Biological Control **17,** 203–217 (2000)
doi:10.1006/bcon.1999.0799, available online at http://www.idealibrary.com on IDEAL®

Evaluation of the Entomopathogenic Fungi *Beauveria bassiana* and *Paecilomyces fumosoroseus* for Microbial Control of the Silverleaf Whitefly, *Bemisia argentifolii*[1]

S. P. Wraight,*[,2] R. I. Carruthers,† S. T. Jaronski,‡ C. A. Bradley,‡ C. J. Garza,‡ and S. Galaini-Wraight‡[,3]

**USDA-ARS, Subtropical Agricultural Research Center, Weslaco, Texas 78596; †USDA, Western Regional Research Center, Albany, California 94710; and ‡Mycotech Corporation, Butte, Montana 59702*

Received December 31, 1998; accepted October 18, 1999

Collaborative research was conducted at the USDA-ARS Subtropical Agricultural Research Center in southern Texas to assess the microbial control potential of *Beauveria bassiana* and *Paecilomyces fumosoroseus* against *Bemisia* whiteflies. Laboratory assays demonstrated the capacity of both pathogens to infect *Bemisia argentifolii* nymphs on excised hibiscus leaves incubated at relative humidities as low as 25% at 23 ± 2°C (ca. 35% infection by *B. bassiana* and *P. fumosoroseus* resulted from applications of 0.6–1.4 × 10^3 conidia/mm^2 of leaf surface). In small-scale field trials using portable air-assist sprayers, applications at a high rate of 5 × 10^{13} conidia in 180 liters water/ha produced conidial densities of ca. 1–2.5 × 10^3 conidia/mm^2 on the lower surfaces of cucurbit leaves. Multiple applications of one isolate of *P. fumosoroseus* and four isolates of *B. bassiana* made at this rate at 4- to 5-day intervals provided >90% control of large (third- and fourth-instar) nymphs on cucumbers and cantaloupe melons. The same rate applied at 7-day intervals also provided >90% control in zucchini squash, and a one-fourth rate (1.25 × 10^{13} conidia/ha) applied at 4- to 5-day intervals reduced numbers of large nymphs by >85% in cantaloupe melons. In contrast to the high efficacy of the fungal applications against nymphs, effects against adult whiteflies were minimal. The results indicated that both *B. bassiana* and *P. fumosoroseus* have strong potential for microbial control of nymphal whiteflies infesting cucurbit crops.

***Key Words: Beauveria bassiana; Paecilomyces fumosoroseus;* entomopathogenic fungi; *Bemisia argentifolii;* silverleaf whitefly; relative humidity; microbial control.**

INTRODUCTION

Paecilomyces fumosoroseus (Wize) Brown & Smith (Hyphomycetes) is one of the most important natural enemies of whiteflies worldwide (Lacey *et al.*, 1996), and reports of strong epizootic potential against *Bemisia* and *Trialeurodes* spp. in both greenhouse and open field environments (Fang *et al.*, 1983; Osborne and Landa, 1992; Carruthers *et al.*, 1993; Lacey *et al.*, 1996) have stimulated a number of commercial development efforts. In Mexico, a product (Pae-Sin) containing aerial conidia of *P. fumosoroseus* is produced by Agrobiológicos del Noroeste, S. A., of Culiacán, Sinaloa, and marketed for both field and greenhouse applications (Torres and Cárdenas, 1996). A similar product (Bemisin) has been developed by PROBIOAGRO, S. A., of Acarigua, Venezuela (R. Pereira, personal communication). United States and European efforts, on the other hand, have focused almost exclusively on development of blastospore or blastospore/mycelium-based preparations (including PFR-97 and PreFeRal) for control of whiteflies on greenhouse and other protected crops (Eyal *et al.*, 1994b; Bolckmans *et al.*, 1995; Jackson *et al.*, 1997; K. Bolckmans, personal communication).

Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes) is one of the most ubiquitous and extensively studied of the entomopathogenic fungal species and is the active agent in many products currently in use and under development worldwide (see reviews by Feng *et al.*, 1994; Jaronski, 1997; Wraight and Carruthers, 1999). Its commercial potential derives from the combined attributes of exceptional mass culture capacity, desiccation stability, compatibility with widely used formulation ingredients, including oils, emulsifiers, and surfactants, high activity against a variety of agricultural pests, and essentially no toxicity or infectivity toward vertebrates (Bradley *et al.*, 1992; Feng *et al.*, 1994; Jaronski, 1997; Goettel *et al.*, 1997). Although *B. bassiana* is not an important natural enemy of whiteflies, nymphal stages of *Bemisia* are highly susceptible

[1] Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

[2] Present address: USDA-ARS, U.S. Plant, Soil, and Nutrition Laboratory, Ithaca, New York 14853.

[3] Present address: 9 Redwood Lane, Ithaca, New York 14850.

1049-9644/00 $35.00

to this pathogen (Wright and Chandler, 1995; Eyal *et al.*, 1994a; Wraight *et al.*, 1998).

Bemisia argentifolii Bellows & Perring is a key pest of arid agriculture. In the United States, infestations have been most severe in the desert southwest. The capacity to operate under hot, dry conditions is therefore an important consideration in the assessment of whitefly biological control agents. This is especially true with the entomopathogenic fungi that must obtain moisture from the environment to germinate and penetrate their hosts. Earlier investigators reported that *P. fumosoroseus* required high-humidity conditions (>85% RH) to infect whiteflies (Osborne and Landa, 1992; Landa *et al.*, 1994).

Because of the moisture requirements of entomopathogenic fungi, bioassays designed to assess their maximum potential virulence have invariably been conducted under constant high-humidity conditions in containers with minimal or no ventilation or at least with an initial postinoculation period of high humidity (usually a convenient 24 h). The assays reported in our screening study (Wraight *et al.*, 1998) were conducted with a 24-h period of moisture-saturated conditions. However, it is well known that temperature and humidity conditions at the leaf surface may differ substantially from ambient conditions (see Willmer, 1986), and an increasing number of studies indicate that, while fungi do require moisture for development, sufficient moisture exists within the microhabitat of many insect hosts or within the microenvironment of the host's body surface to support infection essentially independent of ambient moisture conditions (Ferron, 1977; Riba and Marcandier, 1984; Ramoska, 1984; Marcandier and Khachatourians, 1987; Fargues *et al.*, 1997b).

In a recent paper, we reported results of a screening program that identified numerous isolates of *B. bassiana* and *P. fumosoroseus* with potential as whitefly control agents (Wraight *et al.*, 1998). Here, we report results of follow-up laboratory and small-scale field experiments that further explored the microbial control potential of these pathogens. The research on *B. bassiana* and *P. fumosoroseus* reported here was conducted with three principal objectives: (1) to determine if these fungi are capable of infecting *B. argentifolii* nymphs under conditions of low humidity, (2) to compare the field efficacy of *B. bassiana* and *P. fumosoroseus*, and (3) to identify one or more promising isolates for further development. Because of its importance as a natural regulator of whitefly populations, it was originally anticipated that research and development efforts would focus on *P. fumosoroseus*. However, because mass production of this pathogen proved difficult (Mycotech Corp., unpublished data in Wraight *et al.*, 1998), small quantities of only one strain of *P. fumosoroseus* were produced for limited testing.

MATERIALS AND METHODS

The laboratory and field studies reported here were conducted at the USDA, ARS Subtropical Agricultural Research Center (SARC) located in the Lower Rio Grande Valley of southern Texas and were the result of an extensive collaboration between the USDA and Mycotech Corp. of Butte, Montana under a Cooperative Research and Development Agreement.

