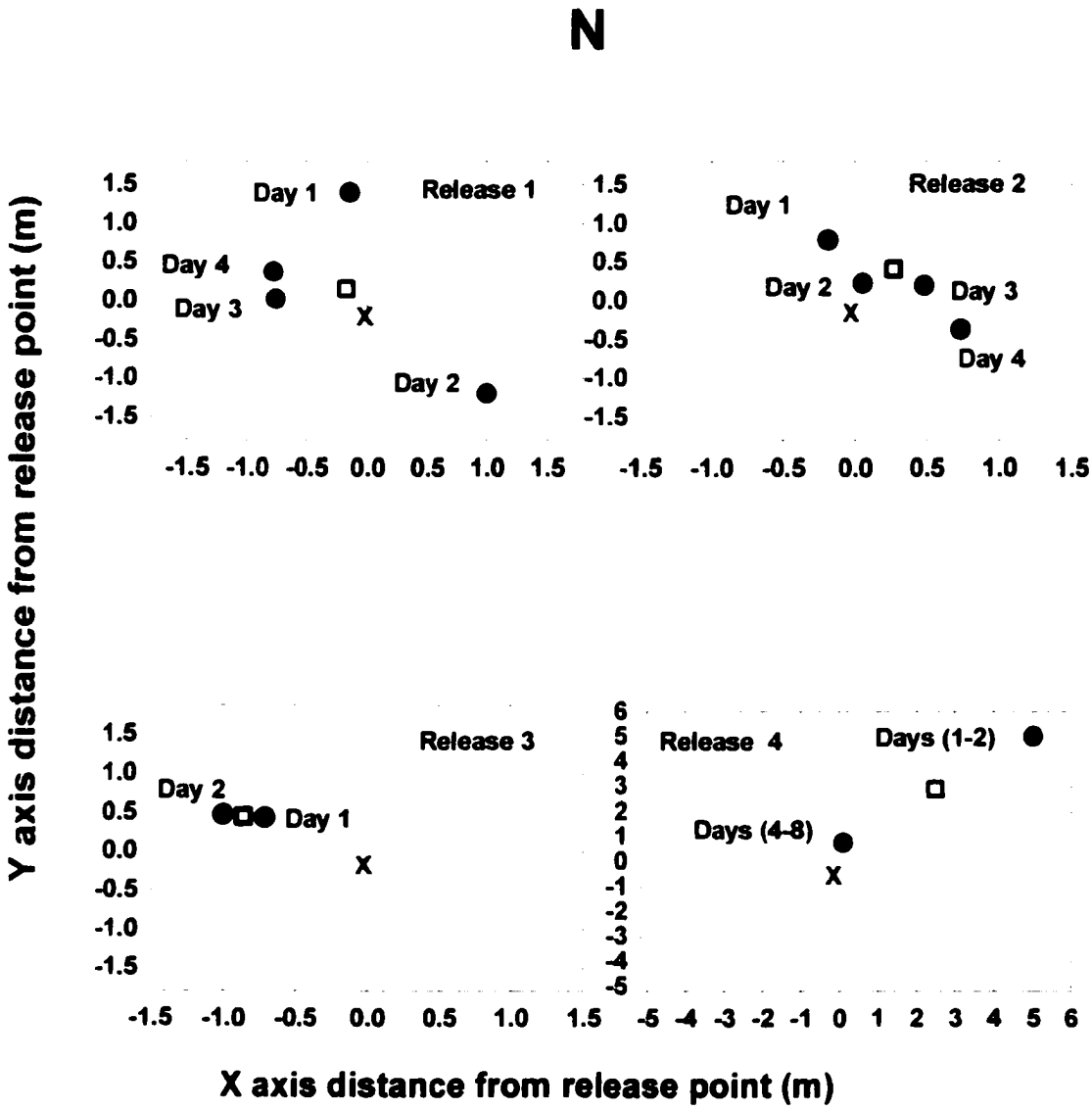


Fig. 4.8. Daily and average displacements of *E. eremicus* recaptures for each release of the dispersal experiment. Central releases point (at, 0,0) marked by **X**. Average displacement of recaptures marked by **□**.

4.3.4 Fitting Recapture Data

Linear regressions of recapture data with the diffusion model provided significant fits to the data with high coefficients of determination (R^2) for all of the releases except release number 3 (Table 4.1, Figs. 4.9, 4.10). The regression of recaptures for release 3 was not significant ($P > 0.16$) so the parameter estimate for B from this release was not used in estimating its mean value. For release 3, there were only 22 recaptures on 10 traps at just 4 distances (Figs. 4.6, 4.9, 4.10) , which appears to have resulted in too few points for fitting the model (Figs. 4.9 & 4.10). The best fit to the data was provided by the negative square-root model (Eq. 4.3) that had slightly higher R^2 values than the diffusion model (Eq. 4.4), and the exponential model (Eq. 4.2), but differences in R^2 were not statistically significant (Table 4.1, ANOVA, $F = 1.66$, df = 2, $P = 0.27$).

Fitting the data to the recapture distribution predicted by the diffusion model indicates that the model fits the data well with no serious outliers with the exception of release 1, where there appears to be an outlier at the 1 m trap distance (Figs. 4.9 & 4.10). Removing recaptures at the 1 m distance and refitting the model provided a better fit (R^2 of 0.93 versus 0.85). During the course of conducting these experiments, I observed that the wasps tended to fly out from the release area without a definite directional path, increasing their distance with ever widening circular movements. I speculated that the 1 m traps might be capturing a disproportionate share of wasps because of this initial behavior. To eliminate the possibility of "over-trapping" too near the release point (Turchin 1998), I removed the 1 m traps for release 4 to see if a better fit could be

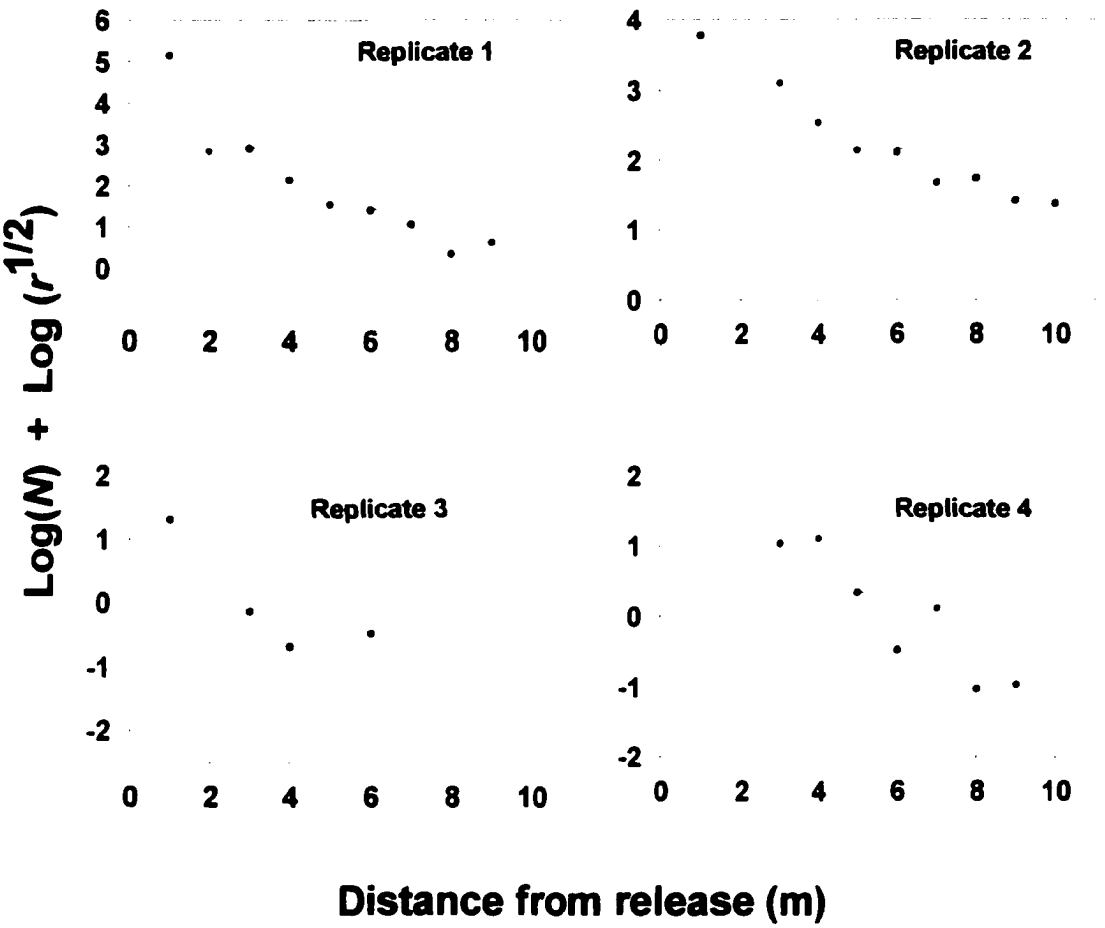


Fig. 4.9. Plot of Log(N) + Log($r^{1/2}$) versus distance from release from linear regressions of Eq. 4.6, Log(N) + Log($r^{1/2}$) = Log(A) $-r/(B)$, for estimation of parameters A and B. The solid lines are the predicted values for the regression equation, data points are the values of Log(N) + Log($r^{1/2}$) where N is the average number of *E. eremicus* per trap at distance r from the release point. See text for details.

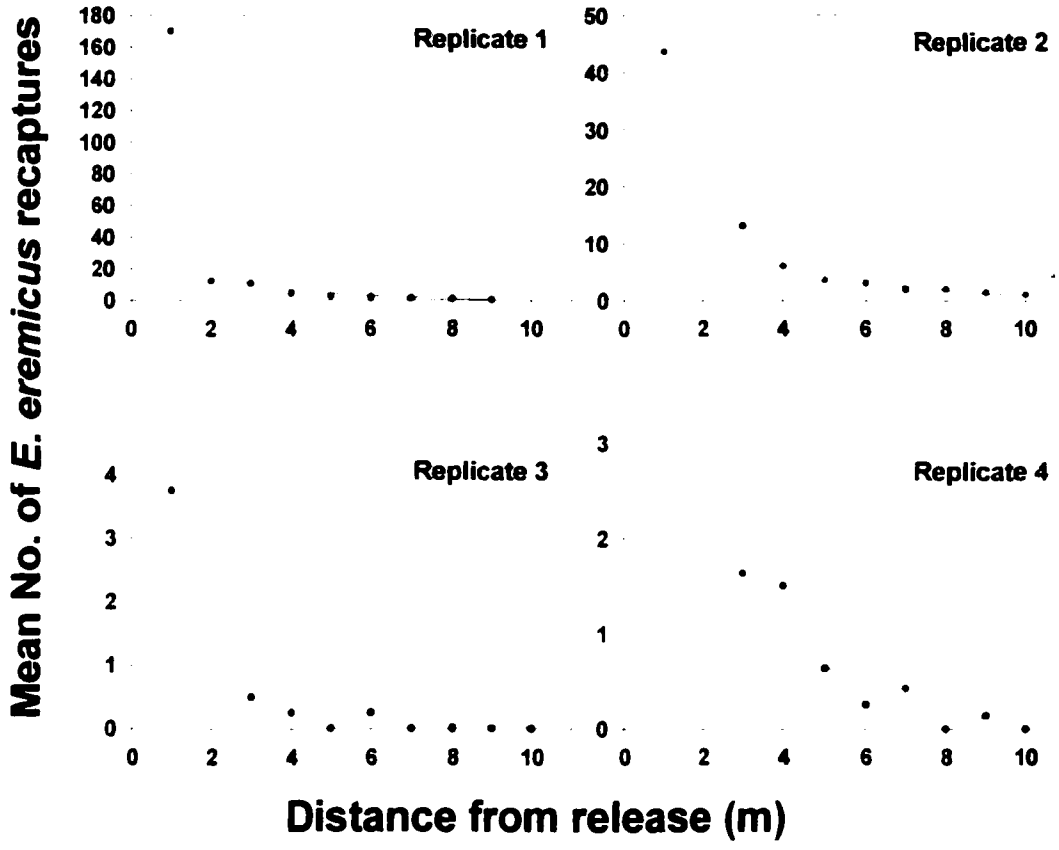


Fig. 4.10. Recapture with distance curves for each release. The data points are the average recaptures at each distance. The solid lines are fitted curves to Eq. 4.3. using the parameter estimates for A and B for each release.

obtained. If fewer wasps were recaptured in the center of the grid, longer distance dispersers may have had a chance to disperse farther changing the form of the dispersal curve. This did not appear to be the case as the fit to the model for release 4 was similar to the other releases (Fig. 4.9, Table 4.1).

4.3.5 Estimating Dispersal Distances with the Diffusion Model

Using the estimates of B for releases 1, 2, and 4, within field dispersal distances were calculated with Eq. 4.7. An estimated one half of the released wasps traveled 4.4 m or less in a time interval of 4 to 8 d (Table 4.2), 95% of the wasps traveled 14.8 m or less during the same time interval (Table 4.2). For all releases, an estimated 1 to 11 % of the wasp population left the trapping arena (Table 4.2).

4.3.6 Estimating the Diffusion Coefficient

Two different laboratory flight chamber estimates of mean flight time were available for estimating the loss rate (δ) in order to estimate the diffusion coefficient (D) with Eq. 4.5. (flight chamber data from two sets of experiments with wasps flown in the D. Byrne laboratory, University of Arizona by J. Blackmer and D. Cross and D. Bellamy and D. Byrne, flight chamber details described in Blackmer and Phelan 1991). The two values available were of mean values for flights by both sexes: 11.7 and 20.6 min. Wasps were allowed to fly until they rested for one min after landing before terminating the observations. Wasps would often resume flight after stopping briefly, but wasps do not appear to fly again after stopping flight for 1 min (D. Bellamy, University of Arizona,

Table 4.2. Estimates of the radius of a circle enclosing various proportions of dispersal distances of *E. eremicus* after point release in a cotton field. Calculated with Eq. 4.7 using estimates of *B* from each release.

Release	Trapping interval (d)	Proportion enclosed	Estimated Radius (m)	95% confidence interval
1	4	0.50	2.35	[2.05-2.80]
		0.67	3.40	[2.96-4.10]
		0.95	7.80	[6.80-9.30]
		0.99	11.80	[9.50-14.0]
2	5	0.50	4.40	[4.00-5.00]
		0.67	6.40	[5.80-7.20]
		0.95	14.80	[13.50-16.20]
		0.99	22.00	[18.70-23.90]
4	8	0.50	3.10	[2.65-3.90]
		0.67	4.55	[3.80-5.60]
		0.95	10.50	[8.70-12.80]
		0.99	15.00	[12.90-18.80]

personal communication). Using the mean flight times of 11.7 and 20.6 min, with the estimates of B (from each of the three statistically significant releases, Table 4.1), the respective estimated means (± SEM) for D are 0.71 ± 0.25 and 0.40 ± 0.14 m^2/min.

4.4 Discussion

4.4.1 Mobility of *E. eremicus*

Though it is known that at least one of the released wasps dispersed to a distance of 82 m in 1 day, the majority only moved a few meters or less. To obtain accurate estimates when fitting recapture data to diffusion models, it is important that the proportion of insects that move beyond the recapture grid is small, otherwise parameter estimation could lead to an underestimate of the extent of dispersal (Kareiva 1983, Turchin 1998). It appears that this was not a concern as the calculations of the median dispersal distances indicate that between 1 to 11% of the wasps moved beyond the recapture grid which requires extrapolation of the distance traveled for only a small proportion of the released wasps. Extrapolation of the recaptures with distance curve beyond the recapture grid can be justified because the formula for the diffusion model is derived from a consideration of the mechanisms determining the shape of the recaptures with distance curve and does not rely on fitting the data to an empirical model (Turchin and Thoeny 1993). It should be noted that these calculations are dependent on the observed recapture pattern, if very many wasps (greater than the estimated 1 to 11%) flew up and beyond the recapture grid escaping detection then the experiments may have underestimated the true spatial scale of dispersal. It is possible for a population of insects to be subdivided into two groups that have two distinct dispersal behaviors (see Byrne et al. 1996), one engaging in trivial or foraging flight the other in longer distance migratory flight. It is unknown if such behavior occurs in *E. eremicus* , it would have

been necessary to have a more extensive trapping grid at greater distance from the release point as well as traps outside of the field to monitor for longer distance fliers.

The estimated median dispersal distance of between 2.4 to 4.4 m (in 4 to 8 d) suggests that *E. eremicus* moves only a short distance within fields after release. The diffusion rate (D) was estimated to be between 0.40 – 0.71 m^2/min. In order for this estimate to be considered accurate, there are a number of assumptions that must be made. These are: 1) that an insignificant number of wasps engaged in long distance flight by flying up and away from the grid; 2) that high levels of predation or other sources of mortality did not significantly affect δ; 3) that the flight chamber estimates are accurate representations of the time that wasps fly in the field and that second long flights in the field are as rare as they appear to be in the flight chamber (D. Bellamy, University of Arizona, personal communication).

For the first assumption, the proportion of wasps that engaged in long range flight was not estimated. However, lower recaptures on the 2 m versus 1 m traps suggests that relatively few wasps were flying above the canopy. Also there were few recaptures outside of the grid on the transect traps though the recapture frequency of these traps was expected to be low due to the area dilution effect (Turchin 1998). There is some evidence from flight chamber studies that some wasps may fly as long as an hour or more (D, Bellamy, University of Arizona, Tucson, AZ and J. Blackmer, USDA-ARS, Western Cotton Research Laboratory, Phoenix AZ, personal communication) which probably translates into a dispersal distance much greater than the ones observed in this study. However, it is not clear what kind of flight *E. eremicus* engages in while in the flight

chamber. In the flight chamber without hosts or plants, *E. eremicus* may undergo longer flights than in a field with both plants and hosts present. If such differences in flight behavior exist, flight in the flight chamber may represent migratory flight (defined as directional movement unresponsive to environmental cues such as hosts or plants, Kennedy 1985, Dingle 1996) while in a cotton field, they may engage in trivial or foraging flight (responsive to resource cues such as hosts or plants, movement is halted when resources are encountered, Dingle 1996). While *E. eremicus* with previous host and plant experience briefly respond to a green light cue (an environmental cue that simulates a whitefly host plant) in the flight chamber (J. Blackmer, USDA-ARS, Western Cotton Research Laboratory, Phoenix AZ, personal communication) it is not known if *E. eremicus* responds to such cues in the field. Though laboratory assays have shown that *E. eremicus* responds to cues such as whitefly honeydew on a leaf (arrestment responses, Shimron et al. 1992, as *Eretmocerus* sp. since confirmed to be *E. eremicus*, Dan Gerling, Tel Aviv University, Israel, personal communication) and attraction to host volatiles has been shown for a closely related species (*E. mundus*, Heinz and Parrella 1998). Certainly the laboratory studies represent a different environment from what is found in the field, where both plants and the presence of hosts are expected to affect movement rates. A refinement of these experiments to estimate within field dispersal rates would include a much more extensive examination of the vertical flight distribution of released wasps to estimate what proportion of the population engages in flights above the canopy. Using a more extensive trapping grid at greater distance could help confirm or refute that only a few wasps flew more than a few meters.

For assumption 2, it is not known how much predation occurs on adult *E. eremicus* in a field situation though at times, in at least some systems, predation of aphelinids can be significant (in almond orchards predation on *Aphytis* spp. can be a significant mortality source, see Hempel et al. 1996). However, in the present experiment, since large numbers of parasitoids were released and significant numbers were recaptured, it seems unlikely that high enough levels of predation could have occurred to significantly change the loss rate (δ). For the last assumption, as noted above, flight chamber estimates of flight duration may not represent a realistic value for the field. Wasps in the field may be able to fly longer times than those in the flight chamber because host feeding (for females) and feeding on honeydew and other sugar sources available in the field may extend the capacity for flight. However, it is believed that the flight times from the flight chamber represent the average flight time that wasps can undergo in the field as few wasps engaged in any significant flight when attempts were made to fly them again. Though this must be considered a tentative conclusion, as more attempts of flying wasps for multiple flights are needed to confirm this (D. Bellamy, University of Arizona, personal communication).

Given the uncertainty about the above assumptions, the estimated range of values of the diffusion rate (D) of 0.40 – 0.71 m^2/min must be regarded as tentative. A sensitivity analysis of the effects of variation in the loss term (δ) on values for D in Eq. 4.5 shows that varying δ by ± 30% (using both of the loss terms based on 11.7 and 20.6 min flight times) results in a range of values for D between 0.28 – 0.93 m^2/min, suggesting that relatively large changes in δ has a small effect on estimates of D.

