EFFECT OF PARASITOID RELEASE PATTERN ON WHITEFLY (HOMOPTERA: ALEYRODIDAE) CONTROL IN COMMERCIAL POINSETTIA

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ABSTRACT

Under commercial poinsettia production conditions we compared two patterns of parasitoid release for the aphelinid whitefly parasitoid *Eretmocerus eremicus* Rose and Zolnerowich. We compared the currently used pattern of a fixed weekly release number (3 females per plant per week) to an experimental pattern in which more parasitoids were released early in the crop (wks 1-8), followed by a lower number (wks 9-17), with the seasonal release average still being 3 female parasitoids per plant per week. We further compared the outcome of these two treatments (fixed and variable) to a low release rate (1 parasitoid per pl per wk) of *Encarsia formosa* Gahan, an aphelinid parasitoid widely used for whitefly control in greenhouse crops. In control cages without parasitoid releases, whitefly nymphal densities reached 15-32 live nymphs per leaf, which was 7 to 16-fold greater than the acceptable level at crop harvest. In cages in which parasitoid releases were made, whitefly nymphal densities were suppressed 99.8%, 96.8% and 50.9% by fixed-rate *E. eremicus*, variable-rate *E. eremicus*, and low-rate *E. formosa* treatments, respectively. In greenhouse populations, the final densities of live whitefly nymphs per leaf were significantly higher in the *E. formosa* treatment than the two *E. eremicus* treatments. Releases of low numbers of *E. formosa* provided commercially acceptable control in only one of two greenhouses. There was no difference between the fixed and variable release rate treatments of *E. eremicus*, indicating that whitefly suppression was not increased by concentrating the release of this parasitoid early in the crop.

Key Words: *Eretmocerus eremicus, Encarsia formosa, Bemisia argentifolii*, poinsettia, biological control, variable release rate, augmentative release, evaluation, cost, greenhouses

RESUMEN

Dos patrones de liberación de parásito para *Eretmocerus eremicus* Rose y Zolnerowhich fueron comparados bajo condiciones de producción comercial de poinsettia (Flor de Pascua). Comparamos el patrón actualmente usado de numero de liberación semanal fija (3 hembras por planta por semana) a un patrón experimental en el cual más parásitos fueron liberados temprano en el cultivo (semanas 1-8), seguido por un numero menor (semanas 9-17), con la liberación estacional promedio aun siendo 3 parásitas hembras por planta por semana. Adicionalmente comparamos el resultado de estos dos tratamientos (fijo y variable) a una baja incidencia de liberación (1 parásito por planta por semana) de *Encarsia formosa* Gahan, un parásito usado extensamente para control de la mosca blanca en cultivos de invernadero. En jaulas de control sin liberación de parásito, densidades de ninfas mosca blanca alcanzaron 15-32 ninfas vivas por hoja, que fue entre 7 y 16 veces mayor que el nivel aceptable al momento de cosecha del cultivo. En jaulas en las cuales liberación de parásito ocurrió, las densidades de ninfas mosca blanca fueron suprimidas 99.8%, 96.8, y 50.9% por *E. eremicus* en tratamientos fijo, variable, y tratamientos de baja incidencia de *E. formosa*, respectivamente. En poblaciones de invernadero, las densidades finales de ninfas vivas de mosca blanca por hoja fueron significativamente mayores en el tratamiento de *E. formosa* que en los dos tratamientos de *E. eremicus*. Liberaciones de bajas cantidades de *E. formosa* proveyeron control aceptable en solo uno de dos invernaderos. No hubo diferencia entre los tratamientos fijo y variable de *E. eremicus*, indicando que supresión de mosca blanca no incremento como causa de concentrar la liberación de este parásito temprano en el cultivo.

Silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) (Homoptera: Aleyrodidae) is an important foliar pest of poinsettia (*Euphorbia pulcherrima* Willd. ex Koltz.) (Byrne et al. 1990; Bellows et al. 1994; Hoddle & Van Driesche 1996). The principal parasitoid species used for its control have been *Encarsia formosa* Gahan and *Eretmocerus eremicus* Rose and Zolnerowich (Hymenoptera: Aphelinidae) (Drost et al. 1996, Hoddle & Van Driesche 1996; Rose & Zolnerowich 1997).