Fungal Preparations

All conidia were produced at the Mycotech laboratory in Butte, Montana using proprietary solid-substrate culture methods and ingredients (see Bradley *et al.*, 1992). Conidial preparations of *P. fumosoroseus* and *B. bassiana* consisted of unformulated (technical) powders containing 9–16 $\times$ 10^{10} conidia/g. Strain GHA of *B. bassiana* was also formulated as a clay-based wettable powder (WP) containing 4.4 $\times$ 10^{10} conidia/g (63% active ingredient). In the following descriptions, the fungus preparations used in each test were identified by Mycotech lot numbers indicating the year, month, and day of production; conidial viability determined at the beginning of each laboratory test and over the course of each field trial is also indicated (in most cases >90%). All preparations were stored at 4°C and retained their initial viabilities for the duration of the study.

Conidial concentrations in the unformulated and wettable powders were determined using standard (improved Neubauer) hemacytometers. Conidial suspensions were prepared by introducing 10–30 mg powder into glass vials containing 10 ml deionized water with 0.01% Silwet-L77 (OSi Specialties, Inc., Tarrytown, NY), adding 1 g glass beads (2-mm diameter), and shaking vigorously by wrist action for 2 min. The vial was then immersed in an ultrasonic water bath (55 kHz) until all visible conidial aggregates were dispersed (ca. 1 min). The final suspension (ca. 1 $\times$ 10^8 conidia/ml) was diluted, and conidia were counted at 400$\times$ magnification in six hemacytometer chambers. This process was replicated three times for each conidial powder (the powder was mixed thoroughly between removal of each sample).

Viability of conidia was determined by suspending conidia as described above, but without sonication, plating on agar (1.5%) containing yeast extract (0.5%), incubating at 25°C for 16–18 h, and examining at 400$\times$ magnification. All conidia with visible germ tubes of any length were scored as viable. The amounts of conidial powder necessary to achieve the desired dosages (viable conidia per ha or per mm^2) in a specific spray volume were determined from these conidial concentration and viability data.

Laboratory Tests

A bioassay protocol was designed at SARC in 1992 and is briefly described here (for a complete description see Wraight *et al.*, 1998). The assays utilized third-instar nymphs of *B. argentifolii* (=*B. tabaci* strain B) on excised leaves of *Hibiscus rosasinensis* L. provided by the USDA-APHIS Biological Control Laboratory of Mission, Texas. Conidia suspended in water with Tween 80 (0.01%) were sprayed onto the whitefly-infested leaves in a spray tower. Following treatment, the leaves were transferred to large petri dishes fitted with small reservoirs to hold the leaf petiole, which was embedded in water-saturated cotton. The dishes were enclosed in plastic bags and placed in an incubator at 25 ± 1°C for 24 h with a 16 h daily photoperiod. Subsequently, the dishes were removed from the bags, allowed to air dry, covered with ventilated lids (lids with an 8-cm-diameter hole covered with a fine screen), and returned to the incubator. The treated leaves were incubated ventral surface up to prevent entrapment of humid air at the leaf surface. Electronic sensors located inside sample incubation dishes (between the leaf and the screen cover) indicated a low relative humidity of 25–30%. Water was added to the cotton reservoir daily to maintain the leaves; the reservoir was covered to minimize release of moisture into the dish.

Two laboratory experiments were conducted to evaluate the effects of relative humidity on infectivity of *B. bassiana* isolate 252 (lot 921020; viability 90%) and *P. fumosoroseus* isolates 3594 (lot 921005; viability 89%) and 613 (lot 930913; viability 92%). For these investigations, the above protocol was utilized with the following changes. After inoculation, the dishes in experiment one were incubated on a lab bench under constant light and for which ambient relative humidity ranged from 49 to 54% and temperature from 21 to 24°C. One group of four replicate dishes was left uncovered and exposed to laboratory ambient humidity for the duration of the experiment. The second group was incubated at 100% RH for 24 h (in plastic bags) and then uncovered for 8 days. The dishes in experiment two (six replicates per treatment) were manipulated in the same way, but held in the 25°C incubator described above. Under the conditions of both experiments, nymphs that succumbed to infection rapidly dried on the leaf surface and did not support fungal outgrowth and sporulation (see Wraight *et al.*, 1998).

