PERSISTENCE AND CONTAINMENT OF *METASEIULUS OCCIDENTALIS* (ACARI: PHYTOSEIIDAE) IN FLORIDA: RISK ASSESSMENT FOR POSSIBLE RELEASES OF TRANSGENIC STRAINS

Gregory J. McDermott and Marjorie A. Hoy
Department of Entomology & Nematology
P.O. Box 110620, University of Florida
Gainesville, FL 32611-0620

Abstract

Metaseiulus occidentalis (Nesbitt) is a phytoseiid mite which is commercially available as a biological control agent of spider mites. Genetic manipulation of this phytoseiid species has yielded transgenic strains, but none have been released into the environment. Previous data suggested that *M. occidentalis* could not survive the wet, humid summers in Florida. A non-transgenic strain of *M. occidentalis* was released into field plots in Gainesville on soybean plants infested with the two-spotted spider mite, *Tetranychus urticae* Koch. Populations were monitored from April-October 1994, and weather data were gathered at the release site. Permethrin-treated barrier rows were monitored to determine if the mites dispersed outside the plots, and aerial dispersal was monitored with sticky traps. Predator and spider-mite populations repeatedly crashed during the summer months, and population growth was negatively correlated with rainfall. CLIMEX, a population growth model which uses climatic factors to determine whether a given poikilothermic species can colonize and persist in new geographic areas, also indicated that *M. occidentalis* cannot persist through the wet season in Florida, although it may be able to establish and persist through the fall, winter and spring months.

Key Words: Spider mites, phytoseiid mites, genetic improvement, climate models, biological control, risk assessment

Resumen

Metaseiulus occidentalis (Nesbitt) es un ácaro fitoséido disponible comercialmente como agente de control biológico de ácaros fitófagos. La manipulación genética de esta especie de fitoséido ha producido colonias transgénicas, pero ninguna ha sido liberada al ambiente. La información previa sugiere que *M. occidentalis* no podría sobrevivir el verano lluvioso y húmedo de la Florida. Una colonia no transgénica de *M. occidentalis* fue liberada en parcelas de campo en Gainesville en plantas de soya infestadas con el ácaro de dos manchas, *Tetranychus urticae* Koch. Las poblaciones fueron muestreadas desde abril hasta octubre de 1994; los datos climáticos fueron colectados en el sitio de liberación. Se muestrearon las hileras de barrera tratadas con permetrina para determinar si los ácaros se dispersaron fuera de las parcelas, y la dispersión aérea fue monitoreada con trampas pegagosas. Las poblaciones del depredador y del fitófago colapsaron repetidamente durante los meses de verano; además, el crecimiento de la población estuvo correlacionado negativamente con la lluvia. CLIMEX, un modelo de crecimiento poblacional que usa factores climáticos para determinar cuando una especie poikilotérmica puede colonizar y persistir en nuevas áreas geográficas, indicó también que *M. occidentalis* no puede persistir durante la estación de lluvias en la Florida, aunque podría establecerse y persistir durante los meses de otoño, invierno y primavera.

The western predatory mite, *Metaseiulus* (= *Typhlodromus* or *Galendromus*) *occidentalis* (Nesbitt) (Acari: Phytoseiidae), is an obligatory predator and successful biological control agent of spider mites (Tetranychidae) in vineyards and apple and almond orchards in the western United States (Hoyt 1969, Flaherty & Huffaker 1970, Hoy 1985a). *M. occidentalis* is marketed commercially as a biological control agent and is recognized as having a potentially world-wide role in integrated spider mite control programs (Hoy 1985a,b).

The biology and bionomics of *M. occidentalis* are well known. Numerous life-table studies have examined the effects of different temperatures and prey availability on *M. occidentalis* (Laing 1969, Tanigoshi et al. 1975, Bruce-Oliver & Hoy 1993). Hoy et al. (1985a) demonstrated that hungry adult females display an explicit aerial dispersal behavior in low to moderate wind speeds. Well-fed mites do not show aerial dispersal behavior, indicating that food availability may be a component in stimulating aerial dispersal.