Trials to measure the efficacy of releases of commercial *E. formosa* (*Ef*), the Beltsville strain of *E. formosa* (*Ef*Belt), or *E. eremicus* (*Ee*) on poinsettia have examined constant weekly releases of either one or three females per plant per week (Hoddle & Van Driesche 1996; Hoddle et al. 1996; Hoddle et al. 1997abc; Hoddle & Van Driesche 1999ab). In small greenhouses holding 90 plants, whitefly mortality (1- survivorship from egg to adult) was 99% (Ee and BfBelt, both high rate), 96% (EfBelt, low rate), 95% (Ef, low rate), 92% (Ef high rate) and 88% (Ee low rate) (Hoddle et al. 1997abc).

While these mortality rates may seem uniformly high, the differences among them have practical importance because whiteflies in poinsettia have four generations in the crop cycle, each with high rates of population increase. With no mortality from pesticides or parasitoids, silverleaf whitefly egg-to-adult survival is about 75% (Hoddle et al. 1997abc). Combined with the per female fertility rates that *B. argentifolii* can achieve on poinsettia, this level of survival results in rates of increase of up to 25-fold per generation. With this rate of increase and an initial density on cuttings in the range of 0.1 nymphs per leaf (a typical value), an uncontrolled population has the potential to exceed 12,000 per leaf by the end of the crop (but actually would be constrained below that level by competition, other sources of mortality, and space on the leaf). By comparison, if parasitoid-caused mortality decreased egg-to-adult survival to 1% (99% mortality), whitefly density would decrease over the course of the crop to less than 0.0004 per leaf. Lower levels of parasitoid caused mortality are progressively less effective; 88% mortality, for example, would allow the whitefly population to reach 8.1 nymphs per leaf, an unacceptably high level. Thus small differences in parasitoid-caused mortality in the range observed (88-99%) are critically important in the success or failure of whitefly biological control in commercial crops.

Because differences in mortality rates interact with variation in realized fecundity and sex ratio in ways that cannot be easily predicted, to actually know how well a given parasitoid release rate or pattern works in limiting final whitefly densities, tests must be rigorously conducted under commercial conditions where this technology will be ultimately utilized. In both summer stock plants and fall Christmas crop plants, *E. eremicus* at 3 females per plant per week effectively suppressed silverleaf whitefly (Hoddle & Van Driesche 1999ab). Poinsettias at the end of the crop were acceptable to growers as a source of cuttings or, for the Christmas crop, for sale to retailers (with fewer than 2 live nymphs and pupae per leaf). This commercially acceptable level of control was not achieved with the same release rates of *E. formosa* Beltsville. This release rate, however, was too expensive for grower adoption. Consequently, we chose to investigate whether or not the pattern of parasitoid releases, independent of number released, might be manipulated in ways that increased the level of parasitoid impact on whitefly populations.

We hypothesized that release patterns that concentrated parasitoid releases either in the early or late part of the crop cycle might be more effective for controlling *B. argentifolii*. In small greenhouses, we tested two variable-rate release patterns against a fixed weekly release rate of 3 females per plant. In pattern 1 ("low-high"), parasitoids were released at a low rate in the first half of the crop and then the number was increased once plants were larger (first 1, then 5 parasitoids per plant per week) and in pattern 2 ("high-low") this pattern was reversed (first 5 parasitoids per plant, then 1 in the second half of the crop cycle), concentrating highest parasitoid numbers on the smallest plants, early in the crop.

The argument for potential greater efficacy of release pattern 1 (low-high) was that, since parasitoid foraging efficiency declines as plant size increases (Hoddle et al. 1998), increasing parasitoid release rate in the later part of the crop when plants are largest might compensate for this decline in per parasitoid efficacy. Also, at lower release rates progeny production by *E. eremicus* within the greenhouse increases because fewer parasitized hosts die from multiple ovipositions and host feeding (see Hoddle 2000 for a review of this argument). The argument for greater efficacy of release pattern 2 (high-low) was that using higher release rates when plants were small might virtually exterminate the whitefly population, leaving too little time before crop sale for whiteflies to recover to damaging levels.