Compared to values of the diffusion rate, D that have been estimated for other insects, *E. eremicus* appears to be at the lower end on the scale of mobility. For example, southern pine beetle, *Dendtroctonus frontalis*, has a value of D three orders of magnitude greater at 1500 m^2/min (Turchin, 1998). The only other estimate of D for a parasitoid of which I am aware is for *Anagrus epos* Girault, (Corbett and Rosenheim, 1996b) which has an estimated value of D of 523 m^2/d. Unfortunately, due to differences in experimental design, it is not possible to compare Corbett and Rosenheim's (1996b) estimate of D with the value for *E. eremicus* (the estimate for *A. epos* is in units of m^2/d versus m^2/min for *E. eremicus*, without estimates of how long *A. epos* can fly in a day, it is not possible to convert these values to the same units). However, it is possible to compare the estimated median dispersal distances of both species. From Corbett and Rosenheim (1996b), I estimated that the median dispersal distance of *A. epos* was 92.3 m/2d (from Fig. 7, Corbett and Rosenheim 1996b) versus 2.4 to 4.4 m (in 4 to 8d) for *E. eremicus*, suggesting that the dispersal rate of *A. epos* is 42 to 154 times more rapid (converting both estimates to a per day basis gives this range of values).

It should be kept in mind that these estimates for median dispersal distances and D probably represent non-migratory foraging movements within the relatively homogenous environment of a cotton field with hosts present. Movement outside of the field, in different crops, without hosts or with greater host density may result in quite different estimates of movement. It is unknown whether *E. eremicus* undergoes migratory movement though it is suspected for a few other species (e.g. *Anagrus delicatus* Dozier, Antolin and Strong 1987).

4.4.2 Implications for Using Fluorescent Dusts in Mark-Recapture Studies of Small Parasitoids

Marking *E. eremicus* with fluorescent dust did not affect either the mortality or flight duration of wasps that were marked with a small, but detectable, amount of dust. Total emergence (which was greater than the emergence that occurred just during the trapping period) of dust treated parasitoid pupae ranged from 14 to 55%. The 14% figure for release three, was very low which resulted in few wasp recaptures. It appears that the poor emergence was due to low quality material that had been stored too long rather than an effect of the dust. The higher figure of 55% is within the normal range of emergence of unmarked wasps in the field. It was not possible to determine if the dust had a negative effect on emergence because there was no control group of untreated pupae. In a study where braconid fruit fly parasitoids were marked with fluorescent dust, Messing et al. (1993) found that the marking wasp pupae improved adult emergence over that from unmarked pupae.

Mortality of wasps that failed to leave the release container ranged between 56 to 92% because there was too much dust on their bodies. This did not affect the results of the dispersal experiment as these wasps never entered the dispersing population. Based on results from laboratory flights of marked wasps, the marking-longevity test, and my own observations, those wasps that were able to walk or fly out of the release container behaved normally and lived a life-span similar to unmarked wasps. Dusted wasps that walk up to the rim and sides of the release container after emergence appear to behave similarly to

wasps that are not dusted (walking around the container, resting and grooming prior to flight, personal observation) so it appears that while some experimentation and care with dusting is needed to reduce the mortality of wasps in the container, the basic technique is sound and probably can be put to use with other small parasitoids. It should be noted, that the "dusted" wasps I observed behaving and flying normally, only have a minute speck of dust on their body. The mark is not visible without the use of a microscope at 50X power in a darkened room and a UV light. Even with this setup, it can take up to a minute to find the mark.

The use of fluorescent dust to mark insects in mark-recapture studies has been used successfully on numerous species. Insects marked in this way include aphids (Turchin and Kareiva 1989, Thomas et al. 1997), bark beetles (Cook and Hain 1992, Turchin and Thoeny 1993), sweetpotato whitefly (Byrne et al. 1996), flea beetles (Kareiva 1981), western corn rootworm (Naranjo 1990, Oloumi-Sadeghi and Levine 1990) and *Drosophila* (Crumpacker 1974). The majority of studies found no or only minor effects of dust on mortality. Those studies that examined flight or movement also noted no or only minor effects of using dust (c.f. Naranjo 1990, Cook and Hain 1992, Thomas et al. 1997).

I could find only three studies where parasitic Hymenoptera have been marked in this manner. In a mark-release recapture study, Corbett and Rosenheim (1996b) marked *Anagrus epos*, an aphelinid parasitoid of grape leafhopper. They did not measure any effects of the marking technique on either mortality or flight but speculated that if there were any negative effects they may have underestimated the diffusion rate. Garcia-Salazar and Landis (1997) tested the effects of dust application technique and dose on survival, flight

ability, and marking efficacy on *Trichogramma brassicae* Bezdenko. They found no differences in mortality between dusted and undusted wasps. The highest dust treatment affected the ability to fly and concluded that there was a trade-off between marking efficiency and flight behavior (the highest dust treatment had the best marking efficiency). They also found that wasp grooming behavior decreased the number of marked wasps over time. Overall, they concluded the technique was viable for marking to study dispersal in small insects such as *Trichogramma.*

Messing et al. (1993) marked two species of braconid parasitoids of tephritid fruit flies and found that marking increased mortality about 3 to 4 fold and decreased dispersal. However, the mortality they measured was of wasps that stayed in the release containers and did not become part of the dispersing population. For studying dispersal, the key question is what is the effect on those wasps that are able to survive and disperse? In any event, most of these wasps probably died from an over-application of dust. I calculated that they used 30 to 40 times the quantity of dust per pupae that I did. The key to successfully using fluorescent dust to mark small parasitoids is to apply a minimum amount to the puparia which allows them to pick up a small amount when they eclose. Even though I used lesser amounts of dust than Messing et al. (1993), I still observed high levels of mortality of emerged wasps. It is not possible to directly compare these dust mortality results with those of Messing et al. (1993) because I did not include a control to measure the effects of dust on mortality. From observation it was clear that I ended up killing wasps with too much dust, these wasps were observed helplessly twitching on the bottom of the release container heavily coated with dust. As I have pointed out this did not affect the dispersal

experiment as enough wasps were able to move up to the lip of the container and fly, unaffected by the small amount of dust on their bodies. My use of the dust marking technique would have been improved if I had conducted a dust dose response study which would have found the minimum dose of dust with the lowest mortality and highest marking efficiency (e.g. Garcia-Salazar and Landis 1997). This would be most important if parasitoid supplies were limited and one needed to minimize the mortality due to the marking technique. Messing et al. (1993) also conclude that using dust to mark the braconids in their system is not useful because it affected their dispersal though they only indirectly measured their ability to fly. They noted that fewer marked wasps were captured on sticky cards relative to released unmarked wasps but they did not indicate whether they controlled for the effects of the dust mortality previously mentioned. Garcia-Salazar and Landis (1997) observed similar effects in their testing of marked *T. brassicae* flight. In the highest dust treatment, there were fewer wasps (dry application treatment) that flew relative to the control (and other dust treatments) and concluded that their dry dust application technique may not be useful for marking small flying insects. However, in their analysis, they did not directly compare the flight performance (e.g. mean flight time in a flight chamber) of the dusted wasps (capable of flight) with the unmarked wasps. The reduced number of dusted wasps that flew was a result of some wasps being treated too heavily with dust. The fact that none of the other dust treatments negatively affected flight ability suggests that their basic technique is sound as long dust application rates are not too high.

Based on the results of Corbett and Rosenheim (1996b), Garcia-Salazar and Landis (1997), and these results reported here, it appears that marking parasitic Hymenoptera with

fluorescent dust is a viable method for studying their dispersal. Its advantages are that it is an inexpensive, technically simple method that if used properly, results in few to no negative impacts and a high marking efficiency. Its disadvantages are that it is time consuming to detect the marks in the laboratory and the mark may be lost with time in the field (e.g. in two of the releases, I estimated approximately 45% of the recaptured wasps had lost the mark while in the field). The difficulty of detecting marks may be improved by using traps that allow easier examination of wasps free of glue (or suction traps; see Byrne et al. 1996, e.g. non-sticky traps such as the CC-trap; see Chu et al. 1997). More study is needed to determine at what rate wasps lose their mark in the field. Different species may lose their marks at different rates so this should be examined for each system of interest. Depending on the life-span of the parasitoid and the nature of the study the mark may last long enough to remain effective. A model study of this kind was conducted by Hagler and Naranjo (1996) where they compared two marking techniques in a field setting. Hagler and Naranjo (1996) found that the duration of the mark on coccinellid predators marked with fluorescent dust remained high for about a week after mark-release after which it declined rapidly to low levels. In contrast, a mark applied by using an immunoglobulin marking technique remained at high levels for four weeks, a clearly superior retention time. However, if one is only interested in following beetle dispersal for a short period of time; using the simpler marking method may be sufficient.

4.4.3 Implications for Biological Control Strategy

The practical significance of my findings is that estimates of mobility can be used to devise release patterns for augmentative biological control in cotton. For example, a single release of parasitoids made on 20 m centers should provide good coverage in the field as wasp dispersal would fill in the gaps between release points (an estimated 99% of the population moved ≈12 to 22 m in 4 to 8 d). Multiple releases could be made using a greater distance between release points using an offset pattern for subsequent releases to ensure adequate coverage of the field.

More research is necessary in order to use information on mobility to make definitive release strategy recommendations. For example, because of the possibility of density dependent interactions between parasitoids (e.g. Kareiva 1981, Turchin 1998) higher release rates may result in greater dispersal distances than the ones estimated in this study. It would be useful to conduct mark-recapture experiments where the release number was manipulated to determine if there was any effect on the diffusion rate or on medial dispersal distances. The diffusion rate of parasitoids may also be affected by the distribution and density of hosts. Because host arrestment (Shimron et al. 1992) and attraction to host volatiles (Heinz and Parrella 1998) have been shown for *Eretmocerus* spp., higher host density could result in slower diffusion rates (e.g. Kareiva and Odell 1987). This probably is not an important factor to consider when devising release strategies because most often initial release of parasitoids will be made when whitefly are low in order to establish a population in time to provide control (but see, Simmons and Minkenberg 1994, and Appendix A). Experimental releases where host density is explicitly manipulated could

help uncover whether such interactions are important. Also it would be important to know exactly what kind of searching strategy that the wasps use to find hosts, and whether they begin dispersing again after first encountering hosts. Plant architecture may also affect parasitoid movement (Burbutis and Koepke 1981, Andow and Prokrym 1990). Varieties of cotton with taller or denser growth may restrict parasitoid movement more than shorter or less dense varieties of cotton. Finally, these estimates of mobility apply only for this particular habitat type. Movement outside of the cotton crop or within native vegetation may be quite different. Movements in these habitats are likely to be much longer and in the absence of hosts (one can speculate) may be migratory.

Application of the diffusion framework to study movement could be extended to include evaluations of new biological control agents. When selecting which agents to use before mounting an expensive long term effort in either classical or augmentative biological control, it would be desirable know how they move and respond to prey density under realistic field conditions (Kareiva 1990a, Kareiva 1990b). An agent with high dispersal capability would be best suited for a program in classical biological control as high dispersal should lead to rapid establishment (Caltagirone 1981, Corbett and Rosenheim 1996b). A less mobile agent may be better suited for augmentative release as it would be more likely to remain in the field where control is needed. Another consideration is the current interest in using beneficial plantings as refugia to harbor natural enemies (c.f. Pickett and Bugg 1998). Knowing how far the agents are likely to move can be used in designing the placement of refugia in relation to the target crop (Corbett and Plant 1993, Corbett and Rosenheim 1996a).

Admittedly, at the beginning of a new biological control program, new agents may be in too short of supply to make open field releases to measure their dispersal; yet in this study statistically significant results were obtained by the release of as little as 7,564 individuals. Decisions often need to be made on which agents to collect overseas so it may not be possible to test the agents directly in the target agroecosystem in the new environment. However, even limited information on dispersal would be useful and might feasibly be collected in the native land of the agent. For example, weed biological control agents are often field tested for non-target effects in their native land (van Driesche and Bellows 1996). With the addition of simple small scale dispersal studies into existing pre-screening protocols, much more information could be gained that should be useful in the evaluation process. For natural enemies of insect pests, many programs forgo extensive pre-screening evaluations beyond determining non-target effects and release programs are implemented with the hope that the best agents will survive and become established. In these cases, since field releases are to be made anyway, designing the releases so that experimental information on movement can be collected would be of great value (Kareiva 1990a).

Finally, due to the increasing scrutiny of biological control programs for possible non-target effects on native species (Simberloff and Stiling 1996, Louda et al. 1997, Strong 1997) it would be prudent to collect information on dispersal. Much of the concern about non-target effects is related to the potential for movement into new ecosystems not originally targeted in the initial release (c.f. Miller and Aplet 1993, Simberloff and Stiling 1996, Strong 1997). Diffusion coefficients (which can be estimated in small scale field studies) are important parameters in invasion models used to predict the long term rate of

spread of invasive species (Andow et al. 1993, Kot et al. 1996). Such models could be used to predict the probability of spread of a new biological control agent to new ecosystems where non-target species are at risk. Obtaining quantitative estimates of dispersal would be a very useful addition to the risk-assessment process and would help make biological control a more predictive endeavor.

CHAPTER 5

PARASITOID-HOST SPATIAL RELATIONSHIPS

5.1 Introduction

Effective natural enemies released in augmentative biological control programs must search out and extinguish prey early in the season when they are more rare and their distribution is patchy (Kareiva 1990b, O'Neil 1990, Andersen and Kareiva 1993, Heinz and Parrella 1998). Pest distributions are generally considered to be heterogeneous (and whitefly in particular) (Hassell 1982, Taylor 1984, Tonhasca et al. 1994, Naranjo and Flint 1995), yet few studies have considered how natural enemies respond to variation in pest distribution in agricultural settings (Kareiva 1990b, Corbett and Plant 1993). Recent research on the efficacy of biological control agents has identified the rate at which parasitoids discover and aggregate to prey patches as one of the most important attributes of an effective biological control agent (Kareiva and Odell 1987, Murdoch and Stewart-Oaten 1989, Kareiva 1990b, O'Neil 1990). While it was beyond the scope of the current experiment to directly test this theory, an attempt was made to measure the degree to which *E. eremicus* responds to variation in host density. These data will be useful for comparisons to other parasitoid species under consideration as biological control agents. They may also suggest how effective *E. eremicus* is at discovering different sized patches of prey and how likely it is that it will aggregate strongly enough to suppress whitefly populations.

In this experiment, whitefly infested cotton plants were placed in a cotton field for a short period of time to observe the pattern of *E. eremicus* attack as a function of host

density. The object was to determine if there is any spatial density dependence in the pattern of parasitoid attack on *B. tabaci* nymphal density. An important feature of this experiment was that the plants with the target hosts (or sentinel hosts) were left in the field for a short period time so that the patterns of parasitism observed would reflect only aggregative effects of the searching parasitoid population and not temporal patterns of parasitism generated by an increase in numbers due to reproduction. Studies of spatial patterns of parasitism have been criticized for confounding spatial and temporal patterns of parasitism (Rosenheim 1989, Cronin and Strong 1990, Turchin 1990). Because the spatial scale at which host-parasitoid interactions are examined may influence the results (Heads and Lawton 1983, Walde and Murdoch 1988), patterns of parasitism were examined at two spatial scales; among leaves and among plants.

5.2 Methods

Two experiments using greenhouse grown cotton with whitefly as sentinel hosts were conducted on 28 August and 11 September, 1995. To produce the sentinel hosts, five weeks before the start of each experiment, 100 plants of short staple cotton variety 'Deltapine 5415' were started in a greenhouse in 3.8 liter plastic pots filled with artificial media (Redi-Earth [tm], Grace-Sierra). Plants were grown between 29 to 41° C and 25 to 70% rh, with natural light. Ten days before the start of each experiment plants were exposed to adult whiteflies that were allowed to oviposit for 24 h. Adults were then removed with insect vacuums. Although it was desired that there be variation in whitefly density within and between plants, there was no manipulation of oviposition to achieve this. Based on previous experience, natural variation in whitefly activity within the cages would produce the desired variation in host density. Plants were taken to the field when leaves had second and third instar whitefly. This is the stage preferred for oviposition by *E. eremicus* (Headrick et al. 1995, Headrick et al. 1996).

Plants were left in the pots and planted into an untreated cotton field of short staple variety 'Deltapine 5415'. The pots were dug into the cotton row alongside of the existing cotton so that the tops of the pots were flush with the soil level. A replicate plot consisted of 25 plants arranged in a row, spaced at 0.5 m intervals to form a plot with dimensions of 12 m by ≈0.5 m (width of cotton plant). There were 10 m between replicate rows, and the start of each row was located 10 m from the edge of the field. In experiment 1, there were three replicate rows. In experiment 2, there were two replicates for a total of 75 and 50 plants respectively. At the start of each experiment, the plants

had an average height of 0.5 m. The height of the existing plants in the field was approximately 1.0 m.

To ensure that there would be some foraging parasitoids present during the experiment *E. eremicus* pupae were released in adjacent rows 1 meter on both sides of the plot row. Parasitoids were placed in 0.47 liter paper ice cream containers that were wrapped with Teflon™ coated tape (SureFire Insect Barrier Tape, Consep, Inc, Bend, Oregon) to protect them from ants and placed in the shade of the cotton row. For experiment 1, an estimated total of 10,600 adult parasitoids (or approximately 5,300 females) were released alongside of the three replicate rows for a release rate of 63 parasitoids/m^2. For experiment 2, an estimated total of 8,800 adult parasitoids were released alongside the two replicate rows for a release rate of 79 parasitoids/m^2. To estimate the number of parasitoids released per unit area the plot size of each replicate was calculated to be 56 m^2. The actual number of released parasitoids per square meter was probably much less than these estimates because the median estimate of their dispersal distance (2.4 to 4.4 m in 4 to 8 d, Chapter 4) was greater than the width of these plots.

Plants were watered daily and left in the field for seven days to allow naturally occurring and released female parasitoids of *E. eremicus* to oviposit. At the end of this exposure period, the plants were brought back to the greenhouse and were kept for 20 d to allow parasitoid development to occur. All of the leaves of each plant were examined using dissecting microscopes at 30x and counts were made of all emerged adult *B. tabaci*, emerged *E. eremicus*, *B. tabaci* pupae parasitized by *E. eremicus*, and *B. tabaci*

parasitized by *Encarsia* spp. The percentage of parasitism was calculated by dividing the number of pupae that were parasitized by *E. eremicus* or had parasitoid emergence holes, by the sum of emerged *B. tabaci* pupae and pupae parasitized by *E. eremicus* and *Encarsia* spp. (or from which parasitoids had emerged). The proportion of discovered leaves per plant was calculated as the number of leaves with one or more parasitoids of *E. eremicus* divided by the total number of leaves.

For statistical analysis, emerged whitefly and parasitoid counts were log transformed, and the percentage of parasitism and the proportion of discovered leaves were arcsine transformed. Least squares linear regression (Freund and Littell 1991) was used for analysis of each experiment to determine the relationship between host density: and the number of parasitoids; and the percentage of parasitism. Both of these relationships were analyzed among plants and among leaves to determine if there was an effect of scale. The relationship between the proportion of discovered leaves and the number of emerged whitefly per plant was also analyzed with least squares regression.

Three additional field collected samples (from nearby untreated fields on the same farm) were analyzed for comparison to these data. On each sample date, leaves containing appropriate stages for counting parasitoids and emerged whitefly were collected. On 18 August, a 25 ha field was sampled in six 5 x 5 m plots each separated by 100 m from other plots. At each location, a random sample of 40 leaves was collected for a total sample of 240 leaves. From the 0 release plot of the 1995 release experiment (embedded within a 22 ha field, see Chapter 3 for details), a random sample of 340 leaves was collected on 8 September. On 12 September, a random sample of 30

leaves was collected from six 5 x 5 m plots each separated by 100 m. The relationships between host density and parasitism, and host density and the number of parasitoids per leaf were analyzed with least squares regression as described above, with the only difference being that these data were examined only at the scale of the leaf.

5.3 Results

5.3.1 Parasitoid-Host Spatial Relationships on Sentinel Host Plants

For experiment 1, only 60 of the initial 75 plants survived to be censused for the numbers of whitefly and parasitoids. Flooding (from field irrigation) for an extended period of time caused 13 of the plants in replicate 2 and one of the plants in replicate 3 to drop their leaves. The emerged whitefly density on leaves ranged from 0 to 2266, with a mean (± SEM) of 62 ± 5. The percentage of parasitism ranged from 0 to 100%, with a mean (± SEM) of 3 ± 0.4%. On plants, the emerged whitefly density ranged from 178 to 8587 with a mean (± SEM) of 1200 ± 154. The percentage of parasitism ranged from 0 to 8%, with a mean (± SEM) of 2 ± 0.2%.