Muma & Denmark (1970) do not list *M. occidentalis* among the species of phytoseiid mites occurring in Florida, and Denmark (Personal communication) indicated no subsequent records of *M. occidentalis* are available to indicate this species has since established in Florida, despite numerous commercial importations. Hoying & Croft (1977) examined literature and museum specimens and, aside from one report from eastern Wisconsin and one specimen from southern Alberta, Canada, found no reports of *M. occidentalis* occurring east of the Rocky Mountains.

A number of phytoseiid species, including *M. occidentalis*, have been genetically manipulated via artificial selection to produce pesticide-resistant or non-diapausing strains (Hoy 1992). Genetic manipulation using recombinant DNA techniques could improve the efficiency of genetic manipulation of biological control agents by reducing the time required to identify variability upon which to select, and by providing genes which do not occur naturally in the species. Presnail & Hoy (1992) used a maternal microinjection technique to transform *M. occidentalis* with a plasmid containing the ß-galactosidase gene (lac Z construct) from *Escherichia coli* under the control of the *Drosophila melanogaster* Meigen heatshock 70 promoter. Because so much is known about the biology of *M. occidentalis*, it is an ideal arthropod to use for evaluating the risks of releasing transgenic arthropods into the environment.

The U.S. Department of Agriculture, Agricultural Biotechnology Research Advisory Committee (ABRAC) (1991) provides guidelines for risk assessment in transgenic releases. Our tests aim to answer the following questions that are raised in the ABRAC guidelines: 1) What is the organism's potential to establish itself in the accessible environment? 2) What is the potential for monitoring and control in the accessible environment?

If *M. occidentalis* cannot permanently establish in Florida due to its inability to survive the unfavorable summer climate, then Florida could be an ideal site for experimental transgenic releases. Experimental plots could be maintained throughout the favorable fall, winter, and spring months, with the summer climate serving as an additional safe-guard against accidental establishment. This study examines the ability of non-transgenic *M. occidentalis* to persist in Florida through the unfavorable wet summer months in experimental field plots. The plots were also designed to determine whether *M. occidentalis* can be contained within the experimental plots and kept from dispersing aerially. In addition, a climatic model is used to determine the likelihood of *M. occidentalis* establishing and persisting in Florida.

Persistence

Sixty to seventy-five pinto bean seeds were planted (3:2 potting soil to vermiculite mixture) in eight liter pots. A total of 75 pots were arranged into five plots, with each plot containing three rows of five pots. Plots were laid out on an east-west axis at a University of Florida field station in Gainesville, FL. The rows were spaced 60 cm apart, and the plots were placed 152 cm apart (Fig. 1). Single pots of beans were placed in line with each of the rows between each of the plots to act as "trap" plants between the plots. On March 27, 1994 (Julian day 86; all subsequent dates refer directly to days of the Julian calender), when the bean plants had reached the 3 to 5-leaf stage, the center row of each plot was infested with *Tetranychus urticae* (Koch) by laying cut foliage containing *T. urticae* atop the uninfested potted plants. As the cut foliage dried, the *T. urticae* adults transferred to the green foliage. On day 92, a 10-leaf subsample was taken from each plot to determine the approximate density of *T. urticae*. Three paraffin-coated paper disks containing adult *M. occidentalis* females were spaced equally along the center row of each plot to approximate a 20:1 spider mite to predator ratio.

On day 113, two pots were removed from each row and replaced with two new pots of bean plants. The foliage from the pots that were removed was cut and laid over the new plants to allow the predators and *T. urticae* present on the cut foliage to transfer to the new foliage. From that point, two new pots were cycled into each row every two weeks, and the pots were rearranged so that the oldest pot was in the center of each row, flanked by the two newest pots. All new plants were sprayed with carbaryl (1.1 kg a.i./ha) one day before placement into the field to eliminate other predators and herbivores. The strain of *M. occidentalis* used (COS) is resistant to carbaryl, sulfur, and organophosphorus insecticides (Hoy 1984).