In fall 1995, we ran a trial with *E. eremicus* in small greenhouses (holding 90 plants) (Hoddle et al. 1999*)* to test the pest control value of these variable release patterns. We found that pattern 2 (high-low) resulted in 75% fewer live whitefly nymphs and pupae per plant at the end of the crop (week 14) than did pattern 1 (low-high) (Hoddle et al. 1999).

The main goal of the study presented here was to directly compare, under fully realistic production conditions, the better of these two variable *E. eremicus* release patterns (high-low) directly against the fixed released pattern. By design these two treatments have the same release rate of *E. eremicus*, allowing us to isolate any effects due to the single factor of parasitoid release pattern. We also took advantage of available greenhouses in this same trial to pursue a second goal, to assess the efficacy of a low release rate (one parasitoid per plant per week) of *E. formosa*. We did this because (1) the low rate of this species did perform reasonably well (causing 95% mortality) in earlier small greenhouse trials; (2) published work indicated that this parasitoid when used at

low rates might actually be more, not less, effective than high rates due to the effects of mutual interference among parasitoids (Hoddle et al. 1997a; Hoddle 2000); and (3) producers recommend use of low release rates of the species in European flower crops, but few trials in North America exist to support this recommendation.

Materials and Methods

Study Site and Experimental Design

The study was conducted in commercial greenhouses in western Massachusetts (Fairview Farms, Whately, MA), for seventeen weeks between 15 August and 6 December, 1996. Six plastic hoop houses (each identical in size and construction, with dimensions of 4.8 × 29.3 m) were used for the principal treatments.

Three treatments were examined, each randomly assigned to a whole greenhouse with two replications: (1) a fixed release rate of three females of *E. eremicus* per plant per week; (2) a variable release of *E. eremicus*, with five females released per plant per week for the first eight weeks of the trial (August 16-October 4) and one female per plant per week for the last nine weeks (October 11 to December 6); and (3) the commercial strain of *E. formosa* released at one female per plant per week. A seventh greenhouse, in which the grower used pesticides for whitefly management, was also examined 12 times between 26 August to 18 November to provide a further comparison to whitefly suppression levels seen in the biological control treatments.

In each test greenhouse (except for the chemical control greenhouse), two cages (95 μm mesh over PVC frames with dimensions 153 × 92 × 117 cm) were used to isolate five pots, each with three poinsettia plants, as controls. One cage in each greenhouse (designated "control for treatment") received no treatments of any kind to suppress whiteflies. The other cage (designated "control for caging effect") received the same parasitoid treatment as the greenhouse in which it was placed. Initial densities of whitefly nymphs and pupae in cages were manipulated to match those in test greenhouses (see below).

Crop Composition and Management

In each greenhouse, a poinsettia crop was established on August 15, 1996 using 1500 plants, all 'Freedom' varieties (1140 red, 200 white and 160 pink per house) from Paul Ecke Ranch (Encinitas, CA). These plants were planted in soilless media, three stems per 20-cm dia pot. Numbers of plants in each house remained constant until November 27, at which time removal of colored plants for Christmas sale began. Numbers of parasitoids released during the last three weeks of the trial were reduced as needed to keep the parasitoid release rate per plant constant. Plants placed in cages were selected from plants in each test greenhouse at the start of the experiment, choosing plants so that the average density of whitefly nymphs on plants put in cages was the same as that of the whole greenhouse.

All whiteflies found on plants were *B. argentifolii* and were assumed to have entered the greenhouses on leaves of rooted cuttings purchased from suppliers.

All plants in all greenhouses were treated with the fungicide thiophanate methyl+etridiazole (Banrot 40WP®, Scotts Sierra Crop Protection Co., 1411 Scottslawn Rd., Marysville, OH 43041) to control root rot on 18 August and 21 September. Plants in the chemical control greenhouse were treated with imidacloprid (Marathon®) on 12 September. No insecticides were used in any of the six test greenhouses, except in one of the two greenhouses in which *E. formosa* was released. In this single greenhouse, insecticide smokes (Fulex Dithio®, sulfotep, Fuller Systems, Inc., Woburn, MA 01801) were applied on 14 and 27 November to reduce numbers of adult whiteflies before sale.