For experiment 2, a total of 46 plants of the initial 50 plants survived to be censused. The emerged whitefly density ranged from 0 to 1965 per leaf, with a mean (± SEM) of 117 ± 6. The percentage of parasitism ranged from 0 to 100%, with a mean (± SEM) of 5.4 ± 0.5%. At the scale of the plant, the emerged whitefly density ranged from 411 to 10434 per plant with a mean (± SEM) of 3100 ± 316. The percentage of parasitism ranged from 0.2% to 9%, with a mean (± SEM) of 3 ± 0.3%.

Among leaves, analysis with least squares regression indicated that there were significant positive linear relationships between the number of *E. eremicus* pupae and emerged whitefly density for all five replicates (Table 5.1, Fig. 5.1). Analysis of parasitism data indicated there were three cases out of five replicates where significant regressions were obtained (replicates 1,4 and 5, Table 5.2, fig. 5.2). However, the values of R^2 were low (0.02 to 0.05) so very little variation is accounted for by the relationship

Table 5.1. The relationship between emerged whitefly density and number of *E. eremicus* pupae among leaves from five replicates of the two density experiments in 1995. Data are from the counts of all parasitoids and emerged whitefly of all of the leaves of the sentinel host plants. Parasitoid and whitefly leaf density data were log transformed before analysis with least squares regression.

Date of Experiment	Replicate	R^2	P	Slope	Intercept	No. of leaves
28 August	1	0.30	0.0001	0.20	-0.07	406
28 August	2	0.16	0.0001	0.12	-0.03	156
28 August	3	0.25	0.0001	0.21	-0.10	449
11 September	4	0.38	0.0001	0.34	-0.11	604
11 September	5	0.29	0.0001	0.25	-0.11	535

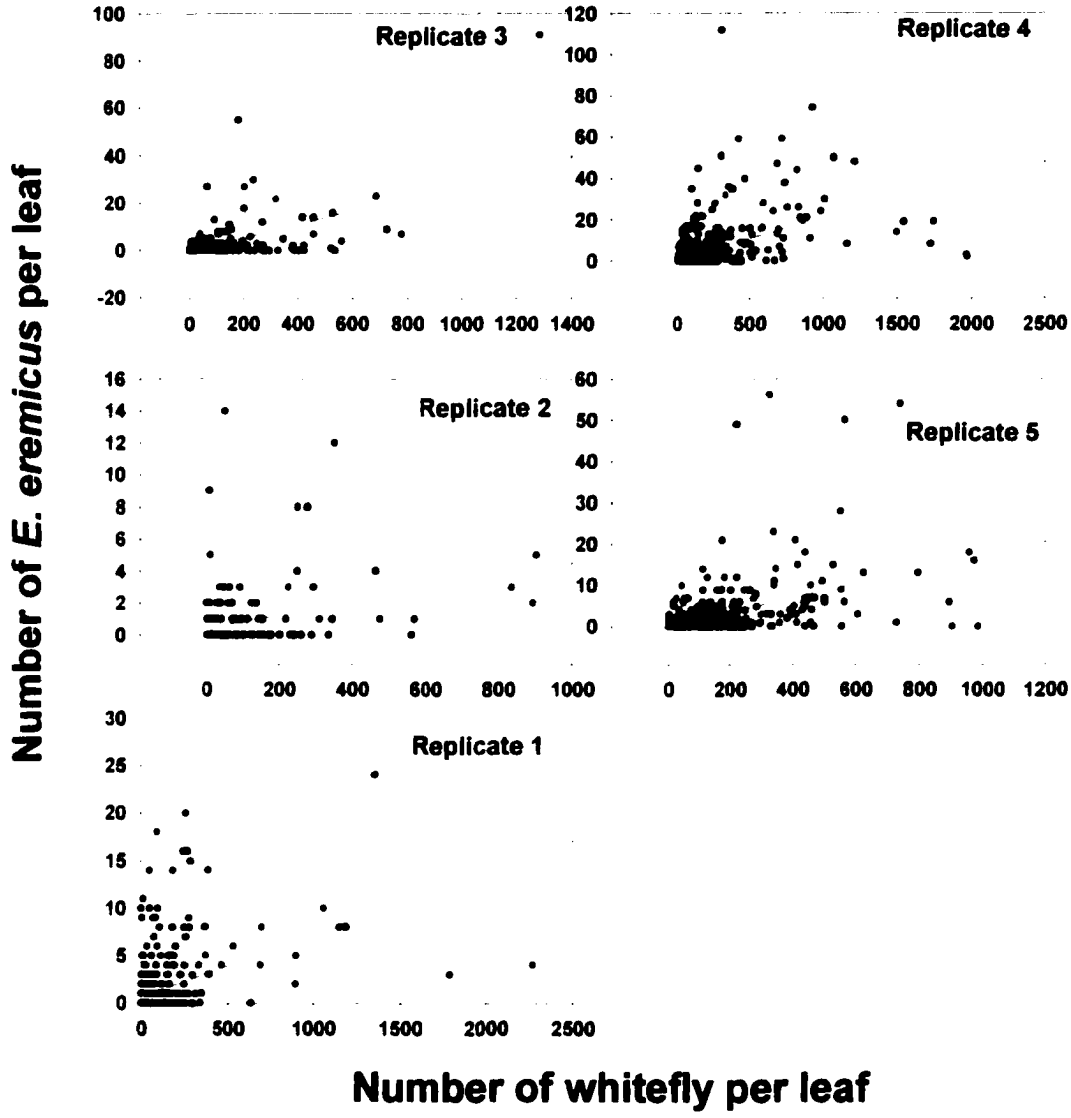


Fig. 5.1. The number of *E. eremicus* pupae per leaf of the sentinel host plants versus the number of emerged whitefly per leaf in density experiments 1 and 2. Curves are fitted least-squares regression lines, see text for regression statistics.

Table 5.2. The relationship between emerged whitefly density and parasitism among leaves from five replicates of the two density experiments in 1995. Data are from the counts of all *E. eremicus* and emerged whitefly of all of the leaves of the sentinel host plants. Parasitism data were arcsine transformed, and whitefly densities were log transformed before analysis with least squares regression.

Date of Experiment	Replicate	R^2	P	Slope	Intercept	No. of leaves
28 August	1	0.02	0.01	-0.04	0.16	406
28 August	2	0.01	0.16	-0.03	0.11	156
28 August	3	0.00	0.99	0.00	0.08	449
11 September	4	0.06	0.0001	-0.08	0.31	604
11 September	5	0.02	0.0003	-0.05	0.19	535

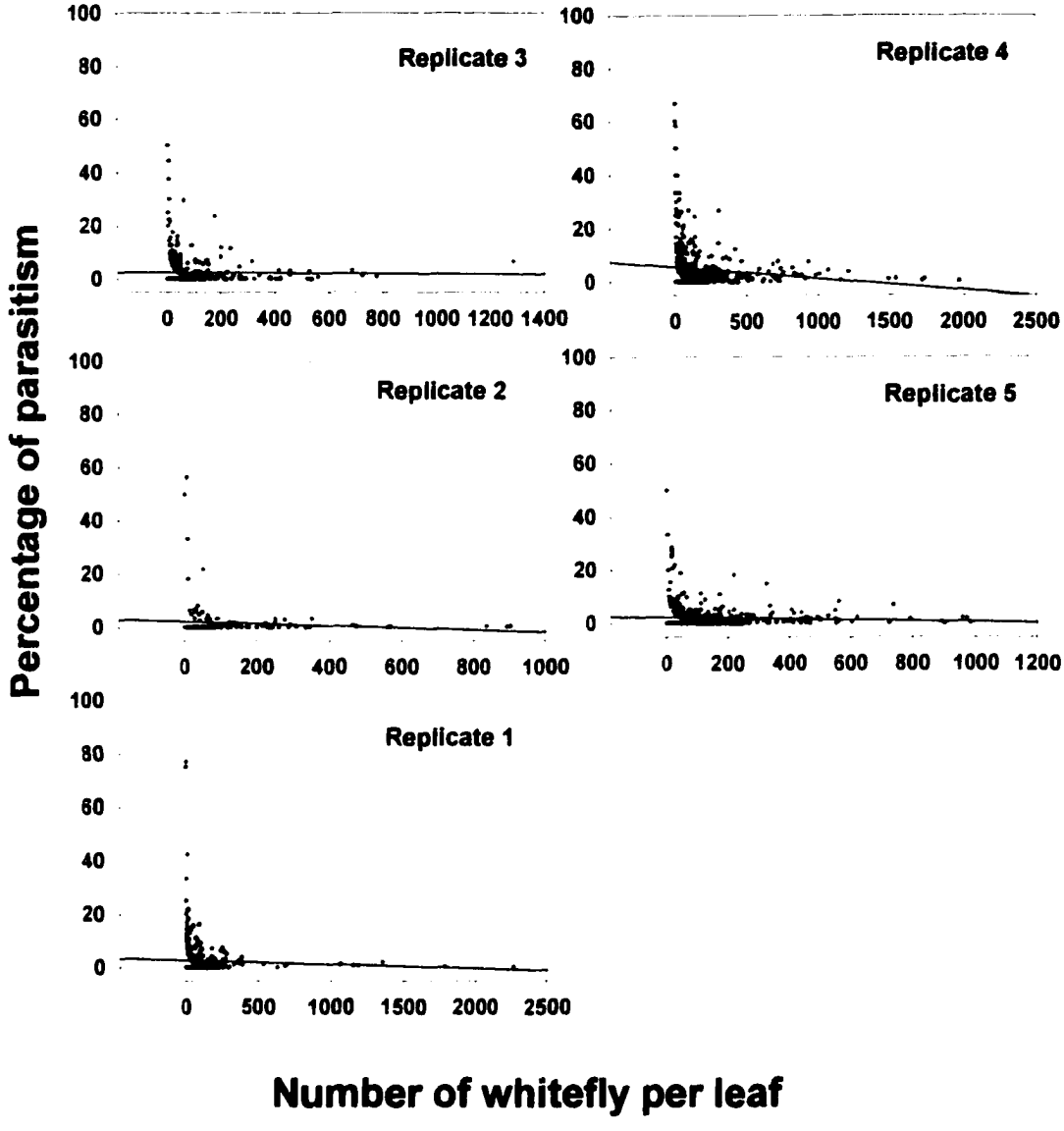


Fig. 5.2. The percentage of parasitism per leaf versus the number of emerged whitefly per leaf on the sentinel host plants the 1995 density experiments 1 and 2. Curves are fitted least-squares regression lines, see text for regression statistics.

between whitefly density and the percentage of parasitism. In cases where significance was found, inverse density dependence is suggested as the slopes were negative (Table 5.2).

Among plants, significant positive linear relationships were also observed between the number of *E. eremicus* pupae and emerged whitefly density per plant for all replicates (Table 5.3). None of the regressions of parasitism on host density were significant (Table 5.4).

Least squares regression analyses of the proportion of discovered leaves data versus plant host density for all five replicates, resulted in a highly significant positive regressions indicating positive density dependent relationships (Table 5.5, Fig 5.3).

Table 5.3. The relationship between emerged whitefly density and the number of *E. eremicus* pupae among plants from five replicates of the two density experiments in 1995. Data are from the counts of all parasitoids and emerged whitefly of all of the leaves of the sentinel host plants. Parasitoid and whitefly density per plant were log transformed before analysis with least squares regression.

Date of Experiment	Replicate	R^2	P	Slope	Intercept	No. of plants
28 August	1	0.33	0.005	0.88	-1.52	25
28 August	2	0.34	0.06	1.10	-2.53	11
28 August	3	0.45	0.0003	1.53	-3.52	24
11 September	4	0.33	0.003	0.75	-0.65	24
11 September	5	0.48	0.0004	1.0	-1.86	22

Table 5.4. The relationship between emerged whitefly density and parasitism among plants from five replicates of the two density experiments in 1995. Data are from the counts of all parasitoids and emerged whitefly from all of the sentinel host plants. Parasitism data were arcsine transformed, and whitefly densities were log transformed before analysis with least squares regression.

Date of Experiment	Replicate	R^2	P	Slope	Intercept	No. of plants
28 August	1	0.004	0.78	0.01	0.10	25
28 August	2	0.01	0.79	0.02	0.03	11
28 August	3	0.12	0.09	0.08	-0.12	24
11 September	4	0.09	0.15	-0.06	0.41	24
11 September	5	0.002	0.85	0.01	0.11	22

Table 5.5. The relationship between emerged whitefly density and the proportion of discovered leaves among plants from five replicates of the density experiments in 1995. Data are the proportion of discovered leaves and the emerged whitefly density from all of the sentinel host plants per replicate. Proportion of discovered leaves were arcsine transformed, and whitefly densities were log transformed before analysis with least squares regression.

Date of Experiment	Replicate	R^2	P	Slope	Intercept	No. of plants
28 August	1	0.28	0.008	0.43	-0.62	25
28 August	2	0.39	0.04	0.38	-0.61	11
28 August	3	0.48	0.0001	0.60	-1.21	24
11 September	4	0.32	0.004	0.35	-0.29	24
11 September	5	0.34	0.005	0.40	-0.60	22

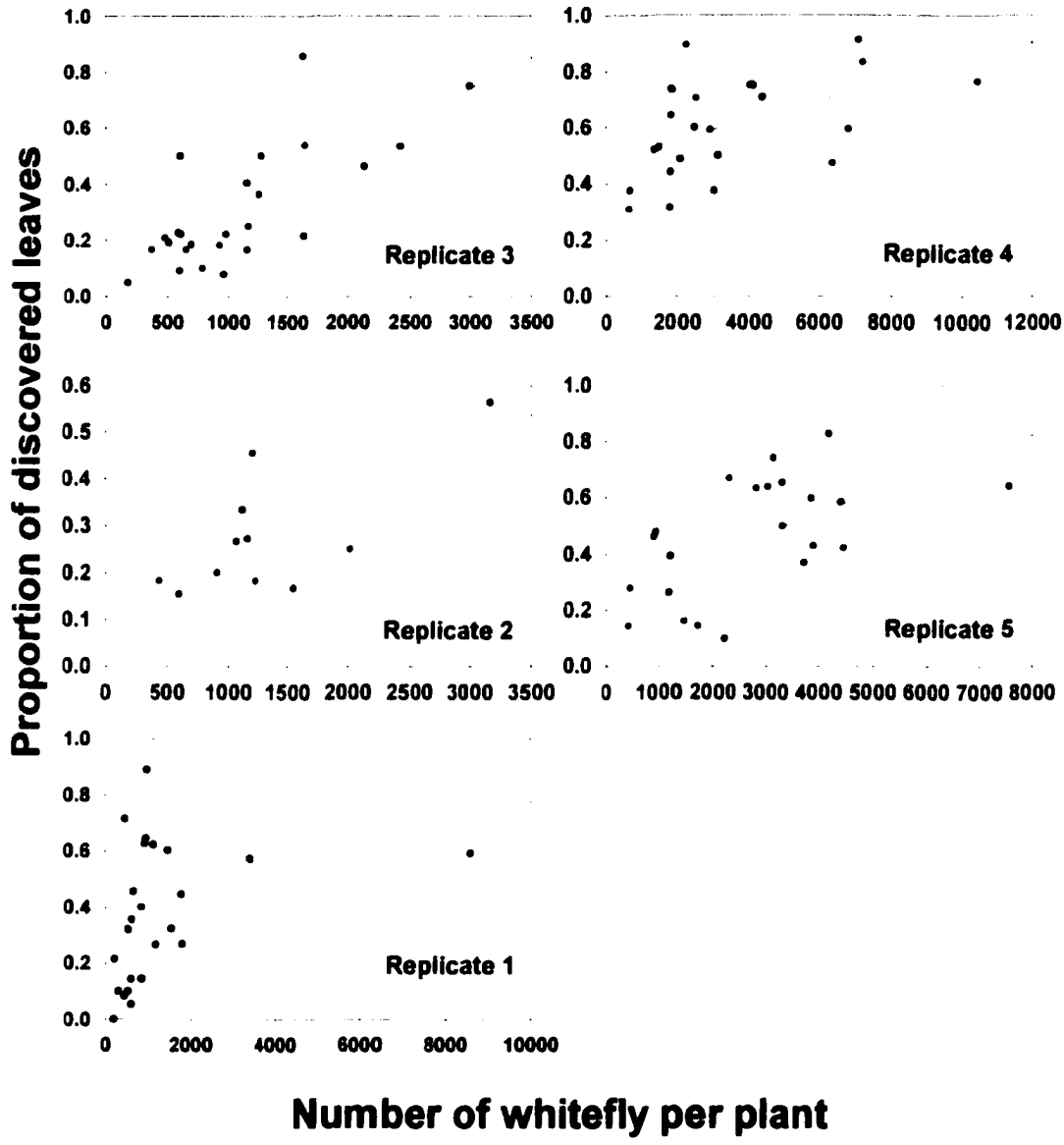


Fig. 5.3. Proportion of discovered leaves per plant versus the total number of emerged whitefly on the sentinel host plants for density experiments 1 and 2. Curves are fitted least-squares regression lines, see text for regression statistics.

5.3.2 Parasitoid-Host Spatial Relationships on Leaf Samples from Cotton Field

For the 18 August sample, emerged whitefly density on leaves ranged from 0 to 95, with a mean (± SEM) of 7.0 ± 0.6. The percentage of parasitism ranged from 0 to 50%, with a mean (± SEM) of 4 ± 1%. For the 8 September sample, emerged whitefly density ranged from 0 to 82 per leaf, with a mean (± SEM) of 6.6 ± 0.5. The percentage of parasitism ranged from 0 to 100%, with a mean (± SEM) of 22 ± 2%. For the 12 September field sample, emerged whitefly density ranged from 0 to 130 per leaf, with a mean (± SEM) of 12.0 ± 1.1. The percentage of parasitism ranged from 0 to 100%, with a mean (± SEM) of 30 ± 2%.

Analysis of the relationship between the density of *E. eremicus* and emerged whitefly on samples from cotton fields revealed significant positive linear relationships for all three of the samples (Table 5.6). The analysis of the parasitism versus host density data only one significant regression for the 8 September sample (Table 5.7). This relationship, which suggests inverse density dependence, was highly significant but had a low R^2 (Table 5.7). Furthermore, graphical analysis of the residuals from least squares regression revealed a poor fit so it appears that there is little evidence of an inverse density relationship.

Table 5.6. The relationship between emerged whitefly density and number of *E. eremicus* pupae among leaves from 3 sample dates from untreated cotton fields in 1995. *E. eremicus* and whitefly densities were log transformed before analysis with least squares regression.

Sample date	R^2	P	Slope	Intercept	No. of leaves
18 August	0.09	0.0001	0.18	-0.06	249
8 September	0.08	0.0001	0.23	0.14	340
12 September	0.32	0.0001	0.60	0.06	187

Table 5.7. The relationship between emerged whitefly density and parasitism among leaves from 3 sample dates from untreated cotton fields in 1995. Parasitism data were arcsine transformed, and whitefly densities were log transformed before analysis with least squares regression.