Plots were sampled once weekly starting on day 99. Five leaves were sampled from each plant for a total of 25 leaves per row per plot. Each 25-leaf sample was placed into a paper bag, chilled and taken to the laboratory, where a mite brushing machine was used to brush the mites from each 25-leaf sample onto a glass plate. Numbers of all stages of *M. occidentalis* and *T. urticae* were counted under a dissecting microscope, and the mean number of mites per leaf was determined for each row in each plot.

Following a population crash, plants were re-infested with spider mites on day 142, and *M. occidentalis* was added again on day 148. Sampling resumed on day 155. Additional *T. urticae* and *M. occidentalis* were added on day 197, and *T. urticae* only were added on day 225. Weekly sampling continued through day 281 (October 8, 1994). To determine if mite populations would rebound on their own, a subset of two pots per plot were removed on day 197 and replaced with new bean plants. The removed pots were placed in a new location with one new pot for each two pot subset. These five new plots were not re-infested, but fresh pots were cycled in each week.

Containment

The two outside rows of each plot, as well as the trap plants between the plots were sprayed with permethrin (0.06 kg a.i./ha) every two weeks starting on day 92. *T. urticae* is unaffected by permethrin at this rate, but the *M. occidentalis* strain used in this study is highly susceptible to this insecticide. Thus the outside rows of each plot were designed to act as barrier rows to dispersal by *M. occidentalis*.

Twenty-two 1.8 m cedar stakes were spaced at 1.5-m intervals around the plot (Fig. 1). Each stake held three 185 mm × 78 mm plexiglass plates coated with a thin
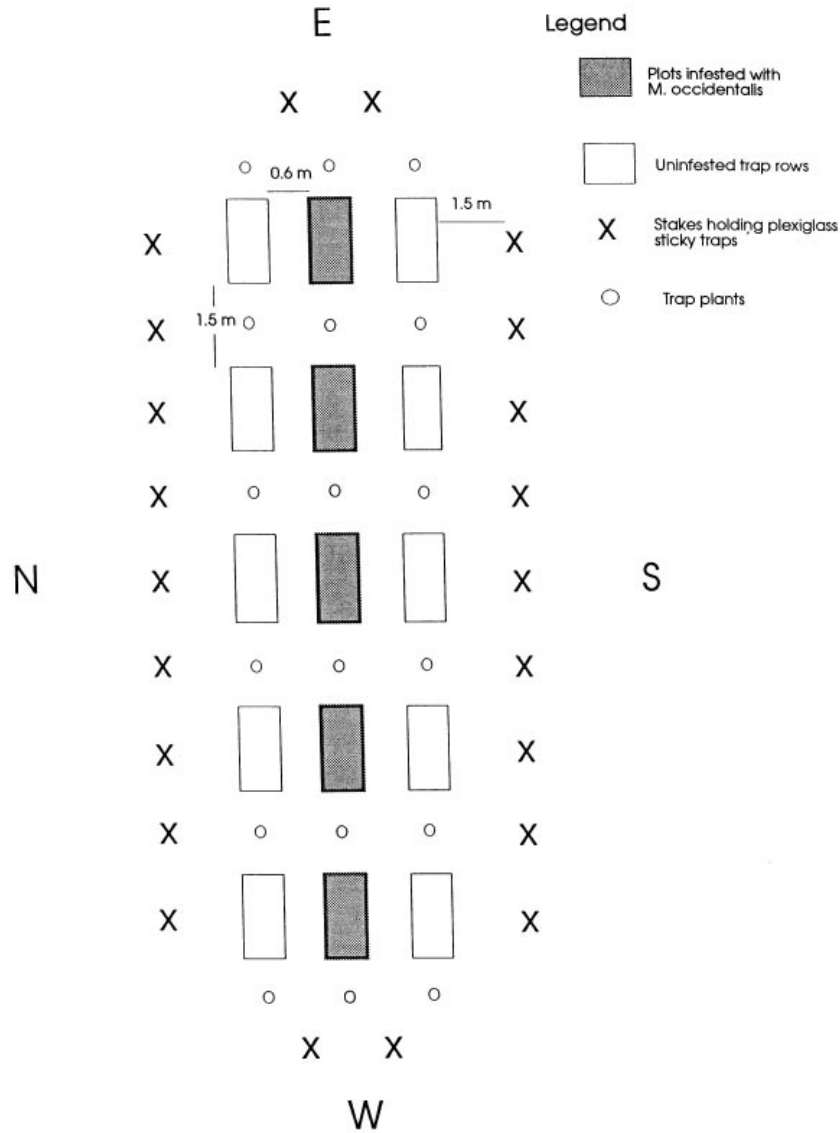
Fig. 1. Schematic diagram of field plot layout showing the center row infested with *M. occidentalis* and *T. urticae*, uninfested barrier rows and trap plants, and plexiglass sticky traps.