Population Sampling

Whitefly densities in each of the six test greenhouses at the start of the trial were determined by examining all the leaves of 100 freshly potted cuttings with a 3.5× head-mounted magnifying device. All live whitefly nymphs, pupae or adults were counted. Because initial whitefly densities were extremely low (0.0083 live stages per cutting), cohorts of *B. argentifolii* nymphs were created using a laboratory colony and infested plants were placed in each house to augment the population by an estimated 1.0 nymph per plant (=1500 *B. argentifolii* nymphs added per greenhouse). To achieve this, six infested plants were introduced into each greenhouse. Each infested plant had three leaves on which cohorts of *B. argentifolii* nymphs had been created by using leaf cages to confine groups of 5-6 pairs of adult whiteflies. Each cohort initially consisted of 105 eggs. Previous lifetable estimates (Hoddle et al. 1997abc) suggest that 80% of these eggs would hatch and this method was thus equivalent to adding 1512 nymphs per house. Plants were placed in greenhouses on 19 August and leaf cages were immediately removed from one infested leaf per plant. Leaf cages were removed from the second and third leaves on each infested plant on August 23 and 29. Staggered removal of leaf cages was intended to promote whitefly survival and enhance the establishment of a whitefly population despite ongoing parasitoid releases. In each of the control cages in each test greenhouse, one plant with one infested leaf bearing 19 eggs was added (being the equivalent of 15 nymphs, allowing for

the estimated 80% hatch). Cages thus received the same increase of one nymph per plant as did the whole greenhouses.

Growth of whitefly populations in test greenhouses and cages was measured by counting whiteflies on each of 3 leaves (one lower, one middle and one upper) on each of 90 randomly selected plants each week from each greenhouse. In cages, 8 plants were chosen at random for sampling, resulting in 24 leaves being examined per cage per week. Sampling was nondestructive, with whitefly numbers being counted with the aid a head mounted optical magnifier, as with the counts on cuttings. For each leaf, numbers were recorded of live nymphs, live pupae, dead nymphs, dead pupae, parasitized nymphs, whitefly pupal exuviae, whitefly exuviae bearing parasitoid emergence holes, and adult whiteflies.

Parasitoid Sources and Sampling

Eretmocerus eremicus. This parasitoid was initially supplied by Beneficial Insectary (Oak Run, CA) and after week 7 by Koppert Biological Systems, Inc. (Berkel en Rodenrijs, The Netherlands, through the North American office in Romulus, MI). *Eretmocerus eremicus* was received as loose parasitized *Trialeurodes vaporariorum* (Westwood) nymphs from the supplier and the number needed for release was calculated by assuming a 60% emergence rate and a 50/50 sex ratio (as determined in other trials, Hoddle & Van Driesche 1999b; Van Driesche et al. 1999). We weighed 10 aliquots of parasitized nymphs to estimate the number of parasitoid pupae in 0.02 g. We then weighed the quantity of parasitized *T. vaporariorum* nymphs needed to treat greenhouses or cages in view of plant number present and desired parasitoid release rate. Actual emergence rates and sex ratios were measured in the course of the trial (see below) and used to calculate the actual release rate achieved.

Eretmocerus eremicus was deployed by placing parasitized *T. vaporariorum* nymphs (not glued to cards) in styrofoam release cups (6 cm tall, 5.5 cm wide at bottom, 8.5 cm wide at top), which had the bottoms cut out and replaced with organdy (mesh 0.9 μm) to allow for drainage. Cups were attached 10 cm above the canopy to wooden stakes (50 cm long) placed in the potting media. In each biological control greenhouse receiving this parasitoid, there were 15 release cups distributed evenly throughout the crop. Since watering was done via overhead hoses, workers were asked to avoid wetting release cups.

Encarsia formosa. This thelytokous parasitoid species was supplied by Applied Bionomics (Sidney, BC, Canada) as parasitized fourth instar nymphs of *T. vaporariorum* glued to release cards, with 200 parasitoids per card. Based on earlier trials and producer's advice, we assumed that 50% of pupae would emerge as adults. Numbers of cards needed per greenhouse were then calculated using these estimates of numbers per card, the emergence rate, and the number of plants per greenhouse or cage.