Field Trials

The aerial conidia of *B. bassiana* and *P. fumosoroseus* are extremely hydrophobic, and miscibility is an important problem. During our investigations, we discovered that organosilicone surfactants were greatly superior to many commonly used wetting agents (e.g., Tween 80) for producing aqueous suspensions of conidia (Wraight and Bradley, 1996). In all of the field trials described below, Silwet-L77 was used at concentrations of 0.01–0.04%. Even using this effective wetting agent, however, preparation of the spore suspensions required vigorous shaking (for ca. 1 min) in a sealed container with a minimum 25% headspace.

All field applications were made using hand-held, single-nozzle, atomizing (air-assist) sprayers: either a backpack, motorized mist blower (Solo Model 412; Kleinmotoren GMBH, Sindelfingen, Federal Republic of Germany,) or an electrostatic spray gun (ESS Spray gun Model BP-4; Electrostatic Spraying Systems, Inc., Watkinsville, GA) connected to a compressed-air line with an operating pressure of 4.92 kg/cm^2 (70 psi). The spray nozzle was carried near ground level and directed at a right angle to the row. Each row was sprayed twice, once from each side. Spray volume was 234–280 liters/ha.

Conidial deposition was quantified on square plastic cover slips (22 mm) attached to the ventral surfaces of leaves. The slips were pierced by a single pin which was passed through the leaf and embedded in a small (1-cm) cube of dense polyethylene foam. Leaves selected for sampling were those fully exposed to the sun, with the midrib and lateral veins as nearly parallel to the ground as possible. Following the spray application, the slips were allowed to dry before collection and then stored and counted as time allowed. For quantification, the slips were mounted in a drop of lactic acid (85%) containing acid fuchsin (1 mg/ml). Enumeration was achieved using the protocol described by Wraight *et al.* (1998). Viability of conidia was checked routinely during all field trials by collecting samples of the residual spray suspensions from the sprayer reservoirs and incubating on yeast extract agar as previously described.

Significantly different rates of conidial deposition were recorded for the various fungal isolates during the initial field trial (see Results). Since the viability-dependent dose adjustments were similar for all of the different fungi (adjusted for 93–95% viability), these findings suggested significant inaccuracies in the initial determinations of conidial concentrations in the technical powders. Additional hemacytometer samples were therefore processed to verify the concentrations. Estimates changed significantly, and adjustments were made in preparing the spray suspensions for subsequent field trials.

Furrow irrigation was applied every 2–3 weeks in the absence of rain. Air temperature and relative humidity were recorded hourly using portable electronic data loggers (Omnidata International, Inc., Logan, UT) maintained in each test field. Sensors were situated over the center of a row at canopy level. Each sensor was fixed beneath the center of a piece of polystyrene foam insulation (38 × 38 × 2 cm) supported by four stakes and maintained in a horizontal position. The logger

was held between two similar sheets of foam situated just above the sensor. This shelter was designed to shield the logger and sensors from sun and rain without trapping air or interfering with lateral air movement.

Unless otherwise indicated, each treatment of a trial was applied to four replicate plots arranged in a randomized complete block design (RCBD). The replicate plots measured four rows × 9.1 m, with rows spaced 102 cm apart. Each trial included a spray (carrier) control and, with the exception of the honeydew melon trial, an untreated control. All applications were made during the day; sprays were usually initiated early in the morning to take advantage of cool, calm conditions.

Cucumber and cantaloupe melon trials (1994). Four isolates of *B. bassiana* were selected for field testing on the basis of combined attributes of high virulence in laboratory bioassays (Wraight *et al.,* 1998) and excellent sporulation under industrial-scale production conditions (Mycotech Corp., unpublished data). These included isolate 648, originally isolated from *Leptinotarsa decemlineata* (Say), isolate 657 from *Diuraphis noxia* (Mordvilko), isolate MLC8 from *Paraclemensia acerifoliella* (Fitch), and a proprietary isolate designated GHA. Isolate 612 of *P. fumosoroseus* from *Bemisia argentifolii* was selected on the basis of its consistently high virulence (Wraight *et al.,* 1998). Additional information on the origins of these isolates is presented by Wraight *et al.* (1998). These fungi were evaluated in four small-scale field trials during the spring and fall planting seasons in 1994. Research fields of spring cantaloupe melons (variety "Perlita") and cucumbers (variety "Poinsett 76") were planted on 14 February. For the fall trials, the same varieties of melons and cucumbers were planted on 23 August and 15 September, respectively.