layer of gear oil. Plates were suspended on hooks set approximately 165, 110, and 54 cm above the ground. Plates were removed once a week, labeled as to the height above ground, and geographical axis to the plot, and taken to the lab. Plates from like heights and axes were placed into trays and soaked in tap water and automatic dish-

washing detergent to loosen material stuck to the grease. The slurry from the trays was then filtered through a fine mesh screen, and the contents were examined under a dissecting microscope to determine if any *M. occidentalis* were stuck to the plates, which would indicate that *M. occidentalis* was dispersing aerially.

Weather Data

Meteorological data was gathered from the site by a Campbell CM-10 datalogger and weather station (Campbell Scientific, Logan, UT). Temperature, precipitation, relative humidity, and wind speed and direction were recorded every 10 min. Maximums, minimums and totals for each 24-h period were compiled. Data were downloaded roughly once a week.

The CLIMEX Model

A computer climate modeling system was used to determine the likelihood of *M. occidentalis* surviving and establishing in Florida. CLIMEX is a computerized climate matching system which uses biological data to predict the potential relative abundance and distribution of poikilothermic animals in a given geographic area (Sutherst & Maywald 1985). The CLIMEX model utilizes climatic data from around the world, along with what is known of the biology and distribution of a given species to determine that species' potential to survive and proliferate in a given environment.

The CLIMEX model calculates an Ecoclimatic Index (EI) which utilizes weekly temperature, moisture, and daylength indices, and yearly cold, dry, heat, and wet stress indices. The EI, scaled between 0 and 100, is determined by the following equation:

$$EI = 100(\Sigma GI)/52 \times [(1\text{-}CS) \times (1\text{-}DS) \times (1\text{-}HS) \times (1\text{-}WS)$$

where CS, DS, HS, and WS are yearly cold stress, dry stress, heat stress, and wet stress indices scaled between 0 and 1. GI is the weekly population growth index, which is the product of the weekly temperature, moisture, and daylength indices.

The derivations of these indices are described in more detail in Sutherst & Maywald (1985).

Optimal and upper and lower threshold temperatures for *M. occidentalis* population growth were obtained from the literature (Tanigoshi et al. 1975) and used in the model. Unknown moisture parameters and threshold indices were then systematically altered until a distribution map approximating the known distribution of *M. occidentalis* in western North America was achieved (Hoying & Croft 1977). The model then graphed the predicted population growth curves for *M. occidentalis* populations in Jacksonville and Tampa, FL. These cities were chosen as they are the two cities closest to Gainesville that are included in the model's meteorological database.

RESULTS

Persistence

Both species remained in the plots at relatively stable levels (at a roughly 28:1 prey:predator ratio) through the month of April (Fig. 2). The mean densities of *M. occidentalis* and *T. urticae* for each sampling date crashed at the beginning of May (day 127), corresponding to a storm on day 124 that dumped 80.7 mm of rain in 5 h. Spider mite populations were reduced four- fold, to just over 1.5 *T. urticae* per leaf. Predator

mite densities were cut in half, lowering the prey:predator ratio. The food supply for *M. occidentalis* continued to decline over the subsequent 7-d period, and only two male *M. occidentalis* found in the entire 125 leaf sample from all five plots on day 141.