Encarsia formosa were deployed in greenhouses by hanging the necessary number of manufacturer's release cards (15 per greenhouse) (bearing parasitized *T. vaporariorum* nymphs) on plants throughout the crop.

Verification of Release Rates. To verify our assumptions concerning the adult emergence rates for each parasitoid species, percentage emergence of pupae of each parasitoid species was determined weekly throughout the trial. No parasitoids emerged from our shipments before being placed in the test greenhouses. In each greenhouse, emergence cards (for *E. formosa*) or release cups (for *E. eremicus*) were collected and taken back to the laboratory after a one week exposure in the test greenhouses. For each parasitoid species, one hundred and fifty whitefly nymphal cadavers were selected randomly from the material on the release card or in the release cups and the number of successfully emerged parasitoids was determined based on observation of parasitoid emergence holes.

The proportion of emerging adults of *E. eremicus* that were female was determined on eight dates throughout the test. On each date, 100-200 pupae were removed from orders received from suppliers and held in the laboratory in glass vials at 22°C for emergence. Seven to ten days later, one hundred adults were randomly selected and sexed.

Information on emergence rate, sex ratio (*E. eremicus* only), and number of pupae placed in greenhouses was used to calculate the release rate achieved in each greenhouse in each week.

Statistical Analysis

The densities of live whitefly nymphs per leaf at harvest were compared among treatments with a nested ANOVA and treatment means separated by use of Tukey's studentized mean separation test (at $P = 0.05$).

Results

Release Rates of Parasitoids and Quality Control

Eretmocerus eremicus. Parasitoid emergence, summed over all dates within each greenhouse, varied among greenhouses from 46.1-58.2% and averaged 53.5% ± 1.9 (SE) overall. The percentage of adult *E. eremicus* emerging from pupae held after receipt in the laboratory that were female ranged among dates from 39 to 58%. The seasonal average, based on 800 parasitoids, was 48.1% ± 2.2 (SE).

Because the proportion of *E. eremicus* that emerged successfully (53.5%) was less than what we assumed (60%), insufficient pupae were placed in some greenhouses on some dates to achieve the

intended release rate. Actual release rates (females parasitoids per plant per week) averaged 3.8 (replicate one, 3.1 ± 0.6; replicate two, 4.4 ± 0.6) and 0.8 (replicate one, 0.8 ± 0.5; replicate two, 0.8 ± 0.7) for the variable release rate treatment and 2.8 (replicate one, 2.7 ± 0.2; replicate two, 2.8 ± 0.2) for the fixed rate, rather than 5 and 1, and 3 as intended (Fig. 1).

Encarsia formosa. The commercial strain of *E. formosa* had seasonal average emergence rates of 44.9 and 44.5% for the two test greenhouses, for a grand seasonal average of 44.7 ± 2.5 (SE). Numbers of pupae per card averaged 199.6 ± 7.2 (SE). Numbers of female parasitoids per plant actually emerging in the greenhouse were 1.2 ± 0.1 (SE) in each test greenhouse.

Whitefly Population Trends in Caged Controls

Whitefly nymphal populations in cages in which no treatments were made increased rapidly in density after mid November in the four greenhouses receiving *E. eremicus* releases (Fig. 2A, B). Whitefly nymphal populations in control cages in the two greenhouses receiving *E. formosa* releases increased in late November, but then declined in December (Fig. 2C). In cages in which *E. eremicus* releases were made (constant and variable), whitefly nymphal densities remained below 1 nymph per leaf during this same period, reflecting a high level of whitefly suppression (Fig. 2A, B). In cages in which *E. formosa* was introduced, nymphal densities in late November exceeded 4 nymphs per leaf (Fig. 2C). Comparison of whitefly densities at harvest in cages with and without parasitoid releases showed that nymphal densities per leaf in control cages were reduced 99.9% by the fixed release rate treatment of *E. eremicus*, compared to 96.8% for the variable (high-low) release rate treatment of the same species and 50.9% for the low release rate of *E. formosa*.