Applications during the spring season were made using the electrostatic air-assist sprayer. For the fall trials, conidia were applied using the motorized mist blower. In the first trial (spring cucumbers), five applications each of *B. bassiana* isolates GHA (lot 921114; viability 94%), 657 (lot 940318; viability 95%), 648 (lot 930924; viability 93%), and MLC8 (lot 940321; viability 94%) and *P. fumosoroseus* isolate 612 (lot 940228; viability 95%) were made at intervals of 4–5 days at a rate of 5×10^{13} conidia/ha. In the second trial (spring cantaloupes), it was not possible to initiate treatments until after the appearance of many late-instar nymphs. Considering the severe pest pressures at the time (whitefly outbreak conditions) and the great potential for rapid whitefly development on spring melons, two applications were made at a high rate of 1×10^{14} conidia/ha, followed by four applications at 5×10^{13} conidia/ha, all at 4-day intervals. The trial used the same technical powders as were used in the spring cucumber trial; however, only two *B. bassiana* isolates were tested (isolates GHA and 657). The third trial (fall cantaloupes) examined the efficacy of five applications of *P. fumosoroseus* isolate 612 (lot 940322; viability 91%) and *B. bassiana* isolates GHA (lot 921114; viability 96%) and 657 (lot 940318; viability 96%) made at intervals of 4–6 days at a rate of 1.25×10^{13} conidia/ha. The application rate in this trial was substantially reduced in an effort to confirm the essentially equal virulence of the isolates observed in the spring trials. An experimental clay-based wettable powder formulation of strain GHA was prepared for the fourth trial (fall cucumbers) using technical powder of lot 940919 (viability 81%). Efficacy of this formulation was compared to that of the unformulated 940919 technical powder; seven applications were made at 5-day intervals at the high rate of 5×10^{13} conidia (1.12 kg WP)/ha. Conidial suspensions were prepared using Silwet at 0.01% in the spring trials and 0.04% in the fall trials.

Honeydew melon and zucchini squash trials (1995–1996). A new batch of the WP formulation of strain GHA (lot 950402; viability 95%) was tested in early 1995 in an abbreviated trial to demonstrate efficacy on another variety of melons. Honeydew melons (variety "Green flesh") were planted in late February. As in the previously described trials, plots were organized in a RCBD. However, in this case, there were only three replicate plots per treatment and each plot consisted of only two 9.1-m rows. Only two applications were made (6 days apart) at the high rate of 5×10^{13} conidia in 280 liters water/ha using the motorized mist blower. Conidia were suspended with 0.02% Silwet.

A trial with similar objectives was conducted in spring 1996 in a commercial field of zucchini squash (variety "President") planted on 9 February near Donna, Texas. Six applications of the wettable powder (lot 950402; viability 94%) were made at weekly intervals at a per-ha rate of 5×10^{13} conidia in 234 liters water with 0.03% Silwet. The fungus was applied to two 0.1-ha plots (each 4 rows × 214 m) using the motorized mist blower. Mean whitefly numbers in the fungus-treated areas were compared to those in immediately adjacent areas designated as untreated and spray carrier controls. Treatments were not randomized; the spray control plot (also 4 rows) was situated between the two fungus-treated plots, and the untreated control plot (6 rows) formed the eastern border of the test field.

Sampling Protocols

Leaf samples were collected within 1 day prior to the initial application and at regular intervals of approx 4–7 days thereafter. On each sample date, 10 leaves were collected from arbitrarily selected locations within each replicate plot (usually 2 or 3 leaves/row). In the zucchini trial, 20 leaves were selected in groups of 10 from two different areas in the two fungus-treated plots; 40 leaves were collected from the spray control

plot and 40 from the untreated control plot (also in groups of 10 from four areas in each plot). All samples were unbiased in that lower surfaces of leaves were not viewed prior to selection. After vining, the leaves were collected alternately from the left and right sides of the rows. Collected leaves were held in plastic bags at 4°C prior to processing.