Reinfestation of the plots in late May was done with higher densities of both mite species, although the prey:predator ratio was maintained roughly the same as in the first infestation. Between days 155 and 162, *T. urticae* populations declined 55%, while *M. occidentalis* populations declined 65% (Fig. 2). Sampling on day 162 occurred in a light rain, and 26 mm of rain fell that afternoon, immediately before the sample was taken. More *T. urticae* were added to each plot on day 164, resulting in the population upswings seen for both species in the samples taken on day 169.

Population trends of both species were negatively correlated with increasing rainfall (df = 16, p = 0.0036 [Fig. 3A], df = 16, p = 0.0015 [Fig. 3B]). Percent population change was not calculated for those weeks in which more mites were added to the plots. The subplots that were started on day 170 showed that neither mite population would rebound without reinfestation (Table 1). No *M. occidentalis* were found in this subplot after day 211, although some *T. urticae* persisted at very low densities through the rest of the sampling dates.

Containment

A total of five *M. occidentalis* were found on the 66 plexiglass plates designed to detect aerial dispersal. The mites were found only on two of the 27 sampling dates (Table 2). Three were found on plates on the north side of the plot at the 43 cm level on day 211, and two were discovered on plates on the day 260 sampling date; one on the north
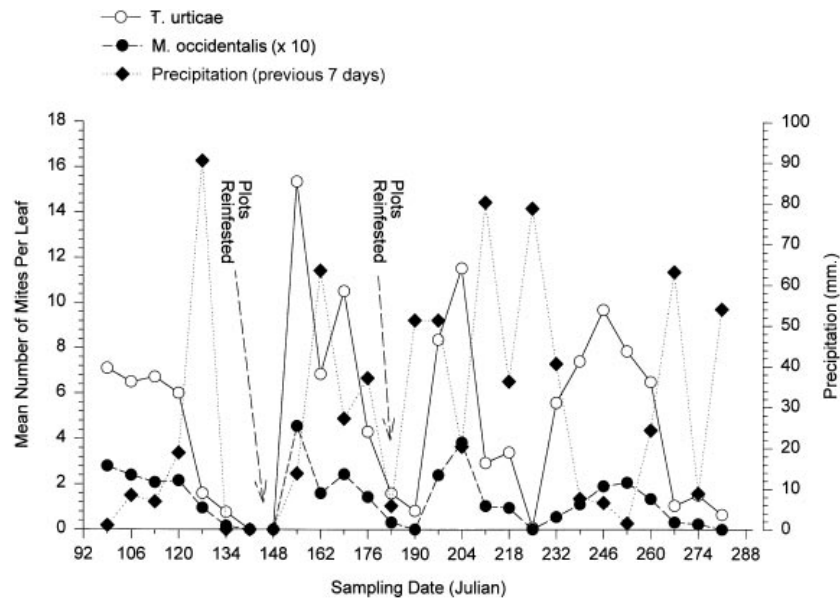