Sample date	R^2	P	Slope	Intercept	No. of leaves
18 August	0.00	0.69	0.02	0.1	249
8 September	0.13	0.0001	-0.40	0.7	340
12 September	0.01	0.24	-0.07	0.6	187

5.4 Discussion

As whitefly density increased, there was a linear increase in the number of *E. eremicus* on the scale of both leaves and plants. For the leaf data, the slopes of these least squares regressions were all less than 1.0 (mean value of 0.22, Table 5.1), which resulted in either inverse density dependence or density independent patterns of parasitism on the scale of leaves. Indeed, at densities lower than 100 emerged whitefly per leaf there were high rates of parasitism (Fig. 5.2). As whitefly densities increased, rates of parasitism decreased to low levels (Fig. 5.2). Mean rates of parasitism ranged from 3 to 5%, far lower than often found in naturally-occurring late summer populations of *B. tabaci* infesting cotton. In these situations rates of parasitism can reach 50 to 80% (see chapter 3 and Gerling 1966, Natwick and Zalom 1984, Bellows and Arakawa 1988). There are two factors that may explain these low rates of parasitism. The first relates to the fact that hosts were exposed to parasitoids for only 7 days. *B. tabaci* nymphs are susceptible to parasitism by *E. eremicus* from the first through most of the fourth instar (Headrick et al. 1996). While the sentinel hosts put into the field were mostly second and third instar whitefly, natural variation in developmental time of nymphs caused a range of hosts from first through fourth instar to be present. Therefore, some of the nymphs may have been susceptible to parasitism for a longer period of time then they were left in the field. Conversely, some hosts with more rapid development, may have developed to a non-susceptible stage (late fourth instar, see references that refer to this phenomena for *E. mundus* on *B. tabaci* Tawfik et al. 1978, Jones and Greenberg 1998) while in the field. The overall effect of these two factors would be to reduce parasitism

rates of the sentinel hosts relative to naturally occurring whitefly. Secondly, though average host densities per leaf were within the range of host densities often encountered in late summer populations of whitefly infesting cotton, these 9 to 17 times higher than the per leaf host density of the plants in the surrounding cotton. Apart from whatever effect high whitefly densities may have on levels of parasitism, low whitefly densities on the cotton in the surrounding field implies that there was a relatively low population of searching parasitoids available to parasitize whitefly (though small release of parasitoids were made. this was no guarantee that these parasitoids would concentrate their attacks on the sentinel hosts). A large native population of parasitoids would require a corresponding large population of whitefly. Indeed, mean levels of parasitism from the surrounding cotton fields were relatively low ranging from 7 to 30%.

Results from examination of the effect of scale on spatial patterns of parasitism show that at the scale of the leaf it was 60% more likely to find an inverse density dependent relationship than not. This relationship was weak as R^2 were low (0.02 to 0.06) which means that host density was only one of the factors that explains variation in values for parasitism. At the spatial scale of plants, the regressions between parasitoid and host density were all statistically significant. The slopes of these regressions averaged about 1.0 (Table 5.3), which suggests that the level of parasitism at the spatial scale of plants was constant or independent of density. Which were the results obtained, as none of the regressions of parasitism on host density were significant at the spatial scale of individual plants. The results for the field samples of leaves paralleled those of the leaves of the sentinel plants with significant regressions (with slopes less than 1.0) of *E. eremicus*

density on host density observed for all replicates. For the parasitism data, there was a significant regression for only one of the three samples of leaves from the cotton plots, which indicated an inverse density dependent relationship.

Results of field studies that examined the effect of scale on patterns of parasitism inverse density dependence are more often found on the smaller scale examined (e.g. leaves, shoots) than on the larger scale (e.g. plants, trees, plots)(Walde and Murdoch 1988). It is not clear why this pattern is often observed in nature or in this experiment. Walde and Murdoch (1988) conclude that in some cases prey were inaccessible at high host densities but mostly the mechanisms are not known. Before making conclusions on the effect of the spatial scale of sampling on patterns of parasitism by *E. eremicus* it will be important to conduct more studies with a greater range of host variation.

There was a significant positive density dependent relationship between the percentage of discovered leaves and host density. This suggests that parasitoids did show an aggregative response to patches of high density, but this was not reflected in the levels of parasitism. Perhaps because there were too many hosts relative to the number of parasitoids searching in the field, dilution of parasitism rates occurred. This result is similar to what Noldus and van Lenteren (1990) found for another aphelinid wasp, *Encarsia formosa*, and its interaction with greenhouse whitefly, *Trialeurodes vaporariorum*. They speculated that at high host density parasitism by *E. formosa* may be limited by either its egg maturation rate or its lifetime egg supply. They also speculated that mutual interference between parasitoids searching on high density patches may have induced dispersal, reducing the overall rates of parasitism. Other factors that

could result in density independent parasitism are when parasitoids use fixed foraging times or fixed number of host encounters to determine the time spent on host patches (van Alphen and Vet 1986). It is unknown what factors may be responsible for the observed density independent patterns of parasitism found for *E. eremicus*. It will be necessary to conduct detailed behavioral studies of its foraging patterns (preferably under field conditions) to determine which mechanisms are important (c.f. Noldus and van Lenteren 1990).

Interestingly, recent work on *Eretmocerus* spp. foraging has suggested two possible mechanisms that could account for the observed pattern of aggregation to patches of high host density. In a laboratory study, Shimron et al. (1992) demonstrated that female *E. eremicus* (as *Eretmocerus* sp. since confirmed to be *E. eremicus*, Dan Gerling, Tel Aviv University, Israel, personal communication) have a density dependent arrestment response to *B. tabaci* honeydew, which led to longer times spent on patches with higher quantities of honeydew. Such a response should lead to an increased probability of parasitizing a host and could cause the pattern of aggregation (as shown by the percentage of discovered leaves data) found in the current experiment. In a choice test with clean and whitefly infested plants using an olfactometer, Heinz and Parrella (1998) showed *E. mundus* is attracted to *B. tabaci* infested plants. It remains to be seen if this attraction is density dependent as this was not examined. However, one can speculate that if such a mechanism exists for *E. eremicus* it could lead to parasitoid aggregation to high density patches.

Hoddle et al. (1998) found density independent spatial relationships in their work on augmentative release of *E. eremicus* in greenhouses against *B. tabaci* infesting poinsettia, *Euphorbia pulcherrima*. For several strains of *E. mundus*, *E. tejanus*, and *E. staufferi*, Heinz and Parrella (1998) also found density independent relationships between parasitism and *B. tabaci* density on cotton and poinsettia. In contrast to these results, Gerling (1986) suggested that the population density of *B. tabaci* infesting cotton fluctuated with the *E. eremicus* population density in a manner that suggested density dependent regulation. However, in Gerling's study (1986) the host-parasitoid relationship was inferred by counting relative adult samples of whitefly and parasitoids rather than directly examining parasitoid and whitefly densities on plants or leaves (c.f. Gerling 1967). So it is possible that factors other than the parasitoid-host interaction was responsible for the observed population changes. Furthermore, unlike the current study, Gerling's study followed population changes over the course of the season, therefore the density dependent relationship found is a temporal one and most likely the result of parasitoid reproduction rather than an aggregative response to host density.

Overall, it appears that density independent spatial relationships between parasitism and host density is a common feature for *E. eremicus* and other *Eretmocerus* spp. These results do not suggest the kind of spatial relationship that it is generally thought necessary for effective augmentative biological control (e.g. van Lenteren and Woets 1988, Kareiva 1990a, Heinz and Parrella 1998). However, at low pest density (when parasitoid release against whiteflies should begin), the speed at which a parasitoid locates and attacks its host is more important for effective pest control than whether is has a positive density

dependent response to host density (van Lenteren and Woets 1988, Waage and Greathead 1988, Kareiva 1990a, Luck 1990). If parasitoids are added to the system in large enough numbers, when whitefly numbers are low, there is an opportunity to control whitefly populations before they increase to unmanageable size (Hoelmer 1996).

In this study, whitefly densities on sentinel plants (at both the spatial scales of leaves and plants) were similar to densities encountered in cotton fields with high levels of whitefly infestations. It is possible that at lower whitefly densities the spatial pattern of parasitism relative to host density may show positive density dependence. O'Neil (1990) argues that studying natural enemy behavior at high host densities may result in an underestimate of their performance at lower host densities. Because of tradeoffs between host searching and feeding (for parasitoids, ovipositing and host feeding), search patterns may change with changing host density. O'Neil (1990) suggests that natural enemy searching and feeding behavior should be studied in the field at low host densities typical of the conditions present when natural enemies must exert control. Future field studies of *E. eremicus* and *B. tabaci* interactions should focus on how quickly released parasitoids find experimentally manipulated different sized patches of prey against a background of low pest density to determine the strength of the aggregative response and their ability to control whitefly before a large population has a chance to grow (c.f. Kareiva and Odell 1987, Kareiva 1990b).

Finally, in this study host feeding was not measured. Numerous studies of *E. eremicus* (and other *Eretmocerus* spp.) feeding behavior have measured high levels of host feeding and have concluded that it is an important source of mortality (Headrick et

al. 1995, Headrick et al. 1996, Heinz and Parrella 1998, Hoddle et al. 1998). Future field studies of *E. eremicus* (as well as other species of *Eretmocerus*) attacking whitefly infesting cotton should attempt to estimate the importance of this mortality source.

CHAPTER 6

SUMMARY OF INVESTIGATIONS ON THE USE OF *ERETMOCERUS EREMICUS* AS AN AUGMENTATIVE BIOLOGICAL CONTROL AGENT AGAINST *BEMISIA TABACI*

6.1 Introduction

In four years of field investigations on the use of *E. eremicus* as an augmentative control agent against *B. tabaci* infesting cotton, I have learned much about the potential and some of the limitations of using biological control against this pest. In this chapter, I highlight the most important findings and describe how they might be used to develop augmentative biological strategies for managing this pest. I also point to some of the areas where knowledge is lacking and what kinds of investigations should prove fruitful to improve prospects for biological control of *B. tabaci*. Finally, I suggest how recent changes in management of *B. tabaci* in the southwestern U.S. agro-ecosystem present new opportunities for the use of biological control.

6.2 Cage Release Rate Studies

Cage release studies in 1992 and 1993 showed that parasitism could be increased and whitefly densities reduced with augmentative releases of mass-reared *E. eremicus*. These studies demonstrated two important findings: 1) that *E. eremicus* could survive and reproduce in the severe climatic conditions present in the hot summers of the southwestern U.S.; 2) they suggested a range of release rates that provided the basis for testing release rates in the open field. With the cages studies in 1992, the maximum

mean parasitism rates achieved were 61% and whitefly densities were reduced to about one fifth of the other treatments. The estimated effective release rate was between 113 to 367 parasitoids per m^2 (or 1.1 to 3.7 million parasitoids/ha). Cotton yield was higher in the highest parasitoid release treatment with an estimated yield (by extrapolation) of 1.3 bales per acre versus 0.5 bales per acre for the no-release treatment.

In the 1993 cage release studies, a wider range of release rates was investigated than in the 1992 cage release studies. The peak rate of parasitism obtained was 79%, which was higher than the 61% observed in the 1992 study. Nonlinear regression analysis, suggested that there was a threshold number of released parasitoids above which parasitism rates did not increase nor were decreases in whitefly densities observed suggesting that further parasitoid release would be ineffectual. From these analyses, effective release rates were estimated to between 77 to 109 parasitoids/m^2 (or 770,000 to 1.1 million parasitoids/ha). Despite observing that there appeared to be a threshold effect of the number of parasitoids released on rates of parasitism, regression analysis of the cotton yield data showed that there was a strong linear effect of number released on yield, which suggested that higher parasitoid release provided a protective effect. This may have occurred because higher release rates eliminated whitefly earlier in the high release cages than in the lower release cages, thus reducing feeding stress allowing the cotton to allocate more resources towards cotton production. Yield was estimated to be 2.5 bales/ac for the highest parasitoid release versus 0.6 bales/ac for the no-release cages.

The overall results from the cage studies showed that parasitoid release can increase parasitism, decrease whitefly density, and increase yield. These studies provided the basis for testing parasitoid release in open field outside of cages.

6.3 Open Field Release Rate Studies

1993 Field Release Experiment. Open field release studies conducted in 1993 suggested that the results from the cages could not easily be applied to the open field (at least not during times when regional populations of whitefly are high because of the migration from nearby crops). A release rate of 786 parasitoids/m^2 (or 7.86 million/ha), which was approximately 3.5 times greater than the highest release rate in 1993 cage experiment, resulted in peak levels of parasitism of 42%; about half of the highest parasitism rate in the highest release cage in 1993. There were no statistically significant differences in parasitism, whitefly density, and yield between release and the no-release control plots.

It appeared that parasitoid release in the open field was not effective because high levels of whitefly immigration from the preceding spring melon crop overwhelmed the capacity of the released parasitoids to control whitefly. It also appeared that many of the released parasitoids left the release plot and spread to the control plots thus reducing the differences between treatments. These above two factors illustrate the key differences between the cage and open field release studies. Because cages eliminate immigration of whitefly and emigration of parasitoids, lower rates of parasitoid release inside of cages

can achieve higher rates of parasitism and reduce whitefly densities more than much larger sized releases in open fields.

1995 Field Release Studies. Because of concerns about the effects of small plot size on parasitoid emigration, a different approach was taken with the open field release experiments conducted in 1995. Release plots were separated from one another by a greater distance than the 1993 study (100 m versus 35 to 85 m) and were embedded within a larger cotton field to provide a more realistic field situation. Another difference of the 1995 study was that it was designed to more accurately answer the question of how many parasitoids should be released per location in a single release while previous studies focused on the total number of parasitoids that were needed for control.

Sentinel hosts were used to more accurately estimate the effects of a single release, as naturally occurring parasitoids would have less opportunity to parasitize hosts that were left in the field for just a short time, thus reducing the possibility of naturally occurring parasitoids obscuring the results of a single release. The sentinel hosts were set out for three days after parasitoid release in the center of a release grid. The percentage of parasitism peaked at 14% in the highest parasitoid release plot on the first day. For the regressions of parasitism versus the number of released parasitoids, there was a statistically significant regression for only one of the three days though there was a trend of declining rates of parasitism over the three days of the experiment. This suggested that parasitoids were leaving the release area or dying over the course of the three days. The percentage of discovered leaves peaked at 78% also on the first day in the highest

release treatment. Similar to the parasitism data there was a non significant trend of decreasing rates of discovered leaves over the course of the experiment. Samples from cotton plants within 1 to 4 m from the central release point were analyzed with nonlinear regression analysis, regressing the percentage of discovered leaves on release number at each of the distances. At the 3 and 4 m distances, there was an asymptote in the percentage of discovered leaves between the release rates of 200 to 400 parasitoids per m^2 (2.0 to 3.0 million/ha) resulting in peak rates of percentages of discovered leaves greater than 80%.

6.4 Summary of Effective Release Rates and Economic Analysis

The results from the cage and open field studies while not definitive, allow tentative estimates of effective release numbers and preliminary economic analyses. Two caveats must be considered concerning the interpretation of these experiments: 1) cage effects may underestimate numbers needed for release because they eliminate immigration of whitefly and emigration of parasitoids; 2) variation in field whitefly densities between years will change the estimates of the number parasitoids needed for release. With the above caveats in mind, considered together the cage and field release studies suggested that release rates between 770,000 to 3,000,000 adult parasitoids/ha would result in high rates of parasitization and reduced whitefly densities.

At current wholesale estimates of $6.00 per 1,000 parasitoids (and using a mean emergence rate of 72%) this would cost between $5,914 to 23,040 to treat one hectare of cotton. The lower value in this range was estimated to be 8 times higher than what

growers in the Imperial Valley of California have spent on whitefly control and still achieve economic returns (based on a highest cost for pesticidal control of $741/ha). Even if it was certain that sufficient control could be obtained with parasitoid release, it is clear that the current costs are far too high to be considered a viable method of pest control.

6.5 Dispersal of *E. eremicus* after Point Release in Cotton

To study within field *E. eremicus* dispersal, a series of mark-recapture experiments were conducted after point release in cotton during 1994 and 1995. Recapture data were fit to a diffusion model, which allowed the estimation of median dispersal distances and the diffusion coefficient (or diffusion rate), which is a standard measure of mobility allowing comparison to the movement rates of other species (Kareiva 1983, Turchin 1998). With these results, it was possible to answer practical questions such as how far apart to place release points. As mobility is a key factor in the efficacy of biological control agents (Kareiva 1990b), the diffusion coefficient can be used to make comparisons between species being evaluated for their potential as biological control agents.

Although the longest observed movement was a female wasp that flew 82 m in one day, the majority moved much less as the estimated median dispersal distance was 2.4 to 4.4 m in an interval of 4 to 8 days. These results suggested that released *E. eremicus* only move a short distance within cotton fields and releases on 20 m centers (an

estimated 99% of the recaptures moved 12 to 22 m in 4 to 8 d) would provide good coverage of parasitoids within a field.

The diffusion rate was estimated to be between 0.40 to 0.71 m^2/min, which appears to be on the lower end of the mobility scale compared to other species. It was not possible to directly compare diffusion rates with *A. epos* the only other parasitoid wasp for which detailed dispersal information is available (c.f. Corbett and Rosenheim 1996b) because of differences in calculation methods. However, median dispersal distances were compared and it was determined that *A. epos* movement may be as much 42 to 154 times more rapid.

It should be noted that these estimates of median dispersal distances and diffusion rates may be specific to the cotton system that was studied. Movement outside of the field, when more or less whiteflies are present, or in other crops may be quite different. It is recommended that the mobility of *E. eremicus* be studied separately for each system of interest. Finally, because the diffusion rate was estimated with laboratory flight data, it should be regarded as a preliminary estimate. In order to increase confidence in this estimate, it will be necessary to verify that the assumptions that were made in estimating the diffusion rate are correct. These assumptions were that only small numbers of wasps engaged in long distance flight up and out of the recapture grid, that high levels of mortality did not effect the loss rate of dispersing wasps, and that the estimates of mean flight time from the flight chamber are accurate representations of the time that wasps fly in the field.

In agreement with other studies where fluorescent dust was used to mark small parasitoids (Corbett and Rosenheim 1996b, Garcia-Salazar and Landis 1997), it was determined that the dust marking technique used in this study is a viable technique for studying dispersal of *E. eremicus*. Laboratory studies showed there were no effects of the dust on either flight time or mortality meeting the requirement that the marking technique not introduce bias into recapture patterns (Southwood 1978, Turchin 1998). Advantages of the technique are that is inexpensive and technically simple to use. Disadvantages are that the mark appears to be lost over time in the field, if the dust is applied in too great of quantity there can be some mortality of wasps upon emergence (but not of wasps that were capable of flight), and that it is time consuming to read the marks under the microscope. It is recommended that dose-mortality studies be conducted comparing mortality rates versus mark retention times for each species of interest.

6.6 Parasitoid-Host Spatial Relationships

In 1995, the relationship between host density and rates of parasitism was studied in a field study with sentinel hosts and samples of cotton leaves with naturally occurring whitefly. The relationship between host density and rates of parasitism was examined on the spatial scale of leaves and plants with least squares linear regression. The goal was to determine if there was any positive spatial density dependence on hosts in patterns of parasitoid attack. Positive spatial density dependent parasitism is thought to be an important characteristic of effective natural enemies used in augmentative biological

control (Waage 1983, Waage and Greathead 1988, Kareiva 1990a, Heinz and Parrella 1998).

For sentinel hosts, analysis of parasitism data at the spatial scale of leaves indicated there were significant regressions observed for three out of five replicates. For the regressions where significance was found, the relationship between parasitism and host density suggested negative density dependence. However, only a small amount of the variation is accounted for by the relationship between parasitism and whitefly density as the values for R^2 were low (0.05 or less). At the spatial scale of plants, none of the regressions of parasitism on host density were significant, which suggested density independent relationships. Because whitefly density was higher on the sentinel host plants than on the on the surrounding vegetation (whitefly density was 10 times higher on the sentinel host plants), it was believed that there may been too many hosts relative to the number of searching adult parasitoids thus diluting parasitism rates. For this reason, the sentinel host data was also analyzed with least squares regression with the proportion of discovered leaves (ratio of leaves per plant with parasitized nymphs divided by the total number of leaves per plant with hosts or parasitized nymphs) versus host density per plant. This measure would be less susceptible to the dilution effect of too few parasitoids relative to the density of whitefly and should show if there were density dependent aggregation of parasitoids to host density. These regressions were all highly significant, indicating positive density dependent relationships.

Analysis of parasitism rates versus whitefly density for three sets of cotton leaf samples (with naturally occurring hosts) showed that only one of the regressions were

significant, which suggested inverse density dependence, while the other non-significant regressions suggested density independent relationships.

Taking all of the data together, there was evidence that *E. eremicus* had a positive density dependent response to whitefly density, as the analysis of the proportion of discovered leaves data showed a linear increase in discovery rates with increasing whitefly density at the scale of plants. However, this density dependent discovery rate did not result in density dependent rates of parasitism. In this experiment it was not possible to rule out a dilution effect of decreased rates of parasitism due to high populations of whitefly. Because other researchers have also found density independent relationships between *E. eremicus* and *B. tabaci* (and other *Eretmocerus* spp.)(Heinz and Parrella 1998, Hoddle 1998), it appears that spatially density independent relationships are common. It would be interesting to determine the mechanism behind these patterns to determine if is based on negative interactions between parasitoids (i.e. mutual interference) or perhaps based on *E. eremicus* oviposition behavior or possibly, negative effects of high amounts of honeydew on leaves with high densities of whitefly.