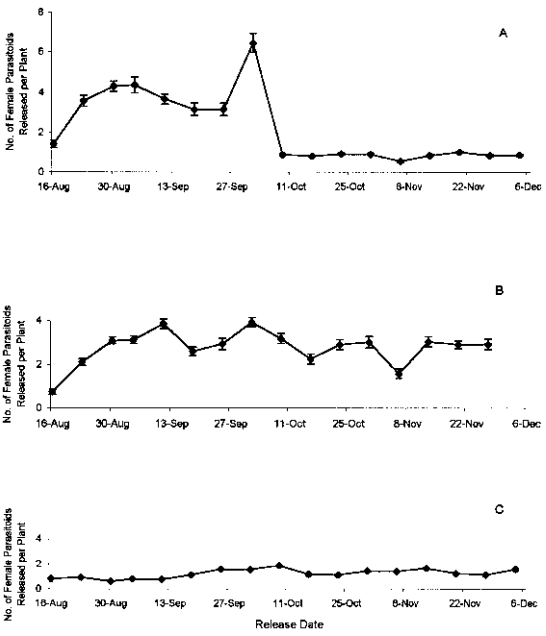

Fig. 1. Average release rates achieved (for two greenhouses each): greenhouses receiving the high-low release rate of *Eretmocerus eremicus* (first 5 females per plant per week, then one female) (A); greenhouses receiving the fixed release rate of *E. eremicus* (3 females per plant per week) (B); greenhouses receiving a low release rate of *E. formosa* (1 female per plant per week) (C).

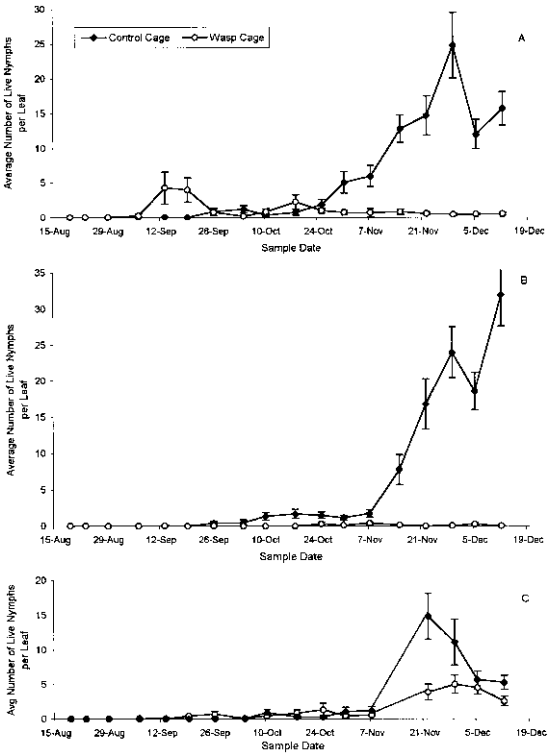

Fig. 2. Trends in average density of whitefly nymphs for control cages (control cage and parasitoid cage) for two greenhouses receiving the variable release rate of *Eretmocerus eremicus* (first 5 females per plant per week, then one female) (A); two greenhouses receiving the fixed rate of *E. eremicus* (3 females per plant per week) (B); two receiving the low rate of *Encarsia formosa* (1 female per plant per week) (C).

Whitefly Population and Parasitism Trends in Trial Greenhouses

Whitefly populations outside of cages in test greenhouses were low in all treatments and remained so throughout the trial (Fig. 3A-E). Densities of live nymphs remained below 1 nymph per leaf in all test greenhouses except in one of the two receiving releases of *E. formosa* (Fig. 3D), in