Fig. 2. Mean densities of *M. occidentalis* and *T. urticae* and cumulative total rainfall for each sampling date.
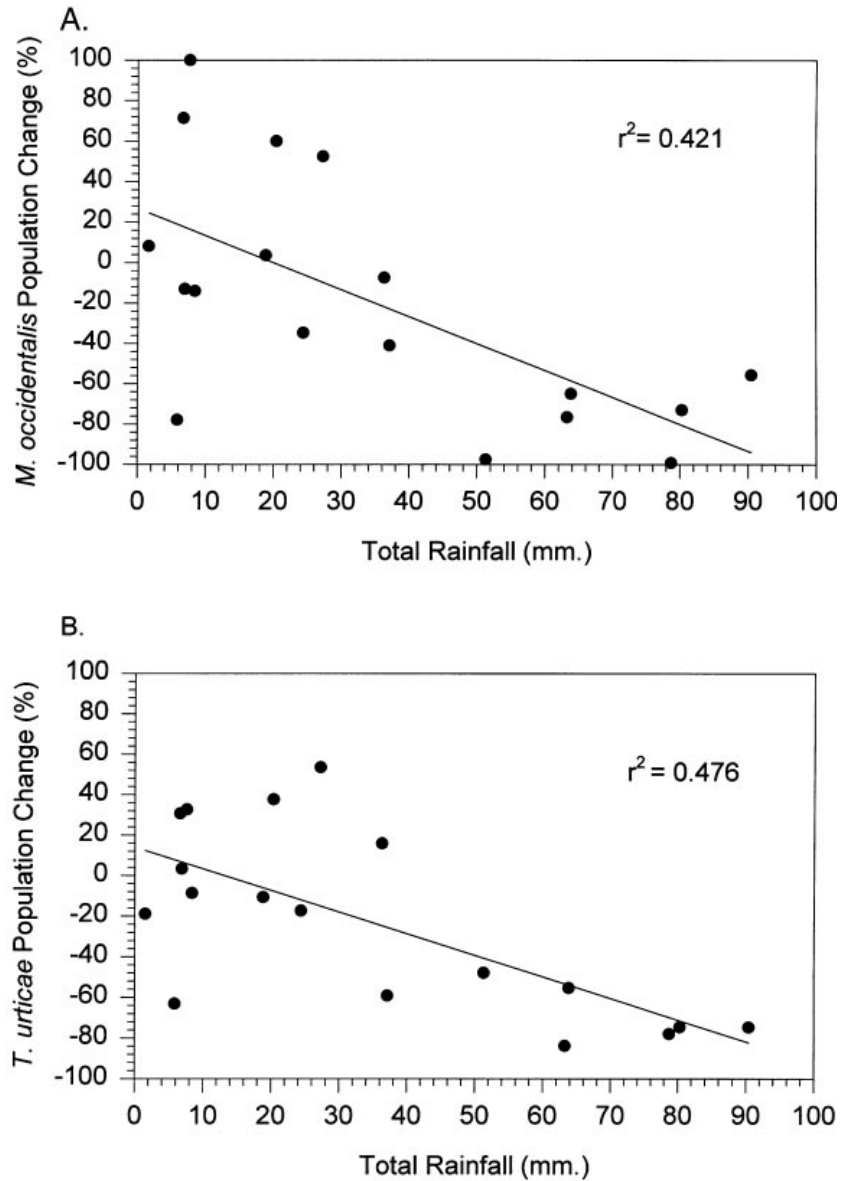
Fig. 3. A. Percent population change of *M. occidentalis* as a function of total rainfall in Gainesville, FL from Julian day 99, 1994 to Julian day 281, 1994. B. Percent population change of *T. urticae* as a function of total rainfall. Population change is calculated weekly, excluding those weeks that mites were released into the plots.

TABLE 1. SUMMARY OF *T. URTICAE* AND *M. OCCIDENTALIS* POPULATIONS FROM SUB-PLOTS ESTABLISHED JULY 16, 1994 (JULIAN DAY 170).

Sampling Date (Julian)	Mean *M. occidentalis* per Leaf	Mean *T. urticae* per Leaf
204	0	1.25
211	0.008	0.86
218	0	0.27
225	0	0.54
232	0	1.01
239	0	0.66
246	0	0.42
253	0	0.20
260	0	0.49
267	0	0.25
274	0	0.12
281	0	0.44

side of the plots at the 54 cm high level, and one on the east side at the 110 cm high level. Spider mites were found on the plates on all sample dates. The prevailing wind direction at the site is from the south-southwest, although easterly winds prevailed during some periods of stormy weather. Calculating the area along each side and each end of the plot to a height of 1.8 m (the height of the stakes holding the plexiglass plates) yields a total area of 61.32 m² around the periphery of the plot. The 66 plexiglass plates cover a total area of 0.952 m², or 1.55% of the peripheral area to a height of 1.8 m. Extrapolating that the five *M. occidentalis* collected on the plexiglass plates represent 1.55% of the total number aerially dispersing within that area, we conclude that aerial dispersal of *M. occidentalis* involved several hundred females. Although this extrapolation may statistically seem of little value, Hoy et al. (1984) used the same type of extrapolation in a California almond orchard to estimate that the numbers of dispersing mites could be in the millions over the same time interval. Thus, the plot management scheme adopted appears to be useful in reducing rates of aerial dispersal.