On each sample date, leaves were collected from a specific node on the main stem, counting from the base of the plant. The initial leaf node selected was that harboring the largest population of newly hatched first-instar nymphs (usually the first or second node of young plants). Once a node was selected, leaves from that node were sampled repeatedly (weekly) until significant numbers of "pupal" exuviae were detected (indicating maturation of the nymphal cohort associated with that leaf node). Upon detection of exuviae, a younger node, in this case having the largest number of early- to mid-instar nymphs, was selected for repeated sampling, and the cycle was repeated until the end of the trial. This "punctuated" sampling protocol was developed to assess full efficacy of the spray treatments, as the entomopathogenic hyphomycetes tend to be slow-acting control agents under field conditions.

The sample unit comprised one circular area of 2 cm^2 marked (by indentation) with a cork borer near the base of each leaf on the right side. Counting of high density nymphal populations was facilitated by situating the sample area such that it was bisected by a leaf vein. Nymphs were categorized as small (first and second instars) and large (third and fourth instars, including those in the late "red-eyed pupal" stage). All nymphs found dead (from all causes) were also counted.

Adult whiteflies were counted on young leaves (third node from the growing tip). Counts were made in the field on 10 leaves in each plot during early morning before adults became active. Leaves were selected as described for the nymph samples.

Statistical Analyses

Multiway ANOVAs with orthogonal comparisons were conducted using the JMP statistical software (SAS Institute, 1995). Because the fungal pathogens were slow acting and did not infect significant numbers of egg-laying adults (see Results), early-instar nymphs often infested the plants in great numbers before succumbing to fungal infection. For this reason the analyses and presentation of results are focused on numbers of large nymphs. The mean number of large nymphs/sample area of 2 cm^2 (and mean number of adult whiteflies/leaf) was determined for each sample of 10 leaves collected from each replicate plot. The multiple means obtained (one from each plot) comprised the replicate values subjected to ANOVA. The grand means from the replicate plots are the values presented in the figures.

Because changes in whitefly populations occurred gradually, a simple approach was adopted for evaluating differences in treatment effects. Analyses were conducted on means derived from the total live large nymphs recorded on all leaves collected over a specified period of time from each plot. Treatment means were thus based on four values (one from each plot) regardless of the number of sample dates included in the analysis (excepting the honeydew melon trial with only three replicate plots). In all figures, and in the following Results and Discussion, all references to days indicate days after the initial fungus application. The day or interval of days associated with each *F* value reported in the text identifies which subset of the sample data presented in the figure was included in the ANOVA. Orthogonal comparisons are also identified. Because means were positively correlated with variance, The log $(n + 1)$ transformation was applied to all whitefly numbers prior to ANOVA.

Analyses of mortality data were conducted in similar fashion. Percentage mortality values were derived from the total live and dead whitefly nymphs of all instars counted on all leaves collected from each plot over a specified interval of time. Treatment means comprised the averages of the four percentage values from the four replicate plots. The arcsine transformation was applied to all percentage values subjected to ANOVA.

The conidial counts from the cover slip samples were square-root transformed and subjected to two-factor ANOVA to assess differences associated with the conidial powders (fungal isolates) and application dates.

RESULTS

Laboratory Tests

A moisture-saturated environment was not required for fungal infection of whitefly nymphs on a natural leaf substrate. Mortality caused by both pathogens was an average of 11% higher when inoculated nymphs were held under dry ambient conditions (25–54% RH) during the entire postapplication incubation period than when held under dry conditions following an initial 24-h period of high humidity; however, in each test, the difference was not significant (Table 1).