6.7 Future Prospects for Biological Control

In recent years, regional whitefly populations have declined in the most severely affected areas in Arizona and California (Ellsworth 1999, Palumbo 1999). Lower regional whitefly populations could provide improved prospects for augmentative biological control in cotton by reducing the number of parasitoids needed for release. Reductions in whitefly populations appear to be due, in large part, to the use of several

new whitefly selective pesticides (Ellsworth 1999, Palumbo 1999) which tend to have few to no effects on natural enemies (Jones et al. 1995, Naranjo and Hagler 1997, Simmons et al. 1997b, Naranjo et al. 1998b), further improving the prospects for biological control. Other considerations that should favor the use of augmentative biological control, are increasing concerns about the development of pesticide resistance and changes in pesticide regulatory laws, which provide increased financial incentives for the use of biological control (Parrella et al. 1992, Heinz et al. 1993).

Another factor that could improve the economics of using parasitoid release is the collection and importation of more effective species of *Eretmocerus* from other regions in the world (Goolsby et al. 1998, Hoelmer et al. 1999). The higher fecundity of the exotic species (c.f. Hoelmer 1998) will reduce the cost of parasitoid release relative to the cost of using *E. eremicus*. Several of these species have been introduced into commercial trade and are available for further evaluation and testing.

Finally, recent research has demonstrated that parasitism can be increased and whitefly densities decreased by making augmentative parasitoid releases in melons. In agroecosytems in the desert southwestern U.S., the spring melon crop is where whitefly populations first increase to large size, later going on to infest cotton (Hoelmer 1996). In part, because temperatures are cooler and initial whitefly populations are lower, parasitoids can be released at lower rates in melons than in cotton (c.f. Simmons et al. 1997b). In addition, the value of the melon crop is higher than cotton, which means growers can afford to spend more on pest control. These two factors make the prospects for augmentative biological control of whitefly in melons much more economically

feasible than in cotton. The use of biological control in melons can benefit the later planted cotton by reducing the number of whitefly in melons that can go on to infest cotton, and by increasing the number of parasitoids that can migrate into cotton (Hoelmer 1996). Both of these factors can reduce the overall pest pressure of whitefly in cotton and may make it possible to use lower parasitoid release rates. The use of augmentative biological control in melons also may serve as a model for the use of augmentative releases in cotton in areas where cotton is grown as a monoculture without a preceding melon crop (e.g. cotton grown near Gila Bend , Arizona). In systems where cotton is the first whitefly susceptible crop, cotton may play the same ecological role as spring melons, in the sense that it is the first crop upon which whitefly can reproduce rapidly in warm weather. Without large early population of whiteflies migrating from melons, there will be lower initial infestations in cotton thus reducing the number of parasitoids needed for release.

6.8 Considerations for Future Research

Research is needed in order to improve the quality of mass-reared *E. eremicus*. Emergence rates in the releases rate studies generally ranged from between 56 to 99% with a mean of 72%. Due to the high costs of parasitoids it is clear that emergence rates will need to be increased to improve the economics of using *E. eremicus* in augmentative releases.

It will also be important to determine how much natural enemy induced mortality is necessary to manage whitefly populations. Naranjo and colleagues (1997, 1998a) are

collecting detailed life-table information on sources of whitefly mortality in the cotton system that can be used to estimate the additional mortality needed by augmentative release. This information could be coupled with population growth parameters of *E. eremicus* (and other aspects of its biology) in order to develop a population model to predict various pest density outcomes based on different release strategies. A key question is what is the effect of parasitoid release with and without whitefly migration from surrounding fields? This would be useful information in order to gauge the feasibility of pursing an augmentative release strategy in regions where cotton is not preceded by any whitefly susceptible crops. Other strategies designed to reduce the cost of augmentative releases might best first be investigated with a model. For example, if releases were made very early, could lower rates of release be used? Investigating various pest control outcomes based on varying release strategies would be very difficult to conduct in the field, a model could help determine which strategy was most promising and suggest which tactics to test in the field.

Further improvements in augmentative release strategy could be gained by developing economic thresholds for cotton (and other target crops) based on making augmentative releases. Such thresholds exist for pesticide applications (Palumbo et al. 1994, Naranjo et al. 1996) but none exist for augmentative releases of parasitoids. Economic thresholds for natural enemy release must necessarily include attack rates and population growth parameters of the natural enemy.

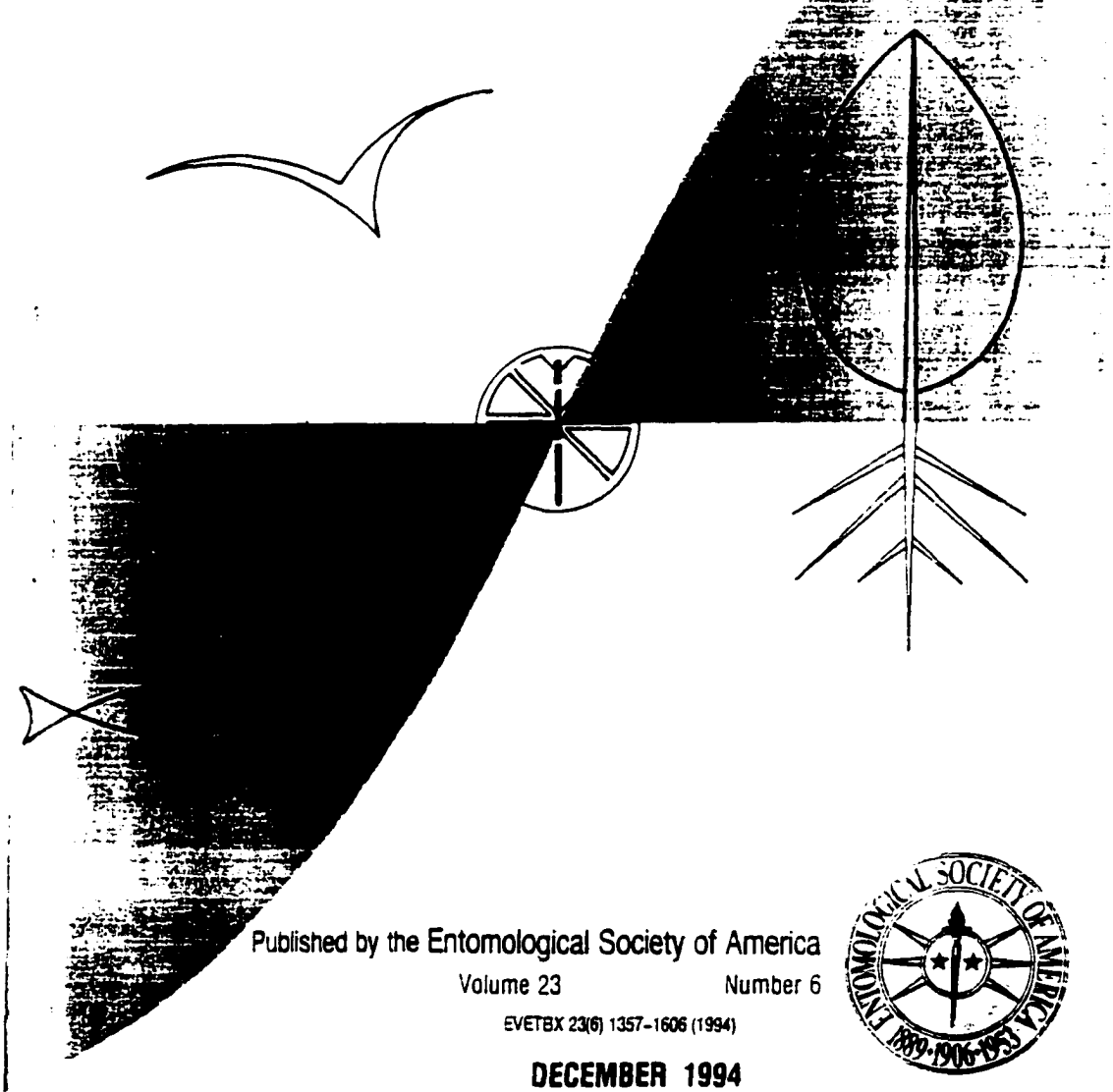
Other areas of investigation that could lead to reduced release costs include the use of new more effective release strategies (e.g. the banker plant strategy, Bennison and

Corless 1993, Goolsby and Ciomperlik 1997, Pickett et al. 1998) and research aimed at reducing rearing costs. Most gains in reducing the costs of augmentative releases have been gained by adopting artificial diets for rearing natural enemies (King and Powell 1992, Parrella et al. 1992). Indeed, many workers argue that development of rearing systems on artificial media are the only way that augmentative release control techniques will be adopted (King and Powell 1992, Parrella et al. 1992). Recently, an artificial diet has been developed for *B. tabaci* and work is underway to adopt these systems for rearing parasitoids (Jancovich et al. 1997, Davidson and Jones 1999, Davidson et al. 1999).

Finally, it would be useful to incorporate information from the dispersal studies with further release rate studies to determine if there are correlations between predicted dispersal distances and patterns of parasitism. This information is needed to design efficacious and efficient release strategies. Because within field dispersal appears to be limited, releasing parasitoids in release patterns that are too close together may result in releasing more parasitoids per unit area than is necessary. While the estimates of median dispersal distances appear to be robust, these should be compared with patterns of parasitism with distance for verification.

JS ISSN 0046 225X

ENVIRONMENTAL ENTOMOLOGY



Published by the Entomological Society of America
Volume 23 Number 6
EVETBX 23(6) 1357–1606 (1994)
DECEMBER 1994

ENTOMOLOGICAL SOCIETY OF AMERICA
9301 Annapolis Road, Lanham, MD 20706-3115
tel. (301) 731-4535 • fax (301) 731-4538
e-mail esa@entsoc.org • http://www.entsoc.org

March 19, 1999

Mr. Gregory S. Simmons
PO Box 3149
Laredo TX 78044-3149

Re: Permission to publish your ESA journal article in your dissertation

Dear Mr. Simmons,

The Entomological Society of America grants you a one-time permission to include a copy of your co-authored article referenced below in your Ph.D. dissertation at the University of Arizona.

> Simmons, G. S. and Minkenberg, O. P. J. M., 1994.
> Field-cage evaluation of augmentative biological control of Bemisia argentifolii (Homoptera: Aleyrodidae) in southern California cotton with the parasitoid Eretmocerus nr. californicus (Hymenoptera: Aphelinidae)
> *Environ. Entomol.* 23(6): 1552-1557.

Furthermore, ESA grants permission to University Microfilms Inc (UMI) to microfilm and publish this dissertation, and will permit UMI to sell or provide single copies of the dissertation, including the copyrighted materials, for scholarly purposes.

Sincerely,

Alan Kahan
Acting Director of Publications

Field-Cage Evaluation of Augmentative Biological Control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in Southern California Cotton with the Parasitoid *Eretmocerus* nr. *californicus* (Hymenoptera: Aphelinidae)

GREGORY S. SIMMONS AND OSCAR P.J.M. MINKENBERG

Department of Entomology, University of Arizona, Tucson AZ 85721

Environ. Entomol. 23(6): 1552–1557 (1994)

ABSTRACT A field cage evaluation of the parasitoid *Eretmocerus* nr. *californicus* as a biological control agent of *Bemisia argentifolii* Bellows & Perring was conducted in Southern California cotton during June through August of 1992. Percentage of parasitism was highest in early August at 61% in the high parasitoid release treatment and was significantly higher than both the low parasitoid release and control treatments. The density of *B. argentifolii* pupae on leaves in the high parasitoid release treatment was approximately one-fifth of the other treatments. Seed cotton yield was significantly higher in the high parasitoid release cage in comparison with those of the low parasitoid release and control cages. We conclude that *E.* nr. *californicus* may be a useful control agent in an augmentative release strategy against whitefly on cotton and is deserving of large-scale, open field studies.

KEY WORDS *Bemisia argentifolii*, *Eretmocerus* nr. *californicus*, augmentative biological control

SINCE 1991, a new species of whitefly, *Bemisia argentifolii* Bellows & Perring, formerly referred to as *Bemisia tabaci* (Gennadius) strain B or poinsettia strain (Bellows et al. 1994), has caused extensive damage to agricultural crops in the southern United States (Faust 1992, Watson et al. 1992, Gruenhagen et al. 1993, Perring et al. 1993). In cotton, this new whitefly causes yield reduction by phloem sap feeding and lint contamination caused by the production of honeydew and associated sooty molds (Natwick 1993, Blua & Toscano 1994). Chemical control of pest species in the genus *Bemisia* has been difficult because of the increasing resistance to pesticides (Byrne et al. 1990, Dittrich et al. 1990, Prahabker et al. 1992) and the difficulty in applying pesticides so that they provide good coverage on the underside of leaves where the nymphs occur (Bellows & Arakawa 1988).

Augmentative biological control, combined with reduced pesticide applications or use of pesticides that are less toxic to natural enemies, may be an important tool for the solution of the *B. argentifolii* problem in desert cotton production. Past records of parasitism by native aphelinid parasitoids of the genus *Eretmocerus* have ranged as high as 60–90% in late summer *Bemisia* spp. populations on cotton (Gerling 1966, Natwick & Zalom 1984, Bellows & Arakawa 1988). That these levels of parasitism were observed when *Bemisia* spp. populations were at their highest and during the time of extreme summer temperatures in the Imperial Valley (mean high temperature in August ≈38°C; range, ≈16–47°C), suggests that parasitoids in the genus *Eretmocerus* may be useful biological control agents. However, these studies were presumably all of *B. tabaci;* since 1991, infestations of *Bemisia* spp. in the Imperial valley have been determined to be all of *B. argentifolii* (Perring et al. 1991, Brown 1992, Faust 1992). *B. argentifolii* appears to be a more severe pest than *B. tabaci* and has a number of different biological characteristics (Costa & Brown 1991, Perring et al. 1993) that may influence its interactions with its natural enemies.

The taxonomic status of many species in the genus *Eretmocerus* is unclear, including those found in southern California. Previous studies of the parasitoids of *Bemisia* spp. found in Imperial County, California, refer to the species variously as *Eretmocerus haldemani* Howard, *Eretmocerus californicus* Howard, *Eretmocerus* nr. *haldemani*, and *Eretmocerus* sp. (e.g., Gerling 1966, 1967, Natwick & Zalom 1984, Gerling 1986, Bellows & Arakawa 1988). It is probable that all of the previous references to these *Eretmocerus* species reared from *Bemisia* spp. in the southern desert areas of California should be regarded as one species, *Eretmocerus* nr. *californicus* (M. Rose, personal communication).

0046-225X/94/1552–1557$02.00/0

This *Eretmocerus* species is a nymphal ecto-endoparasitoid (Gerling 1966) and in culture has a 50:50 sex ratio (O.P.J.M.M., unpublished data). The parasitoid first instar chews a hole and enters the host through the venter and completes its development inside its host, from which the adult wasp emerges (Gerling 1966). Host feeding has been observed in *Eretmocerus* and is an additional source of mortality (Gerling 1990).

To investigate the potential of *E.* nr. *californicus* for use in the biological control of *B. argentifolii*, we conducted field cage studies in cotton in Imperial County, California, during the summer of 1992. Our goal for these cage studies was to determine the feasibility of *E.* nr. *californicus* as a control agent. The field cages provided favorable test conditions of *E.* nr. *californicus* on a small scale by limiting dispersal of released parasitoids and preventing continuous immigration of whitefly. The success or failure of this trial would help determine if larger scale open field studies were warranted.

Materials and Methods

Test plots were planted with short staple cotton ('Deltapine 5461'). Plots were 49 m long by 31 m wide, with 1-m row spacing and maintained using standard agronomic practice for the area. Plantings were made at the USDA–ARS Irrigated Desert Research Station in Brawley, CA (four plots), and at the University of California Desert Research Station in Holtville, CA (eight plots) on 10 April and 12 May, respectively.

Five replications of three treatments each were applied in a randomized complete block design consisting of high parasitoid release, low parasitoid release, and no parasitoid release as a control. Three blocks of the experiment were at the Brawley site and two blocks of the experiment were at the Holtville site.

On 30 May, field cages were erected within plots of cotton at both sites. The cages were 3 m long by 1.83 m wide by 1.83 m high and were constructed of polyester organdy with closures at one end. The cages were built over two rows of cotton and contained a mean ± SEM of 49.6 ± 2.5 plants and 27.8 ± 1.6 plants per cage at the Brawley and Holtville stations, respectively.

The *E.* nr. *californicus* were obtained from *B. argentifolii* on a greenhouse culture of poinsettia at the USDA Western Cotton Research Laboratory in Phoenix, AZ, on 25 April 1991 (provided by G. Butler) and were identified by M. Rose (Texas A&M University, College Station). The population size of this original colony is unknown. In total, 150 adult *E.* nr. *californicus* were collected and introduced to *B. argentifolii* on poinsettia in greenhouses at the USDA–ARS Honey Bee Research Center in Tucson, AZ. In total, 300 adult wasps were collected during the winter of 1992 from the Tucson rearing and sent to Koppert B.V. in Berkel en Rodenrijs, the Netherlands, to start a mass-rearing operation. Parasitoids from Koppert B.V., were shipped and released in the form of parasitized greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), pupae glued onto paper cards. *T. vaporariorum* was used as the rearing host instead of *B. argentifolii*, because mass-rearing procedures of another whitefly parasitoid, *Encarsia formosa*, on *T. vaporariorum* are well established and were easily adapted for rearing *E.* nr. *californicus*.

Releases of *E.* nr. *californicus* commenced on 2 June and continued weekly until 26 July. Cards were attached to stems and were evenly distributed within the lower canopy. The number of parasitoids released was quantified by holding a part of each weekly shipment from the Netherlands in the laboratory at ≈26°C in paper cartons and counting the number of parasitoid emergence holes in the exuviae of parasitized whiteflies after emergence was complete. Because of the variation in shipping times, there were occasions when a large portion of parasitoid emergence occurred in transit. On these occasions, simply counting emergence holes as a measure would overestimate release rates because many adult parasitoids died or were in poor condition before release. To avoid this problem we counted the number of adult parasitoids that emerged during the period the cards were held in the laboratory. This allowed us to estimate a range of release rates; the upper limit of the range was based on the total number of emergence holes per card, which would in some cases reflect the true number of parasitoids released assuming no death of adult wasps that had emerged before release, and the lower limit based on the number of adult wasps that emerged from cards while they were held in the laboratory.

Sampling for *B. argentifolii* and *E.* nr. *californicus* began on 26 June, ≈3 wk after the first parasitoid introduction, and continued at ≈2-wk intervals until harvest. Following Von Arx et al. (1984), random samples were taken from the mainstem leaf node of the leaf that had the highest number of *B. argentifolii* pupae. The node position of this leaf changes as the plant grows and was determined on each sampling date by observation. Von Arx et al. (1984) termed this leaf the most infested leaf and showed that these samples provided mean estimates of pupal density with the lowest coefficients of variation. We sampled *B. argentifolii* pupae, which we consider to be the fourth and final immature stage, because it is easy to differentiate between nonparasitized and parasitized individuals in this stage. Parasitized pupae have an amber colored cuticle and lack the characteristic red eye spots of healthy *B. argentifolii*. On 10 June, we took 10 leaf samples from each cage to estimate *B. argen-*

tifolii levels and parasitism by *Eretmocerus* spp. at the start of the experiment. On 26 June, 40 leaf samples were collected per cage. On 8 and 22 July, 36 leaf samples were collected per cage and, thereafter, 30 leaf samples were collected.

Using dissecting microscopes in the laboratory, we counted *B. argentifolii* pupae, emerged adult *B. argentifolii*, emerged *E.* nr. *californicus*, parasitized *B. argentifolii* pupae, dead *B. argentifolii* pupae, and *B. argentifolii* parasitized by *Encarsia* spp. that were present in the cages in low numbers.

On 26 June and 8 July, we censused one half of a distal sector of the cotton leaf, dividing it parallel to the main leaf veins. This area was estimated to represent 19.45 ± 2.7 cm^2. Thereafter, we censused a 5.0-cm^2 leaf disk taken from random locations within the whole leaf. All counts were transformed into a per square centimeter basis. Percentage of parasitism was calculated by dividing the number of pupae that were parasitized, or had parasitoid emergence holes, by the sum of healthy *B. argentifolii* pupae and pupae parasitized by *E.* nr. *californicus* and *Encarsia* spp. (or from which parasitoids had emerged). The percentage of parasitism was arcsine transformed. *B. argentifolii* pupal counts were log transformed. These transformed variables were analyzed using analysis of variance (ANOVA) (PROC GLM) and treatment means were separated using Tukey's studentized range test (SAS Institute 1989).