TABLE 2. SUMMARY OF *M. OCCIDENTALIS* COLLECTED FROM AERIAL DISPERSAL PLATES. THE DATE LISTED IS THE DATE THE PLANTS WERE COLLECTED; HEIGHT REPRESENTS THE HEIGHT OF THE TOP OF THE PLATE ABOVE THE GROUND; AXIS IS THE GENERAL DIRECTION OF THE PLATE IN RELATION TO THE PLOTS; WIND DIRECTION IS THE PREVAILING WIND DIRECTION IN DEGREES AVERAGED OVER THE PREVIOUS SEVEN DAYS.

Date (Julian)	Number of *M. occidentalis*	Height (cm)	Axis	Wind Direction (°)
211	3	110	N	187°
260	1	54	N	97°
260	1	110	E	97°

Four living and two dead adult female *M. occidentalis* were found on the permethrin-treated trap crops and barrier rows over the course of the experiment. All represented single individuals found on different dates (days 176, 190, 197, 225, 232, 267). No *M. occidentalis* eggs were found on the barrier rows, suggesting that a population had failed to develop there despite the presence of prey. Spider mites had no difficulty dispersing from the infested center row to the outer barrier rows, and mean spider mites per leaf ranged from 0.2 to 19.4 per leaf, so sufficient prey was available to sustain *M. occidentalis* if they had dispersed there.

The CLIMEX Model

The upper portion of each graph shows 30 year average monthly temperatures and precipitation from the CLIMEX meteorological database (Figs. 4A, 4B). The bottom portion of each graph shows the population growth index for *M. occidentalis*. The line labeled "GI" indicates predicted population growth during the year. Population growth is maximized where GI = TI. Figures 4A and 4B indicate that *M. occidentalis* may not enter a winter diapause in much of Florida, and that it can indeed survive the drier and cooler spring, fall, and winter months. Both graphs indicate that *M. occidentalis* populations should decrease to zero in the summer months (July, August, September). The model predicts that populations start to crash earlier in Tampa (mid-June), than in Jacksonville (early July). This could be expected since Tampa has a higher average rainfall for the month of June. The model also predicts that *M. occidentalis* populations could establish earlier in the fall in Tampa (late September) than in Jacksonville (mid-October). This is because September is the wettest month of the year for Jacksonville, while July and August are the wettest months in Tampa. Since *M. occidentalis* does not have a summer estivation to carry it through the stressful months of July, August, and September, the Ecoclimatic Index for both cities is zero, indicating that *M. occidentalis* will not permanently establish in Florida.