Field Trials

All fungal treatments in all trials caused high rates of mortality among nymphal whiteflies. Nymphs killed by the fungi dried rapidly and remained attached to the leaf. Nymphs infected by *B. bassiana* (all isolates) were distinctly red to red-brown. Outgrowth and sporulation of the fungi on killed hosts occurred only during extended periods of rain or late in the trials after many nights of exposure to high-humidity conditions. Hyphal growth and sporulation of *P. fumosoroseus* were visibly

TABLE 1

Mortality of *Bemisia argentifolii* Nymphs Treated with *Beauveria bassiana* and *Paecilomyces fumosoroseus* and Incubated under Different Moisture Conditions at 23 ± 2°C

Fungus/strain	Rate ± SE (*n*) (viable conidia ($\times 10^3$)/mm^2)	Mean % mortality ± SE[a]		ANOVA *P* value
		Incubated 24 h at 100% RH then 8 days at 49–54% RH	Incubated 9 days at 49–54% RH	
Test 1				
P. fumosoroseus ARSEF 3594[b]	1.13 ± 0.120 (8)	67.7 ± 2.80	55.8 ± 8.95	0.247
B. bassiana ARSEF 252[b]	1.35 ± 0.109 (8)	75.1 ± 4.07	73.6 ± 9.72	0.925
Spray control (0.01% Tween 80)	0	8.1 ± 2.74	2.7 ± 0.30	0.428
		Incubated 24 h at 100% RH then 8 days at 25–30% RH	Incubated 9 days at 25–30% RH	
Test 2				
P. fumosoroseus Mycotech 613[b]	1.40 ± 0.363 (12)	53.6 ± 6.14	36.2 ± 5.40	0.065
B. bassiana ARSEF 252	0.59 ± 0.090 (12)	47.9 ± 7.91	34.7 ± 8.58	0.234
Spray control (0.01% Tween 80)	0	8.9 ± 3.36	10.0 ± 4.01	0.891

[a] Mean percentage mortality (±standard error) of *B. argentifolii* nymphs on four and six replicate leaves in tests 1 and 2, respectively (approx 50 third-instar nymphs per leaf).

[b] Strain ARSEF 3594 originally isolated from *B. argentifolii*, McAllen, Texas, 1992; strain ARSEF 252 originally isolated from *Leptinotarsa decemlineata*, Orono, Maine, 1978. Strain Mycotech 613 originally isolated from *B. argentifolii*, Weslaco, Texas, 1993.

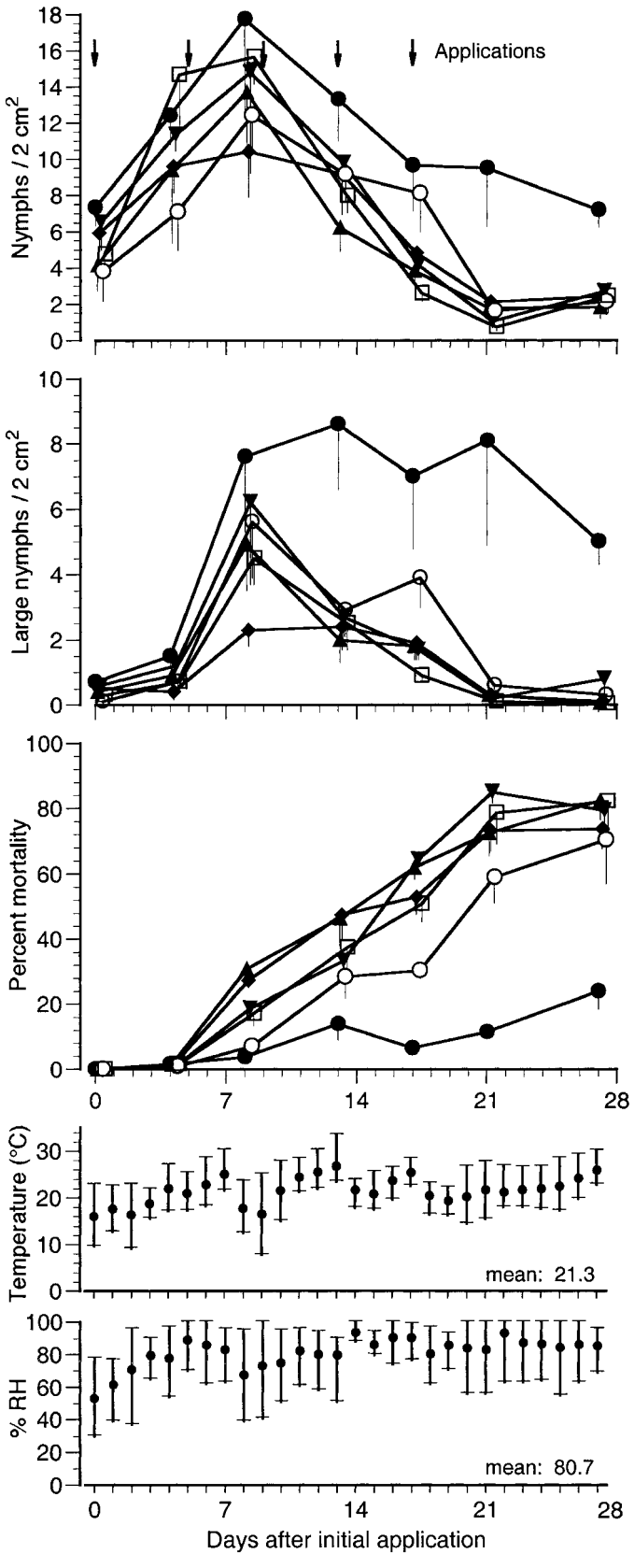
greater and more rapid than that of the *B. bassiana* isolates; however, postmortem development of the various fungi was not monitored or quantified.