Additional samples from the plots of cotton surrounding the cages at the Brawley site were taken for comparison to the results of the cage study. On 27 July, 30 leaf samples were taken from each of the three plots containing the cages and the one additional plot of cotton adjacent to the cage plots. On 21 August, 30 leaf samples were taken from the three plots containing the cages. Whitefly levels and percentage of parasitism were estimated in the same manner as the samples from cages.

Seed cotton yield was estimated by hand picking the lint from all the plants within the cages at the Brawley site. The Holtville site yield was not recorded because the plants produced little cotton as a result of late planting. Yield data were analyzed by ANOVA (PROC GLM) and treatment means were separated using Tukey's studentized range test (SAS Institute 1989).

Results

Combining all dates, we released between 1,649–4,228 parasitoids per high parasitoid release cage and a range of 79–328 per low parasitoid release cage (Table 1). This release rate corresponds to 295–755 parasitoids per square meter in the high parasitoid release cages and 14–59 parasitoids per square meter in the low parasitoid release cages.

Table 1. Estimates of number of *E.* nr. *californicus* released per cage

Release date	High release		Low release	
	No. cards	No. parasitoids	No. cards	No. parasitoids
2 June	8	120– 298	3	45–112
9 June	16	80– 720	3	15–135
19 June	15	432–1,035	—	—
26 June	15	93– 405	3	19– 81
6 July	20	420	—	—
11 July	9	44– 144	—	—
14 July	18	270– 720	—	—
26 July	17	190– 486	—	—
Totals	118	1,649–4,228	9	79–328

At the start of the experiment, the mean ± SEM number of *B. argentifolii* pupae in the high parasitoid release cages was 0.24 ± 0.14 pupae per square centimeter. An ANOVA followed by Tukey's studentized range test indicated that this was significantly lower than the control cages, which had 0.85 ± 0.60 pupae per square centimeter but was not significantly different from the low release cages with 0.56 ± 0.26 pupae per square centimeter ($F = 6.94$; df = 2, 8; $P < 0.001$). Parasitism by *Eretmocerus* spp. was <1% in all treatments at the start of experiment.

Percentage of parasitism in the high parasitoid release cages increased more rapidly than the low and control treatments and was highest with a mean ± SEM of 61 ± 1% on 20 August (Fig. 1). ANOVA followed by Tukey's studentized range test indicated that the percentage of parasitism was significantly higher for all sampling dates relative to both low release and control cages (26 June: $F = 156.84$; df = 2, 8; $P < 0.0001$, 8 July; $F = 573.40$; df = 2, 8; $P < 0.0001$, 22 July; $F = 330.34$; df = 2, 8; $P < 0.0001$, 5 August; $F = 232.57$; df = 2, 8; $P < 0.0001$, 20 August; $F =$

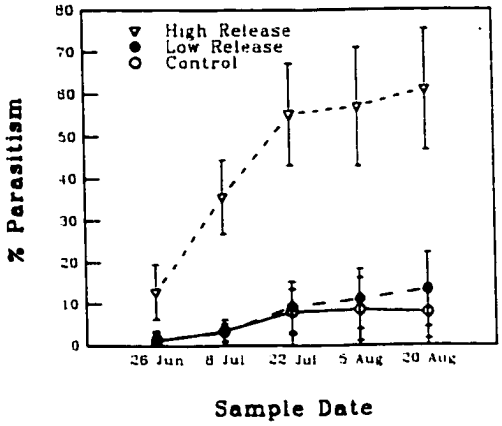


Fig. 1. Mean percentage of parasitism ± SEM (n = 5) for high and low parasitoid release cages and control cages on cotton leaves.

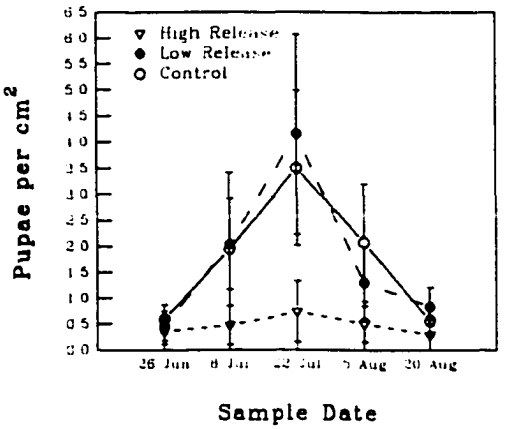


Fig. 2. Mean number ± SEM (n = 5) of *B. tabaci* pupae per square centimeter of cotton leaf for the high and low parasitoid release cages and control cage.

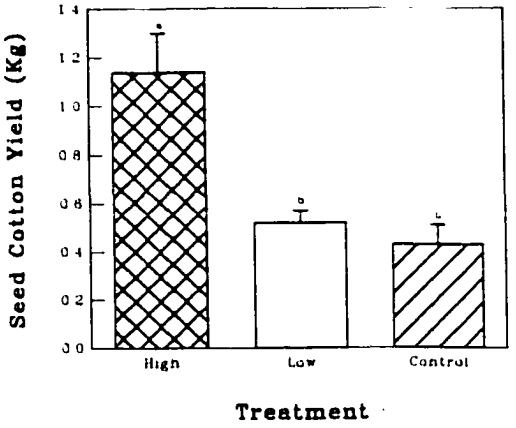


Fig. 3. Mean seed cotton yield ± SEM (n = 3) from the high and low parasitoid release cages and control cages. Treatments with different letters are significantly different ($P < 0.05$) by Tukey's studentized range test.

195.98; df = 2, 8; $P < 0.0001$; Fig. 1). Parasitism in the low parasitoid release cages was highest with a mean ± SEM of 13 ± 7% on 20 August and was not significantly higher than parasitism in the control cages during the entire sampling period (Fig. 1).

ANOVA followed by Tukey's studentized range test indicated that the number of *B. argentifolii* pupae per square centimeter was significantly lower for the high parasitoid release cages than for both low and no release cages for all sampling dates except 26 June, when there was no significant difference between the high and low parasitoid release cages (26 June; F = 13.63; df = 2, 8; $P < 0.0001$, 8 July; F = 73.83; df = 2, 8; $P < 0.0001$, 22 July; F = 114.83; df = 2, 8; $P < 0.0001$, 5 August; F = 46.44; df = 2, 8; $P < 0.0001$, 20 August; F = 34.76; df = 2, 8; $P < 0.0001$). The highest mean ± SEM density of *B. argentifolii* in high parasitoid release cages was 0.74 ± 0.59 pupae per square centimeter on 22 July in contrast to 4.15 ± 1.92 pupae per square centimeter in the low parasitoid release cages and 3.50 ± 1.48 pupae per square centimeter in the control cages (Fig. 2). The number of *B. argentifolii* pupae per square centimeter in the low parasitoid release and control cages were not significantly different for all sampling dates except for 5 August when there were 1.30 ± 0.69 and 2.07 ± 1.13 pupae per square centimeter, respectively (F = 46.44; df = 2, 8; $P < 0.0001$; Fig. 2). After 22 July, there was a decline in number of pupae per square centimeter of leaf sample for all three treatments (Fig. 2).

On 27 July, the percentage of parasitism in the cotton plots surrounding the cages was 14 ± 2% and on 21 August, was 39 ± 4%.

Cotton yield from the high parasitoid release cages was significantly higher than the low parasitoid release and control cages with a mean ± SEM seed cotton yield of 1.15 ± 0.16 kg versus 0.53 ± 0.05 kg and 0.44 ± 0.08 kg, respectively (F = 12.59; df = 3, 6; $P < 0.005$) (Fig. 3).

Discussion

The results show that parasitism of *B. argentifolii* can be increased by augmentative releases of *E.* nr. *californicus*. With the high release levels of parasitoids, we increased parasitism, suppressed whitefly levels, and increased cotton yield.

Declining plant quality may explain the steep decline in the number of *B. argentifolii* pupae per leaf seen in the low parasitoid release and control cages after the 22 July sampling date. This pattern probably parallels what happens in cotton fields when high numbers of whiteflies are present.

We achieved lower average levels of parasitism than found in previous field studies in cotton, where levels of parasitism have ranged from 60 to 90% (Gerling 1966, Natwick & Zalom 1984, Bellows & Arakawa 1988). One caveat about these previous data is that they represent levels of parasitism found in single samples, whereas ours are means from replicate samples from cages. Sample means of parasitism from individual cages were as high as 88% on 5 August, suggesting that high levels of parasitism can be achieved. Also, previous studies were presumably of *B. tabaci* and our study was conducted on *B. argentifolii*. *B. argentifolii* has been shown in laboratory studies to have higher fecundity and faster developmental times than *B. tabaci* (Bethke et al. 1991, Wagner 1993). This could lead to a rapid build up of whitefly population

levels such that *E.* nr. *californicus* lags behind in parasitism levels relative to its performance on *B. argentifolii* populations. Furthermore, the previous studies of *E.* nr. *californicus* reported the highest parasitism levels during the months of September and October. Our study was concluded at the end of August because of mandatory cotton plow-down regulations currently in effect in Imperial County. The levels of parasitism we achieved are within the range found in previous work during the month of August in untreated fields (Gerling 1966, 1967; Bellows & Arakawa 1988). In the plots of untreated cotton surrounding our cages at the Brawley station, we measured levels of parasitism by *E.* nr. *californicus* of 14% in late July and 39% in late August. These observations support our conclusion that we can increase levels of parasitism by *E.* nr. *californicus* by augmentative releases in field cages, as demonstrated when levels of parasitism found in the high parasitoid release cages during July and August were higher than those natural occurring levels of parasitism.

The proper release rate for effective control is difficult to ascertain because it is difficult to estimate the numbers of parasitoids that we released per cage. The low release range of rates appears to be too low because there was no difference between low parasitoid release and control cages in parasitism or whitefly levels. The proper release rate is less than the high release range of rates because the steepest rise in parasitism occurred between 26 June and 8 July. This suggests that the parasitoid releases in June had the greatest effect on parasitism. Coupled with the observation that *E.* nr. *californicus* has a developmental time between 18 and 20 d (Powell & Bellows 1992), it is likely that the first three releases produced the greatest increase in parasitism, suggesting that a release between 632 and 2,053 parasitoids (i.e., 113–367 per square meter or the sum of estimates of the first three releases) would be an effective one. Higher cotton yields were obtained in the high parasitoid release cages. This is 165% higher than the cotton from the control cages. It is difficult to know if this level of whitefly suppression could achieve economic cotton yields. The cages cause the cotton plants to grow differently and may cause the yield to be less than that of normal field grown plants. Furthermore, the cages restrict both *B. argentifolii* migration and *E.* nr. *californicus* dispersal. Unrestricted migration of both whiteflies and their natural enemies may result in different levels of parasitism and whitefly levels than achieved in our study. For these reasons it will be important to conduct field studies outside of cages to determine if these parasitoids will suppress *B. argentifolii* levels in open cotton plots and, moreover, if economic suppression of *B. argentifolii* can be achieved.

Acknowledgments

We thank D. Byrne, S. Naranjo, M. Rose, D. Papaj, M. Taylor, and two anonymous reviewers for their critical reviews of this manuscript; R. Weddle and E. Natwick for assistance in planning; M. Rose for identification of parasitoids; G. Butler for providing the original *Eretmocerus*, C. Brown and staff at the USDA–ARS in Brawley and the staff of the University of California Desert Agriculture station in Holtville for field and logistical support; E. Erickson and L. Gasca of the USDA–ARS Honey Bee Research Center in Tucson for logistical support; J. Diehl for advice on field studies with parasitoids; E. Mendoza for field assistance; and J. van Schelt and Koppert B.V for *Eretmocerus* rearing. This research was supported by the Imperial Valley Whitefly Management Committee and D. Byrne.

References Cited

Bellows, T. S. & K. Arakawa. 1988. Dynamics of preimaginal populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) in southern California cotton. Environ. Entomol. 17: 483–487.

Bellows, T. S., Jr., T. M. Perring, R. J. Gill & D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87: 195–206.

Bethke, J. A., T. D. Paine & G. S. Nuessly 1991. Comparative biology, morphometrics and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. Ann. Entomol. Soc. Am. 84: 407–411.

Blua, M. J. & N. C. Toscano. 1994. *Bemisia argentifolii* (Homoptera: Aleyrodidae) Development and honeydew production as a function of cotton nitrogen status. Environ. Entomol. 23: 316–321.

Brown, J. K. 1992. Biotypes of the sweetpotato whitefly: a current perspective, pp. 665–670. *In* Proceedings, Beltwide cotton conferences, 6–10 January 1992. National Cotton Council of America, Memphis, TN.

Byrne, D. N., T. S. Bellows & M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 227–261. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept, Andover, UK.

Costa, H. S. & J. K. Brown. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with the silverleaf symptom induction. Entomol. Exp. Appl 61: 211–219.

Dittrich, V., S. Uk & G. H. Ernst. 1990. Chemical control and insecticide resistance in whiteflies, pp. 263–285. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept, Andover, UK.

Faust, R. 1992. Conference report and 5-year national research and action plan for development of management and control methodology for the sweetpotato whitefly. Agricultural Research Service publication ARS-107, Beltsville, MD.

Gerling, D. 1966. Studies with whitefly parasites of southern California II. *Eretmocerus californicus* Howard (Hymenoptera: Aphelinidae). Can. Entomol. 98: 1316–1329.

1967. Bionomics of the whitefly parasite complex associated with cotton in southern California (Ho-

moptera: Aleurodidae; Hymenoptera; Aphelinidae). Ann. Entomol. Soc. Am. 60: 1306–132.

1986. Natural enemies of *Bemisia tabaci*, biological characteristics and potential as biological control agents: a review. Agric. Ecosys. Environ. 17: 99–110.

1990. Natural enemies of whiteflies: predators and parasitoids, pp. 147–185. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept, Andover, UK.

Gruenhagen, N. M., T. M. Perring, L. G. Bezark, D. M. Daoud & T. F. Leigh. 1993. Silverleaf whitefly present in the San Joaquin Valley. Calif. Agric. 47: 4–6.

Minkenberg, O., G. S. Simmons, R. Malloy, J. Kaltenbach & C. Leonard. 1994. Biological control of whiteflies on cotton: a reality check, pp. 887–890. *In* Proceedings, Beltwide Cotton Production Conferences, 10–14 January 1994. National Cotton council of America, Memphis, TN.

Natwick, E. T. 1993. Silverleaf whitefly control in cotton using various insecticides in the Imperial Valley of California, pp. 722–727. *In* Proceedings, Beltwide Cotton Production Conferences, 10–14 January 1993. National Cotton Council of America, Memphis, TN.

Natwick, E. T. & F. G. Zalom. 1984. Surveying sweetpotato whitefly in the Imperial Valley. Calif. Agric. 38: 11.

Perring, T. M, A. D. Cooper, D. J. Kazmer, C. Shields, J. Shields. 1991. New strain of sweetpotato whitefly invades California vegetables. Calif. Agric. 45: 10–12.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar & T. S. Bellows, Jr. 1993. Identification of a whitefly species by genomic and behavioral studies. Science (Washington, DC.) 259: 74–77.

Powell, D. A. & T. S. Bellows, Jr. 1992. Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). Environ. Entomol. 21: 651–658.

Prahabker, N., N. C. Toscano, T. M. Perring, G. Nuessly, K. Kido & R. R. Youngman. 1992. Resistance monitoring of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley of California. J. Econ. Entomol. 85: 1063–1068.

SAS Institute. 1989. SAS/STAT user's guide, version 6, 4th ed. SAS Institute, Cary, NC.

Von Arx, R., J. Baumgartner & V. Delucchi. 1984. Sampling of *Bemisia tabaci* (Genn.) (Sternorrhyncha: Aleyrodidae) in Sudanese cotton fields. J. Econ. Entomol. 77: 1130–1136.

Wagner, T. L. 1993. Temperature-dependent development of sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), biotype 'B', pp. 714–718. *In* Proceedings, Beltwide Cotton Conferences, 10–14 January 1993. National Cotton Council of America, Memphis, TN.

Watson, T. F., J. C. Silvertooth, A. Tellez & L. Lastra. 1992. Seasonal dynamics of sweetpotato whitefly in Arizona. Southwest. Entomol. 17: 149–167.

Received for publication 4 November 1993; accepted 28 July 1994.

REFERENCES

Andersen, M. and P. M. Kareiva. 1993. Interactions between imported predators and their prey in patchy environments, *In* K. Kelhung and B. McPheron [eds.], Evolution of insect pests. John Wiley & Sons, New York.

Andow, D. A., P. M. Kareiva, S. A. Levin, and A. Okubo. 1993. Spread of invading organisms: patterns of spread, *In* K. Chung and M. B [eds.], Evolution of Insect Pests. John Wiley & Sons, New York.

Andow, D. A. and D. R. Prokrym. 1990. Plant structural complexity and host-finding by a parasitoid. Oceologia 82: 162-165.

Andow, D. A. and D. R. Prokrym. 1991. Release density, efficiency and disappearance of *Trichogramma nubilale* for control of european corn borer. Entomophaga 36: 105-113.

Antolin, M. F. and D. R. Strong. 1987. Long-distance dispersal by a parasitoid (*Anagrus delicatus*, Mymaridae) and its host. Oecologia 73: 288-292.

Bedford, I. D., R. W. Briddon, J. K. Brown, R. C. Rosell, and P. G. Markham. 1994. Gemini virus transmission and biological characterization of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Ann. Appl. Biol. 125: 311-325.

Bellows, T. S., Jr. and K. Arakawa. 1988. Dynamics of preimaginal populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) and *Eretmocerus* sp. (Hymenoptera: Aphelinidae) in southern California cotton. Environ. Entomol. 17: 483-487.

Bellows, T. S., Jr., T. M. Perring, and D. H. Headrick. 1994. Parasitism of silverleaf whitefly in California crops and weeds., pp. 122. *In* T. J. Henneberry, T. C. Toscano, R. M. Faust , and J. R. Coppedge [eds.], Silverleaf whitefly, (formerly sweetpotato whitefly, strain B) supplement to the five-year national research and action plan., Vol. ARS-125 USDA-ARS, Beltsville, MD.

Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994b. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 87: 195-206.

Bennison, J. A. and S. P. Corless. 1993. Biological control of aphids on cucumbers: further development of open rearing units or "banker plants" to aid establishment of aphid natural enemies. IOBC-WPRS Bull. 16: 5-8.

Bethke, J. A., T. D. Paine, and G. S. Nuessly. 1991. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* (Homoptera: Aleyrodidae) on cotton and poinsettia. Ann. Entomol. Soc. Am. 84: 407-411.

Blackmer, J. L. and P. L. Phelan. 1991. Behavior of *Carpophilus hemipterus* in a vertical flight chamber: transition from phototactic to vegetative orientation. Entomologia Experimentalis et Applicata 58: 137-148.

Blua, M. J. and N. C. Toscano. 1994. *Bemisia argentifolii* (Homoptera: Aleyrodidae) development and honeydew production as a function of cotton nitrogen status. Environ. Entomol. 23: 316-321.

Brown, J. K. 1992. Biotypes of the sweetpotato whitefly: a current perspective, pp. 665-670. *In* D. J. Herber and D. A. Richter [eds.], Proceedings of Beltwide Cotton Production Research Conferences., Vol. 2 National Cotton Council of America, Memphis, TN.

Brown, J. K., D. R. Frohlich, and R. C. Rosell. 1995. The sweetpotato or silverleaf whiteflies: biotypes of *Bemisia tabaci* or a species complex. Ann. Rev. Entomol. 40: 511-534.

Brown, J. K. and P. F. O'Leary. 1994. Whitefly-transmitted geminiviruses. California-Arizona Cotton 30: 20-22.

Burbutis, P. P. and C. H. Koepke. 1981. European corn borer control in peppers by *Trichogramma nubilale*. J. of Econ. Entomol. 74: 246-247.

Butler, G. D., Jr., T. J. Henneberry, and T. E. Clayton. 1983. *Bemisia tabaci* (Homoptera: Aleyrodidae): development, oviposition, and longevity in relation to temperature. Ann. Entomol. Soc. Am. 76: 310-313.

Byrne, D. N. and T. S. Bellows, Jr. 1991. Whitefly biology., Vol. 36. Annu. Rev. Entomol.

Byrne, D. N., T. S. Bellows, Jr., and M. P. Parrella. 1990. Whiteflies in agricultural systems, pp. 227-262. *In* D. Gerling [ed.], Whiteflies: their Bionomics, Pest Status and Management. Intercept, Andover, UK.