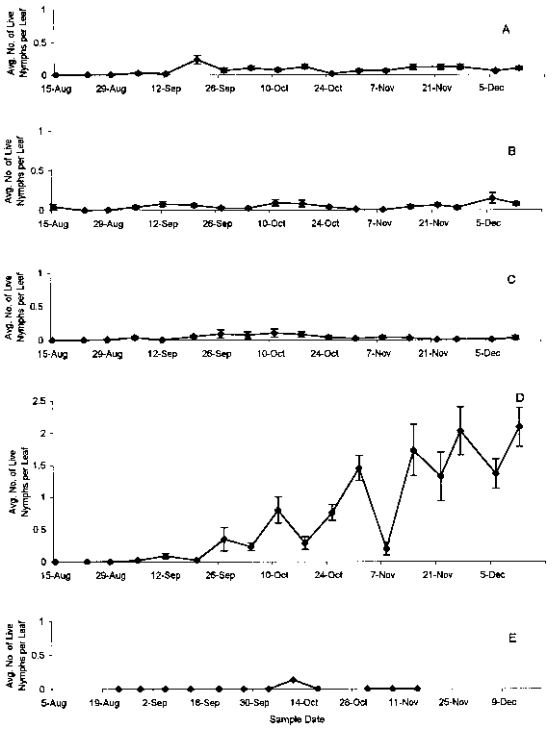

Fig. 3. Trends in density of whitefly nymphs per leaf on plants in greenhouses receiving the variable release rate of *Eretmocerus eremicus* (first 5 females per plant per week, then one female) (A, average of two replicates); the fixed release rate of *E. eremicus* (3 females per plant per week) (B, average of two replicates); the low rate of *Encarsia formosa* (1 female per plant per week) (C, D, replicates different and presented separately); or the chemically-treated greenhouse under grower management (E, one replicate).

which increasing densities exceeded 2 nymphs per leaf at harvest. Pupal and adult whitefly densities in all greenhouses were lower than nymphal densities, but followed similar trends, which are not presented. The trend of quick increase in whitefly nymphal numbers in one *E. formosa* greenhouse (Fig. 3D) prompted the grower to apply insecticidal smokes (Fulex Dithio® [sufotep]) to suppress whitefly adults on plants before sale.

Final densities of live nymphs per leaf were significantly different at harvest among treatments ($F = 27.89$, $df = 2$, $p = 0.0001$) and whitefly nymphal densities in the low rate of *E. formosa* treatment were significantly higher than the two *E. eremicus* treatments, which were statistically similar. Whitefly densities in the chemical control greenhouse were highly suppressed throughout the trial.

Few parasitized whitefly nymphs were encountered in this trial in any of the test greenhouses and we were not able to draw any inferences about the effect of the treatments on parasitism rates. However, low parasitism rates do not imply that total host mortality caused by parasitoids was low. For these parasitoid species, host feeding is often the major cause of host mortality, especially when parasitoid-to-host ratios are high, as would be the case in an augmentative biological control program when hosts are scarce. The effect of mortality from parasitoid host feeding is reflected in lowered host density, which has been analyzed above.

Discussion

We had two objectives with this experiment: (1) to determine if variable release rates of *E. eremicus* would be more effective than a fixed release rate of the same total number of parasitoids released over a complete poinsettia cropping period under commercial growing conditions and (2) to ascertain if a low fixed weekly release rate of *E. formosa* would suppress *B. argentifolii* densities on poinsettia to acceptable levels at time of harvest.

In this trial, there were no statistical differences in whitefly nymphal densities at harvest between greenhouses receiving fixed versus variable rate releases of *E. eremicus*. While starting whitefly densities in these greenhouses were very low, whitefly populations in control cages (in which *E. eremicus* was not applied) increased to high levels (25-35 live nymphs per leaf) by harvest. The absence of such increase in the four *E. eremicus*-release greenhouses (and their associated parasitoid-release cages) was thus due to natural enemy activity. Based on (1) the lack of evidence in this trial supporting the idea that a variable rate increases control and (2) lack of any such effect in a previous trial run in small greenhouses (Hoddle et al. 1999), we conclude that the variable release pattern has no discernible advantage over a fixed release rate pattern and do not recommend its use.

Encarsia formosa released at one parasitoid per plant per week provided inconsistent whitefly control. One of the two greenhouses receiving this treatment developed an increasing whitefly population that at harvest was still relatively low but was increasing enough that the grower intervened with chemical control measures. Because low rates of *E. formosa* have failed to consistently control *B. argentifolii* on poinsettia, we do not recommend use of this parasitoid on this crop in North America.

This trial demonstrates that tactics other than varying the release rates of *E. eremicus* will be needed to make use of this parasitoid both economical and effective. Insect growth regulators found in the laboratory to be compatible with *E. eremicus* (Hoddle et al. 2000) have enhanced the economic feasibility of using this parasitoid in greenhouses, allowing release rates to be reduced by two thirds (Van Driesche et al. 2000).

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