DISCUSSION

Both the CLIMEX model and the field plot data suggest that *M. occidentalis* populations will not survive the summer months in Florida without reintroductions. The CLIMEX model indicates that *M. occidentalis* could establish in Tampa and Jacksonville during the drier fall, winter and spring months, but persistence in Gainesville is only suggested from the experimental data during April. Populations of both *M. occidentalis* and *T. urticae* were negatively impacted by high rainfall (Fig. 2). According to Sutherst & Maywald (1985), the three most important aspects of the climate in determining distribution and abundance of animals are temperature, moisture, and for some species, daylength. Field & Hoy (1986) showed that *M. occidentalis* larvae do not mature well at high relative humidities and that egg hatch is inhibited. Herne (1968) found that immersion of the European red mite, *Panonychus ulmi* (Koch), arrested feeding, oviposition, and molting activities. Klubertanz et al. (1990) suggested that wetted leaf canopy may temporarily retard spider mite population growth. Akinlosotu (1982) and Yaninek et al. (1987, 1996) found that in the absence of significant predators, weather was the greatest limiting factor in cassava green mite (CGM), *Monony-chellus tanajoa* (Bondar), populations in Africa. CGM populations were highest during the dry season and were lowest during the wet season, when precipitation exceeds evaporation (Yaninek et al. 1987). What we observed in this experiment may be a combination of both direct mortality from the rainfall and population decline from the wetted canopy. Most of Florida's summer rains occur in the late afternoon and
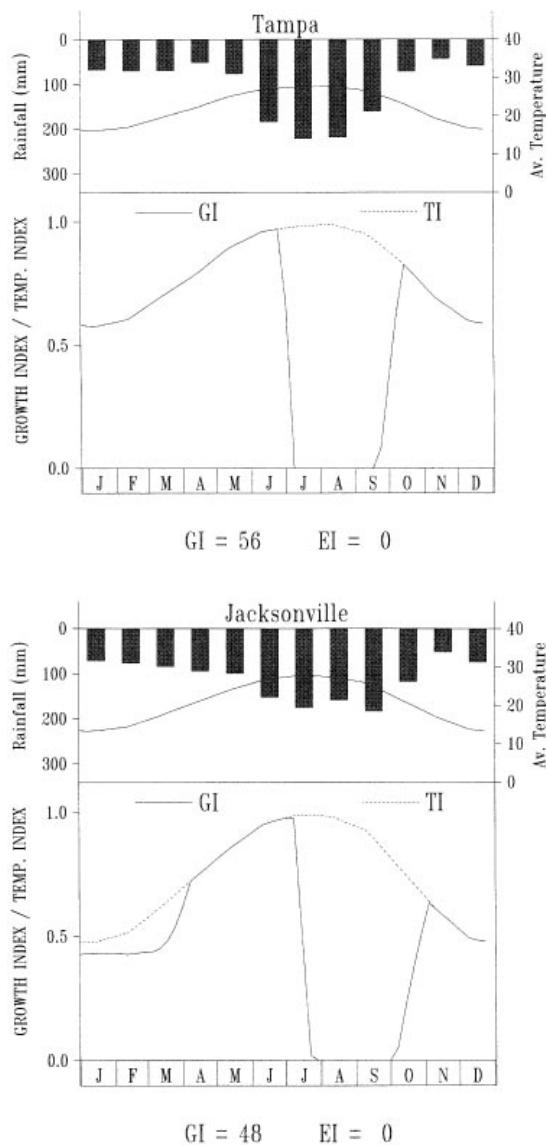
Fig. 4. A. Predicted population growth curves for *M. occidentalis* based on Tampa, Florida meteorological data from the CLIMEX model. B. Predicted population growth curves for *M. occidentalis* based on Jacksonville, Florida meteorological data from the CLIMEX model. Upper portion of graphs shows average monthly temperatures (°C, line) and average monthly rainfall (mm, bars). Bottom portion of graphs depicts Temperature Index (TI) and Growth Index (GI) of *M. occidentalis*. Population growth is maximized where GI=TI. An Ecoclimatic Index (EI) of 0 indicates climatic conditions are not favorable for permanent survival of *M. occidentalis*.

early evening hours and are followed by high nighttime relative humidities peaking at >95% between 3:00 and 6:00 am. A combination of rain and dew can keep the canopy wet for almost all of the evening and nighttime hours.

Results from this study indicate that the permethrin-treated barrier rows did provide an effective barrier to ambulatory dispersal of *M. occidentalis*, although some aerial dispersal did occur. While we recognize that this experimental design may not be optimal from some standpoints, it is very pragmatic for risk assessment studies which require small easily sampled and easily mitigated treatment plots. Although we detected low rates of aerial dispersal, our sampling method undoubtedly underestimated the incidence of aerial dispersal. However, all other evidence suggests that any transgenic *M. occidentalis* that do disperse will be unlikely to permanently establish and persist in Florida.

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