Byrne, D. N. and N. F. Hadley. 1988. Particulate surface waxes of whiteflies: morphology, composition and waxing behaviour. Physiol. Entomol. 13: 267-276.

Byrne, D. N., R. J. Rathman, T. V. Orum, and J. C. Palumbo. 1996. Localized migration and dispersal by the sweetpotato whitefly, *Bemisia tabaci*. Oecologia 105: 320-328.

Byrne, F. J., M. Cahill, I. Denholm, and A. L. Devonshire. 1995. Biochemical identification of interbreeding between B-type and non B-type strains of the tobacco whitefly *Bemisia tabaci*. Biochem. Genet. 33: 13-23.

Caltagirone, L. E. 1981. Landmark examples in biological control. Annu. Rev. Entomol. 26: 213-232.

Chu, C. C., T. J. Henneberry, M. A. Boykin, and A. C. Cohen. 1997. A modified new whitefly trap (CC trap) to increase whitefly adult catches, *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conferences., Vol. 2, Proceedings Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Cook, S. P. and F. P. Hain. 1992. The influence of self-marking with fluorescent powders on adult bark beetles (Coleoptera: Scolytidae). J. Entomol. Sci. 27: 269-279.

Corbett, A. and R. E. Plant. 1993. Role of movement in the response of natural enemies to agroecosystem diversification: a theoretical evaluation. Environ. Entomol. 22: 519-531.

Corbett, A. and J. A. Rosenheim. 1996a. Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape. Ecol. Entomol. 21: 155-164.

Corbett, A. and J. A. Rosenheim. 1996b. Quantifying movement of a minute parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae), using fluorescent dust marking and recapture. Biol. Control 6: 35-44.

Costa, H. S. and J. K. Brown. 1991. Variation in biological characteristics and esterase patterns among populations of *Bemisia tabaci*, and the association of one population with silverleaf symptom induction. Entomol. Exp. Appl. 61: 211-219.

Cronin, J. T. and D. R. Strong. 1990. Density-independent parasitism among host patches by *Anagrus delicatus* (Hymenoptera: Mymaridae): experimental manipulations of hosts. J. of Anim. Ecol. 59: 1019-1026.

Crumpacker, D. W. 1974. The use of micronized fluorescent dusts to mark adult *Drosophila pseudoobscura*. Am. Midl. Nat. 91: 118-29.

Davidson, E. W. and W. Jones. 1999. Successful rearing of parasitoid wasps on *Bemisia argentifolii* cultured on artificial diet, pp. 69. *In* T. J. Henneberry and R. M. Faust [eds.], Silverleaf Whitefly: 1999, Supplement to the five year national research, action and technology transfer plan , second annual review, Albuquerque, NM., Vol. 1999-01, USDA-ARS, Beltsville.

Davidson, E. W., M. D. Lavine, M. Mathews, and D. L. Hendrix. 1999. Improvements to the Artificial Feeding System for *Bemisia argentifolii*, pp. 23. *In* T. J. Henneberry and R. M. Faust [eds.], Silverleaf Whitefly: 1999, Supplement to the five year national research, action and technology transfer plan , second annual review, Albuquerque, NM., Vol. 1999-01, USDA-ARS, Beltsville.

Debach, P. and D. Rosen. 1991. Biological control by natural enemies. Cambridge University Press, Cambridge, U.K.

Denholm, I., M. Cahill, F. J. Byrne, and A. L. Devonshire. 1996. Progress with documenting and combating insecticide resistance in *Bemisia*, pp. 577-603. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia* : 1995 Taxonomy, Biology, Damage, Control and Management. Intercept Ltd., Andover, Hants, UK.

Dennehy, T. J. and L. Williams. 1997. Management of resistance in *Bemisia* in Arizona cotton. Pest. Sci. 51: 398-406.

Diehl, J. W., C. G. Jackson, J. L. Flexner, J. W. Debolt, and W. A. Jones. unpublished manuscript. Augmentative releases of two indigenous parasitoids to control *Lygus* spp. in seed alfalfa.

Dingle, H. 1996. Migration, the biology of life on the move. Oxford University Press, New York.

Dittrich, V., S. Uk, and G. H. Ernst. 1990. Chemical control and insecticide resistance of whiteflies, pp. 263-285. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept, Andover, UK.

Ellsworth, C. P. 1999. Whitefly management in cotton - a historical perspective, pp. 121. *In* T. J. Henneberry and R. M. Faust [eds.], Silverleaf Whitefly: 1999, Supplement to the five year national research, action and technology transfer plan , second annual review, Albuquerque, NM., Vol. 1999-01, USDA-ARS, Beltsville.

Enkegaard, A. 1993. The poinsettia strain of the cotton whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), biological and demographic parameters on poinsettia (*Euphorbia pulcherrima*) in relation to temperature. Bull. Entomol. Res. 83: 535-546.

Faust, R. M. 1992. Conference report and five-year national research and action plan for development of management and control methodology for the sweetpotato whitefly, Vol. ARS-107., USDA-ARS, Beltsville, Md.

Flinn, P. W. and D. W. Hagstrum. 1995. Simulation model of *Cephalonomia waterstoni* (Hymenoptera: Bethylidae) parasitizing the rusty grain beetle (Coleoptera: Cucujidae). Environ. Entomol. 24: 1608-1615.

Fransen, J. J. 1990. Natural enemies of whiteflies: fungi, pp. 187-210. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status and management. Intercept, Andover, UK.

Freeman, G. H. 1977. A model relating numbers of dispersing insects to distance and time. J. of Appl. Ecol. 14: 477-487.

Freund, J. R. and R. C. Littell. 1991. SAS system for regression, second edition. SAS Institute Inc., Cary, NC.

Garcia-Salazar, C. and D. A. Landis. 1997. Marking *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) with fluorescent marker dust and its effect on survival and flight behavior. J. Econ. Entomol. 90: 1546-1550.

Gerling, D. 1966. Studies with whitefly parasites of southern California. II. *Eretmocerus californicus* Howard (Hymenoptera: Aphelinidae). Can. Entomol. 98: 1316-1329.

Gerling, D. 1967. Bionomics of the whitefly-parasite complex associated with cotton in southern California (Homoptera: Aleurodidae; Hymenoptera: Aphelinidae). Ann. Entomol. Soc. Am. 60: 1306-1321.

Gerling, D. 1986. Natural enemies of *Bemisia tabaci*, biological characteristics and potential as biological control agents: a review. Agric. Ecosyst. Environ. 17: 99-110.

Gerling, D. 1990. Natural enemies of whiteflies: predators and parasitoids, pp. 147-186. *In* D. Gerling [ed.], Whiteflies: their Bionomics, Pest Status and Management. Intercept, Andover, UK.

Gerling, D. and V. Kravchenko. 1996. Pest management of *Bemisia* out of doors, pp. 667-680. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia* 1995, taxonomy, biology, damage, control and management. Intercept, Andover, Hants, UK.

Gerling, D. and R. T. Mayer. 1996. *Bemisia* 1995: taxonomy, biology, damage, control and management, pp. v-vi. *In* D. Gerling and R. T. Mayer [eds.], Intercept, Andover, UK,.

Gerling, D., T. Orion, and Y. Delarea. 1990. *Eretmocerus* penetration and immature development: a novel approach to overcome host immunity. Arch. Insect. Biochem. Physiol. 13: 247-253.

Giles, D. K., J. Gardner, and H. E. Studer. 1995. Mechanical release of predacious mites for biological pest control in strawberries. Transactions of the ASAE 38: 1289-1296.

Gill, R. J. 1990. The morphology of whiteflies, pp. 13-46. *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status, and management. Intercept, Andover, UK.

Godfray, H. C. J. 1994. Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton.

Goolsby, J. A. and M. A. Ciomperlik. 1997. Novel method for field delivery of parasitoids in row crops, pp. 144. *In* T. J. Henneberry, N. C. Toscano, T. M. Perring, and R. M. Faust [eds.], Silverleaf whitefly: 1997 supplement to the five-year national action plan: progress review, technology transfer, and new research and action plan (1997-2001)., Vol. 1997-02, USDA-ARS, Beltsville.

Goolsby, J. A. and M. A. Ciomperlik. 1998. Field evaluation of banker plants for field delivery of parasitoids in cucurbit crops, pp. 57. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and D. Kopp [eds.], Vol. 1998-01, USDA-ARS, Beltsville.

Goolsby, J. A., M. A. Ciomperlik, B. C. Legaspi, J. C. Legaspi, and L. E. Wendel. 1998. Laboratory and field evaluation of exotic parasitoids of *Bemisia tabaci* (Gennadius) (Biotype 'B') (Homoptera: Aleyrodidae) in the lower Rio Grande Valley of Texas. Biol. Control 12: 127-135.

Gould, J. R., T. S. Bellows, Jr., and T. D. Paine. 1992. Population dynamics of *Siphoninus phillyreae* in California in the presence and absence of a parasitoid, *Encarsia partenopea*. Ecol. Entomol. 17: 127-134.

Greathead, D. J. 1986. Parasitoids in classical biological control, pp. 289-318. *In* J. K. Waage and D. J. Greathead [eds.], Insect parasitoids. Academic Press, London.

Gruenhagen, N. M., T. M. Perring, L. G. Bezark, D. M. Daoud, and T. F. Leigh. 1993. Silverleaf whitefly present in the San Joaquin Valley. Calif. Agric. 47: 4-6.

Hagler, J. R. and S. E. Naranjo. 1994. Determining the frequency of heteropteran predation on sweetpotato whitefly and pink bollworm using multiple ELISAs. Entomol. Exp. Appl. 72: 59-66.

Hagler, J. R. and S. E. Naranjo. 1996. Using a pest-specific monoclonal antibody library to evaluate potential biological control agents: a case study, pp. 383-399. *In* W. Symondson and J. Liddell [eds.], The ecology of agricultural pests: biochemical approaches. Chapman Hall, London.

Hall, R. W. and L. E. Ehler. 1979. Rate of establishment of natural enemies in classical biological control. ESA Bulletin 25: 280-282.

Hassell, M. P. 1978. The dynamics of arthropod predator-prey systems, Vol. 13. Monographs in population biology. Princeton University Press, Princeton, NJ.

Hassell, M. P. 1982. Patterns of parasitism by insect parasitoids in patchy environments. Ecol. Entomol. 7: 365-377.

Hawkes, C. 1972. The estimation of the dispersal rate of the adult cabbage root fly (*Erioischia brassicae* (Bouche) in the presence of a brassica crop. J. of Appl. Ecol. 9: 617-632.

Headrick, D. H., T. S. Bellows, Jr., and T. M. Perring. 1995. Behaviors of female *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on sweet potato. Environ. Entomol. 24: 412-422.

Headrick, D. H., T. S. Bellows, Jr., and T. M. Perring. 1996. Behaviors of female *Eretmocerus* sp. nr. *californicus* (Hymenoptera: Aphelinidae) attacking *Bemisia argentifolii* (Homoptera: Aleyrodidae) on cotton, *Gossypium hirustum*, (Malavaceae) and melon, *Cucumis melo* (Cucurbitaceae). Biol. Control 6: 64-75.

Heads, P. A. and J. H. Lawton. 1983. Studies on the natural enemy complex of the holly leaf-miner: the effects of scale on the detection of aggregative responses and the implications for biological control. Oikos 40: 267-276.

Heinz, K. M., L. Nunney, and M. P. Parrella. 1993. Toward predictable biological control of *Liriomyza trifolii* (Diptera: Agromyzidae) infesting greenhouse cut chrysanthemums. Environ. Entomol. 22: 1217-1233.

Heinz, K. M. and M. P. Parrella. 1998. Host location and utilization by selected parasitoids of *Bemisia argentifolii* (Homoptera: Aleyrodidae): implications for augmentative biological control. Environ. Entomol. 27: 773-784.

Hempel, G. E., J. A. Rosenheim, and M. Mangel. 1996. Predation on adult *Aphytis* parasitoids in the field. Oecologia 110: 346-352.

Hendrix, D. L., T. L. Steele, and H. H. Perkins, Jr. 1996. *Bemisia* honeydew and sticky cotton, pp. 189-199. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia*: 1995 taxonomy, biology, damage, control and management. Intercept, Andover, Hants, UK.

Henneberry, T. J., D. H. Hendrix, H. H. Perkins, S. Naranjo, H. M. Flint, D. H. Akey, L. F. Jech, and R. A. Burke. 1995. Silverleaf whitefly, sticky cotton, and cotton lint yields, pp. 836-838. *In* D. A. Richter and J. Armour [eds.], Proceedings Beltwide Cotton Production Conferences., Vol. 2, National Cotton Council of America, Memphis, TN.

Henneberry, T. J. and N. C. Toscano. 1997. Current status of silverleaf and sweetpotato whiteflies in the United States. FAO Plant Production and Protection Paper 143, Rome, Italy.

Henneberry, T. J., N. C. Toscano, R. M. Faust, and J. R. Coppedge. 1993. Sweetpotato whitefly: 1993 Supplement to the five-year national research and action plan-first annual review held in Tempe, Arizona, January 18-21, 1993. Vol. ARS-112, USDA-ARS, Beltsville.

Henneberry, T. J., N. C. Toscano, T. M. Perring, and R. M. Faust. 1997. Introduction, brief history of the program and current status, pp. 7. *In* T. J. Henneberry, N. C. Toscano, T. M. Perring, and R. M. Faust [eds.], Silverleaf whitefly: 1997 supplement to the five-year national research and action plan: progress review, technology transfer, and new research and action plan (1997-2001). Vol. 1997-02, USDA-ARS, Beltsville.

Hoddle, M. S., R. G. Van Driesche, J. S. Elkinton, and J. P. Sanderson. 1998. Discovery and utilization of *Bemisia argentifolii* patches by *Eretmocerus eremicus* and *Encarsia formosa* (Beltsville strain) in greenhouses. Entomol. Exp. Appl. 87: 15-28.

Hoelmer, K. A. 1996. Whitefly parasitoids: can they control field populations of *Bemisia*?, pp. 451-476. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia*: 1995 taxonomy, biology, damage, control and management. Intercept, Andover, Hants, UK.

Hoelmer, K. A. 1998. Comparative field cage evaluations of top-performing introduced parasitoids in desert cantaloupes, pp. 68. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and D. Kopp [eds.], Silverleaf whitefly: national research, action and technology transfer plan, 1997-2001; first annual review of the second five-year plan., Vol. 1998-01, USDA-ARS, Beltsville.

Hoelmer, K. A., A. A. Kirk, and G. S. Simmons. 1999. An overview of natural enemy explorations and evaluations for *Bemisia* in the U.S., pp. 689-696. Fifth International Conference on Pests in Agriculture. Assoc. Nat. Prot. Plantes, Montpellier Fr.

Hoelmer, K. A., L. S. Osborne, F. D. Bennett, and R. K. Yokomi. 1994. Biological control of sweetpotato whitefly in Florida., *In* D. Rosen, F. D. Bennett, and J. L. Capinera [eds.], Pest Management in the Subtropics: Biological Control: A Florida Perspective. Intercept Ltd., Andover, UK.

Hoelmer, K. A., L. S. Osborne, and R. K. Yokomi. 1991. Foliage disorders in Florida associated with feeding by sweetpotato whitefly, *Bemisia tabaci*. Fla. Entomol. 74: 162-166.

Hoelmer, K. A., W. J. Roltsch, C. C. Chu, and T. J. Henneberry. 1998a. Selectivity of whitefly traps in cotton for *Eretmocerus eremicus* (Hymenoptera: Aphelinidae), a native parasitoid of *Bemisia argentifolii* (Homoptera: Aleyrodidae). Environ. Entomol. 27: 1039-1044.

Hoelmer, K. A., W. J. Roltsch, and G. S. Simmons. 1998b. Establishment of introduced *Eretmocerus* species in Imperial Valley, CA, pp. 70. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and D. Kopp [eds.], Silverleaf whitefly: national research, action and technology transfer plan, 1997-2001; first annual review of the second five-year plan., Vol. 1998-01 USDA-ARS, Beltsville.

Holmes, E. E. 1993. Are diffusion models too simple? A comparison with telegraph models of invasion. Am. Nat. 142: 779-795.

Hopper, K. R. and R. T. Roush. 1993. Mate finding, dispersal, number released, and the success of biological control introductions. Ecol. Entomol. 18: 321-331.

Horowitz, A. R., G. Forer, and I. Ishaaya. 1994. Managing resistance in *Bemisia tabaci* in Israel with emphasis on cotton. Pestic. Sci. 42: 113-121.

Horowitz, A. R. and I. Ishaaya. 1994. Managing resistance to insect growth regulators in the sweetpotato whitefly (Homoptera: Aleyrodidae). J. of Econ. Entomol. 87: 866-871.

Horowitz, A. R. and I. Ishaaya. 1996. Chemical control of *Bemisia* - management and application., pp. 537-556. *In* D. Gerling and R. T. Mayer [eds.], *Bemisia* : 1995 taxonomy, biology, damage, control and management. Intercept Ltd., Andover, Hants. UK.

Huffaker, C. B., F. J. Simmonds, and J. E. Laing. 1976. The theoretical and empirical basis of biological control, pp. 42-80. *In* C. B. Huffaker and P. S. Messenger [eds.], Theory and practice of biological control. Academic Press, New York.

Jancovich, J. K., E. W. Davidson, and D. L. Hendrix. 1997. Feeding chamber and diet for culture of nymphal *Bemisia argentifolii* (Homoptera: Aleyrodidae). J. Econ. Entomol. 90: 628-633.

Jones, W. A. and S. M. Greenberg. 1998. Suitability of *Bemisia argentifolii* (Homoptera: Aleyrodidae) instars for the parasitoid *Eretmocerus mundus* (Hymenoptera: Aphelinidae). Environ. Entomol. 27: 1569-1573.

Jones, W. A., D. A. Wolfenbarger, and A. A. Kirk. 1995. Response of adult parasitoids of *Bemisia tabaci* (Hom.: Aleyrodidae) to leaf residues of selected cotton insecticides. Entomophaga 40: 153-162.

Kapadia, M. N. and S. N. Puri. 1991. Toxicity of different insecticides against two parasitoids of *Bemisia tabaci* (Gennadius) and their persistence against *Encarsia transvena* (Timberlake). Int. J. Trop. Agric. 9: 81-84.

Kareiva, P. 1990a. Establishing a foothold for theory in biological practice: using models to guide experimental design and release protocols, pp. 65-81. *In* R. R. Baker and P. E. Dunn [eds.], New directions in biological control: alternatives for suppressing agricultural pests and diseases. Alan R . Liss, Inc, New York.

Kareiva, P. 1990b. The spatial dimension in pest-enemy interactions, pp. 213-227. *In* M. Mackauer and L. Ehler [eds.], Critical issues in biological control. Intercept Ltd., Andover, Hants.

Kareiva, P. K. and G. Odell. 1987. Swarms of predators exhibit "preytaxis" if individual predators use area-restricted search. Am. Nat. 130: 233-269.

Kareiva, P. M. 1981. Non-migratory movement and the distribution of herbivorous insects: experiments with plant spacing and the application of diffusion models to mark-recapture data. Ph.D. Dissertation, Cornell University, Ithaca.

Kareiva, P. M. 1983. Local movement in herbivorous insects: applying a passive diffusion model to mark-recapture field experiments. Oecologia 57: 322-327.

Keller, M. A. and W. J. Lewis. 1985. Movements by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) released into cotton. Suppl. Southwest. Entomol. 99-109.

Keller, M. A., W. J. Lewis, and R. E. Stinner. 1985. Biological and practical significance of movement by *Trichogramma* species: a review. Suppl. Southwest. Entomologist 138-155.

Kennedy, J. S. 1985. Migration, behavioral and ecological, pp. 5-26. *In* M. A. Rankin [ed.], Migration: mechanisms and adaptive significance. Suppl. Vol. 27, Contrib. Marine Science.

King, E. G. and J. Powell. 1992. Propagation and release of natural enemies for control of cotton insect and mite pests in the United States. Crop Prot. 497-506.

Kot, M., M. A. Lewis, and P. van den Driessche. 1996. Dispersal data and the spread of invading organisms. Ecol. 77: 2027-2042.

Levin, S. A. 1981. The role of theoretical ecology in the description and understanding of populations in heterogeneous environments. Am. Zool. 21: 865-875.

Littell, R. C., R. J. Freund, and P. C. Spector. 1991. SAS system for linear models, third edition. SAS Institute Inc, Cary, NC.

Louda, S. M., D. Kendall, J. Connor, and D. Simberloff. 1997. Ecological effects of an insect introduced for the biological control of weeds. Science 277: 1088-1090.

Luck, R. F. 1990. Evaluation of natural enemies for biological control: A behavioral approach. TREE 5: 196-199.

Luck, R. F., B. M. Shepard, and P. E. Kenmore. 1988. Experimental methods for evaluating arthropod natural enemies. Annu. Rev. Entomol. 33: 367-391.

Messing, R. H., L. M. Klungness, M. Purcell, and T. T. Y. Wong. 1993. Quality control parameters of mass-reared opiine parasitoids used in augmentative biological control of tephritid fruit flies. Biol. Control 3: 140-147.

Miller, M. and G. Aplet. 1993. Biological control: a little knowledge is a dangerous thing. Rutgers Law Review 45: 285-334.

Murdoch, W. W. and A. Stewart-Oaten. 1989. Aggregation by parasitoids and predators: effects on equilibrium and stability. Am. Nat. 134: 288-310.

Naranjo, S. E. 1990. Influence of two mass-marking techniques on survival and flight behavior of *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae). J. of Econ. Entomol. 83: 1360-1364.

Naranjo, S. E., C. C. Chu, and T. J. Henneberry. 1996. Economic injury levels for *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton: impact of crop price, control costs, and efficacy of control. Crop Prot. 15: 770-788.

Naranjo, S. E. and P. C. Ellsworth. 1999. Mortality factors affecting whitefly populations in Arizona cotton management systems: life table analysis. *In* . [ed.], Cotton: a College of Agriculture Report., Vol. P-113, University of Arizona, Tucson.

Naranjo, S. E., P. C. Ellsworth, and J. W. Diehl. 1997. Partial life table studies of *Bemisia* in cotton fields subject to different management strategies, pp. 71. *In* T. J. Henneberry, N. C. Toscano, T. M. Perring, and R. M. Faust [eds.], In silverleaf whitefly: 1997 supplement to the five-year national research and action plan., Vol. 1997-02, USDA-ARS, Beltsville.

Naranjo, S. E., P. C. Ellsworth, and J. W. Diehl. 1998a. Comparative life table studies of *Bemisia* under different management strategies in cotton, pp. 17. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and D. Kopp [eds.], Silverleaf whitefly: 1997: National research, action and technology transfer plan, 1997-2201; first annual review of the second five-year plan., Vol. 1998-01 USDA-ARS, Beltsville.

Naranjo, S. E. and H. M. Flint. 1994. Spatial distribution of preimaginal *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development of fixed-precision sequential sampling plans. Environ. Entomol. 23: 254-266.

Naranjo, S. E. and H. M. Flint. 1995. Spatial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. Environ. Entomol. 24: 261-270.

Naranjo, S. E. and J. R. Hagler. 1997. Conservation of natural enemies relative to use of insect growth regulators for control of sweetpotato whitefly, pp. 318-323. Cotton: a College of Agriculture Report., Vol. P-108, Cooperative Extension, University of Arizona, Tucson.

Naranjo, S. E., J. R. Hagler, and P. C. Ellsworth. 1998b. Whitefly management in Arizona: conservation of natural enemies relative to insecticide regime, pp. 319-323. Cotton: a College of Agriculture Report., Vol. P-112, University of Arizona, Tucson.

Natwick, E. T. 1993. Silverleaf whitefly control in cotton using various insecticides in the Imperial Valley of California, pp. 722-727. *In* D. J. Herber and D. A. Richter [eds.], 1993 Proceedings Beltwide Cotton Conferences, January 10-14 1993., Vol. 2, National Cotton Council of America, Memphis, TN.

Natwick, E. T. and F. G. Zalom. 1984. Surveying sweetpotato whitefly in the Imperial Valley. Calif. Agric. 38: 11.

Newton, P. J. 1988. Movement and impact of *Trichogrammatoidea cryptophlebiae* Nagaraja (Hymenoptera: Trichogrammatidae) in citrus orchards after inundative releases against the false codling moth, *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Bull. of Entomol. Res. 78: 85-99.

Noldus, L. P. J. J. and J. C. van Lenteren. 1990. Host aggregation and parasitoid behaviour: biological control in a closed system, pp. 229-262. *In* M. Mackauer, L. E. Ehler, and J. Roland [eds.], Critical issues in biological control. Intercept Ltd., Andover, Hants.

Obrycki, J. J., L. C. Lewis, and D. B. Orr. 1997. Augmentative releases of entomophagous species in annual cropping systems. Biol. Control 10: 30-36.

Ohnesorge, B., N. Sharaf, and T. Allawi. 1980. Population studies on the tobacco whitefly *Bemisia tabaci* Genn. (Homoptera; Aleyrodidae) during the winter season. I. The spatial distribution on some host plants. Z. Angew. Entomol. 90: 226-232.

Okubo, A. 1980. Diffusion and ecological problems: mathematical models. Springer-Verlag, Heidelberg-Berlin-New York.

Oloumi-Sadeghi, H. and E. Levine. 1990. A simple, effective, and low-cost method for mass marking adult western corn rootworms (Coleoptera: Chrysomelidae). J. Entomol. Sci. 25: 170-175.

O'Neil, R. 1990. The functional response and the theory of biological control, *In* R. Baker and P. Dunn [eds.], New directions in biological control. Alan R. Liss, Inc, New York.

Onillon, J. C. 1990. The use of natural enemies for the biological control of whiteflies, *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status, and management. Intercept, Andover, UK.

Palumbo, J. C. 1999. A grower initiated model for sustaining chemical efficacy across commodities, pp. 3. *In* T. J. Henneberry and R. M. Faust [eds.], Silverleaf Whitefly: 1999, Supplement to the five year national research, action and technology transfer plan , second annual review, Albuquerque, NM., Vol. 1999-01, USDA-ARS, Beltsville.

Palumbo, J. C., A. Tonhasca, Jr., and D. N. Byrne. 1994. Sampling plans and action thresholds for whiteflies on spring melons, Univ. Arizona IPM Series No. 1., University of Arizona, Tucson.

Parrella, M. P., K. M. Heinz, and L. Nunney. 1992. Biological control through augmentative releases of natural enemies: a strategy whose time has come. Am. Entomol. 172-179.

Pedgley, D. E. 1993. Managing migratory insect pests-- a review. Int. J. Pest Manag. 39: 3-12.

Perring, T. M., A. D. Cooper, R. J. Rodriguez, C. A. Farrar, and T. S. Bellows, Jr. 1993a. Identification of a whitefly species by genomic and behavioral studies. Science 259: 74-77.

Perring, T. M., C. A. Farrar, T. S. Bellows, Jr., A. D. Cooper, and R. J. Rodriguez. 1993b. Evidence for a new species of whitefly: UCR findings and implications. Calif. Agric. 47: 7-8.

Pickett, C. H., W. L. Abel, C. Riccomini, G. S. Simmons, and J. A. Goolsby. 1998a. Recovery and releases of parasites for biological control of Bemisia sp. in the San Joaquin Valley, California, pp. 75. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and D. Kopp [eds.], Silverleaf whitefly: 1997: National research, action and technology transfer plan, 1997-2201; First annual review of the second five-year plan., Vol. 1998-01, USDA-ARS, Beltsville.

Pickett, C. H. and R. L. Bugg. 1998. Enhancing biological control : habitat management to promote natural enemies of agricultural pests. University of California Press, Berkeley.

Pickett, C. H., G. S. Simmons, and J. A. Goolsby. 1998b. Augmentative biological control using transplants, pp. 223-226. *In* M. S. Hoddle [ed.], California Conference on Biological Control, June 10-11, 1998. University of California, University of California, Berkeley.

Plant, R. E. and R. T. Cunningham. 1991. Analyses of the dispersal of sterile mediterranean fruit flies (Diptera: Tephritidae) released from a point source. Environ. Entomol. 20: 1493-1503.

Powell, D. A. and T. S. Bellows, Jr. 1992a. Adult longevity, fertility and population growth rates for *Bemisia tabaci* (Genn) (Hom, Aleyrodidae) on two host plant species. J. Appl. Entomol. 113: 68-78.

Powell, D. A. and T. S. Bellows, Jr. 1992b. Development and reproduction of two populations of *Eretmocerus* species (Hymenoptera: Aphelinidae) on *Bemisia tabaci* (Homoptera: Aleyrodidae). Environ. Entomol. 21: 651-658.

Prabhaker, N., N. C. Toscano, T. M. Perring, G. Nuessley, K. Kido, and R. R. Youngman. 1992. Resistance monitoring of the sweetpotato whitefly (Homoptera: Aleyrodidae) in the Imperial Valley of California. J. Econ. Entomol. 85: 1063-1068.

Quicke, D. L. J. 1997. Parasitic wasps. Chapman & Hall, London.

Roltsch, W. J. and C. H. Pickett. 1994. Silverleaf whitefly natural enemy refuges in Imperial County., pp. 146. *In* T. J. Henneberry, N. C. Toscano, R. M. Faust, and J. R. Coppedge [eds.], Silverleaf whitefly: 1994 supplement to the five-year national research and action plan., Vol. 125, USDA-ARS, Beltsville.

Roltsch, W. J. and G. S. Simmons. 1997. Release and establishment of exotic natural enemies in home gardens and agricultural field refuges in Imperial Valley, CA, pp. 167. *In* T. J. Henneberry, N. C. Toscano, T. M. Perring, and R. M. Faust [eds.], Silverleaf whitefly: 1997 supplement to the five-year national research and action plan: progress review, technology transfer, and new research and action plan (1997-2001)., Vol. 1997-02, USDA-ARS, Beltsville.

Rose, M. and P. Debach. 1991-1992. Biological control of *Parabemisia myricae* (Kuwana) (Homoptera: Aleyrodidae) in California. Israel. J. Entomol. 25-26: 73-95.

Rose, M. and G. Zolnerowich. 1997. *Eretmocerus* Haldeman (Hymenoptera: Aphelinidae) in the United States, with descriptions of new species attacking *Bemisia* (*tabaci* complex) (Homoptera: Aleyrodidae). Proc. Entomol. Soc. Wash. 99: 1-27.

Rosenheim, J. A. 1989. Behaviorally mediated spatial and temporal refuges from a cleptoparasite, *Argochrysis armilla* (Hymenoptera: Chrysididae), attacking a ground-nesting wasp, *Ammophila dysmica* (Hymenoptera: Sphecidae). Behav. Ecol. Sociobiol. 25: 335-348.

Rudd, N. T. and P. B. McEvoy. 1996. Local dispersal by the cinnabar moth *Tyria jacobaeae*. Ecol. Appl. 6: 285-297.

Rudd, W. G. and R. W. Gandour. 1985. Diffusion model for insect dispersal. J. Econ. Entomol. 78: 295-301.

SAS Institute Inc. 1989. SAS/STAT user's guide, version 6, fourth edition, Vol. 1., SAS Institute Inc., Cary, NC,

Shimron, O., A. Hefetz, and D. Gerling. 1992. Arrestment responses of *Eretmocerus* species and *Encarsia deserti* (Hymenoptera: Aphelinidae) to *Bemisia tabaci* honeydew. J. Insec. Behav. 5: 517-526.

Simberloff, D. and P. Stiling. 1996. How risky is biological control ? Ecol. 77: 1965-1974.

Simmons, A. L., L. Williams, III, and T. J. Dennehy. 1997. Investigations of two insect growth regulators against Arizona whitefly populations, pp. 1248-1252. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conferences., Vol. 2, National Cotton Council, Memphis, TN.

Simmons, G. S., K. Hoelmer, R. Staten, T. Boratynski, and E. Natwick. 1997. Seasonal inoculative biological control with parasitoids of *Bemisia* infesting cantaloupe in the Imperial Valley of California: a report on three years of investigations, pp. 170. *In* T. J. Henneberry, N. C. Toscano, T. M. Perring, and R. M. Faust [eds.], Silverleaf whitefly: 1997 supplement to the five-year national research and action plan: progress review, technology transfer, and new research and action plan (1997-2001)., Vol. 1997-02, USDA-ARS, Beltsville.

Simmons, G. S. and O. P. J. M. Minkenberg. 1994. Field-cage evaluation of augmentative biological control of *Bemisia argentifolii* (Homoptera: Aleyrodidae) in southern California cotton with the parasitoid *Eretmocerus* nr. *californicus* (Hymenoptera: Aphelinidae). Environ. Entomol. 23: 1552-1557.

Sivasupramaniam, T. J., T. J. Dennehy, and L. Williams, III. 1997. Management of pyrethroid-resistant whiteflies in Arizona cotton: selection, cross-resistance and dynamics, pp. 1252-1259. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conferences., Vol. 2, National Cotton Council, Memphis, TN.

Sokal, R. R. and F. J. Rohlf. 1981. Biometry, 2 ed. W.H. Freeman & Co., New York.

Southwood, T. R. E. 1978. Ecological methods. Chapman and Hall, London.

Sterling, W., T. Wilson, and F. Gilstrap. 1992. Contaminated experiments: a flaw that underestimates the effects of natural enemies. Am. Entomol. 38:88.

Stern, V. M. and A. Mueller. 1968. Techniques of marking insects with micronized fluorescent dust with especial emphasis on marking millions of *Lygus hesperus* for dispersal studies. J. Econ. Entomol. 61: 1232-1237.

Stern, V. M., E. I. Schlinger, and W. R. Bowen. 1965. Dispersal studies of *Trichogramma semifumatum* (Hymenoptera: Trichogrammatidae) tagged with Radioactive Phosphorus. Ann. Entomol. Soc. Am. 58: 234-240.

Stiling, P. 1993. Why do natural enemies fail in classical biological control programs? Am. Entomol. 31-37.

Stinner, R. E. 1977. Efficacy of inundative releases. Ann. Rev. of Entomol. 22: 515-531.

Strong, D. R. 1997. Fear no weevil? Science 277: 1058-1059.

Strong, D. R., J. H. Lawton, and R. Southwood, Sir. 1984. Insects on plants: community patterns and mechanisms. Harvard University Press, Cambridge.

Summy, K. R., F. E. Gilstrap, W. G. Hart, J. M. Caballero, and I. Saenz. 1983. Biological control of citrus blackfly (Homoptera: Aleyrodidae) in Texas. Environ. Entomol. 12: 782-786.

Sundaramurthy, V. T. 1992. Upsurgence of whitefly *Bemisia tabaci* Gen. in the cotton ecosystem in India. Outlook Agric. 21: 109-115.

Tawfik, M. F. S., K. T. Awadallah, M. Hafez, and A. A. Sarhan. 1978. Biology of the aphelinid parasite *Eretmocerus mundus* Mercet. Bull. Soc. Entomol. Egypte 33-48.

Taylor, L. R. 1984. Assessing and interpreting the spatial distributions of insect populations. Ann. Rev. Entomol. 29: 321-357.

Taylor, R. A. J. 1978. The relationship between density and distance of dispersing insects. Ecol. Entomol. 3: 63-70.

Taylor, R. A. J. 1980. A family of regression equations describing the density distributions of dispersing organisms. Nature 286: 53-55.

Thomas, P. E., S. Marco, and G. Reisenhauer. 1997. Effect of marking aphids with fluorescent powders on virus vectoring activities. J. Agric. Entomol. 14: 187-198.

Tonhasca, A., J. C. Palumbo, and D. N. Byrne. 1994. Aggregation patterns of *Bemisia tabaci* (Homoptera: Aleyrodidae) in response to insecticide applications. Entomol. Exp. Appl. 72: 265-272.

Turchin, P. 1990. Rarity of density dependence or population regulation with lags? Nature 344: 660-663.

Turchin, P. T. 1998. Quantitative analysis of movement, measuring and modeling population redistribution in animals and plants. Sinauer, Sunderland, Mass.

Turchin, P. T. and P. M. Kareiva. 1989. Aggregation in *Aphis varians*: an effective strategy for reducing predation risk. Ecol. 70: 1008-1016.

Turchin, P. T., F. J. Odendaal, and M. D. Rausher. 1991. Quantifying insect movement in the field. Environ. Entomol. 20: 955-963.

Turchin, P. T. and W. T. Thoeny. 1993. Quantifying dispersal of southern pine beetles with mark-recapture experiments and a diffusion model. Ecol. Appl. 3: 187-198.

van Roermund, H. J. W., J. C. van Lenteren, and R. Rabbinge. 1997. Analysis of foraging behavior of the whitefly parasitoid *Encarsia formosa* on a leaf: A simulation study. Biol. Control 8: 22-36.

van Alphen, J. J. M. and L. E. M. Vet. 1986. An evolutionary approach to host finding and selection, pp. 23-61. *In* J. Waage and D. Greathead [eds.], Insect parasitoids. Academic Press, London.

van den Berg, M. A., P. J. Newton, V. E. Deacon, and C. Crause. 1987. Dispersal of *Trichogrammatoidea cryptophlebiae* (Hymenoptera: Trichogrammatidae), an egg parasitoid of the false codling moth, *Cryptophlebia leucotreta* (Lepidoptera: Tortricidae), in an empty habitat. Phytophylactica 19: 515-516.

van Driesche, R. G. and T. S. Bellows, Jr. 1996. Biological Control. Chapman & Hall, New York,

van Lenteren, J. C. 1986. Parasitoids in the greenhouse: successes with seasonal inoculative release systems, pp. 342-374. *In* J. K. Waage and D. J. Greathead [eds.], Insect parasitoids. Academic Press, London.

van Lenteren, J. C., Y. C. Drost, H. J. W. van Roermund, and C. J. A. M. Posthuma-Doodeman. 1997. Aphelinid parasitoids as sustainable biological control agents in greenhouses. J. Appl. Entomol. 121: 473-485.

van Lenteren, J. C. and L. P. J. Noldus. 1990. Whitefly-plant relationships behavioral and ecological aspects, *In* D. Gerling [ed.], Whiteflies: their bionomics, pest status, and management. Intercept, Andover, UK.

van Lenteren, J. C., H. J. W. van Roermund, and S. Sutterlin. 1996. Biological control of greenhouse whitefly (*Trialeurodes vaporariorum*) with the parasitoid *Encarsia formosa*: how does it work? Biol. Control 6: 1-10.

van Lenteren, J. C. and J. Woets. 1988. Biological and integrated control in greenhouses. Ann. Rev. of Entomol. 33: 239-269.

Vet, L. E. M. 1980. Laboratory studies on three *Encarsia* spp. and one *Eretmocerus* sp. (Hymenoptera, Aphelinidae) parasites of the greenhouse whitefly *Trialeurodes vaporariorum* (Westw.) to assess their efficiency as biological control agents. Meded. Fac. Landbouwwet Rijksuniv 45: 555-561.

Von Arx, R. V., J. Baumgärtner, and V. Delucchi. 1984. Sampling of *Bemisia tabaci* (Genn.) (Sternorrhyncha: Aleyrodidae) in Sudanese cotton fields. J. Econ. Entomol. 77: 1130-1136.

Waage, J. K. 1983. Aggregation in field parasitoid populations: foraging time allocation by a population of *Diadegma* (Hymenoptera, Ichneumonidae). Ecol. Entomol. 8: 447-453.

Waage, J. K. and D. J. Greathead. 1988. Biological control: challenges and opportunities. Phil. Trans. R. Soc. Lond. 318: 111-128.

Walde, S. J. and W. W. Murdoch. 1988. Spatial density dependence in parasitoids. Ann. Rev. Entomol. 33: 441-466.

Weisser, W. W. 1997. The influence of migration on dynamics in host-parasitoid and predator-prey systems. Proceedings of the German Society for General and Applied Entomology, Bayreuth, Germany, 18-22 March 1997. 11: 591-599.

Weisser, W. W. and W. Volkl. 1997. Dispersal in the aphid parasitoid, *Lysiphlebus cardui* (Marshall) (Hym., Aphidiidae). J. Appl. Entomol. 121: 23-28.

Williams, L., III, T. J. Dennehy, and J. C. Palumbo. 1997. Defining the risk of resistance to imidacloprid in Arizona populations of whitefly, pp. 1242-1246. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Research Conferences., Vol. 2, Proceedings Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Wolfram, S. 1988. Mathematica: a system for doing mathematics by computer. Addison-Wesley, Redwood City, California, USA.

Yu, D. S. K., J. E. Laing, and E. A. C. Hagley. 1984. Dispersal of *Trichogramma* spp. (Hymenoptera: Trichogrammatidae) in an apple orchard after inundative releases. Environ. Entomol. 13: 371-374.

Zalom, F. G. and E. T. Natwick. 1987. Developmental time of sweetpotato whitefly (Homoptera: Aleyrodidae) in small field cages on cotton plants. Fl. Entomol. 70: 427-431.