Cucumbers (spring 1994). All of the fungal isolates (four *B. bassiana* and one *P. fumosoroseus*) caused substantial reductions in nymphal whitefly populations (Fig. 1). There were no significant differences among numbers of large nymphs from the different fungal treatments (days 8–21 $F_{[4,12]} = 2.66$; $P = 0.085$). Compared to the carrier control, a significant 71% reduction in the mean number of large nymphs in all fungus treatments was recorded within 13 days after the initial application (day 13 orthogonal contrast $F_{[1,15]} = 7.09$; $P < 0.025$), and control reached 96% within 21 days.

Analysis of the percentage mortality data from the second week of the trial suggested significant differences in efficacy among three groups of pathogens: *B. bassiana* strains GHA and 657 > *P. fumosoroseus* 612, and *B. bassiana* 648 > *B. bassiana* MLC8 (days 8–13 $F_{[4,12]} = 5.29$; $P = 0.011$). Within the next week, however, mortality due to the fungi in the first two groups became equal (day 17 $F_{[3,9]} = 2.47$; $P = 0.129$). Mortality due to isolate MLC8 increased more slowly and remained significantly lower until the end of the trial (e.g., when MLC8 is included in the day 17 analysis, $F_{[4,12]} = 11.28$; $P < 0.001$); however, mortality due to this isolate ultimately reached a level statistically equivalent to that of the other isolates (day 27 $F_{[4,12]} = 1.30$; $P = 0.325$). Percentage mortality in the carrier control remained low (<14%) during the first 3 weeks of the trial (through day 21), whereas mortality in the fungal treatments (excluding strain MLC8) averaged 77%.

Cover-slip conidial samples collected during three consecutive spray applications revealed that the amount of fungus applied to the lower surfaces of the cucumber leaves averaged 1.1–2.8 $\times$ 10^3 conidia/mm^2 (Table 2). Although all isolates were presumably applied at the same field rate (5×10^{13} conidia/ha), the conidial depositions differed significantly among isolates ($F_{[4,140]} = 3.99$; $P = 0.004$). The consequence of this result was discussed under Materials and Methods. The rates of conidial deposition were not influenced by application date ($F_{[2,140]} = 1.07$; $P = 0.347$), and there was no significant interaction between application date and isolate ($F_{[8,140]} = 1.74$; $P = 0.095$).

Cantaloupe melons (spring 1994). Isolates GHA and 657 of *B. bassiana* and 612 of *P. fumosoroseus* also caused large reductions in numbers of whitefly nymphs on melons (Fig. 2). In contrast to the results in cucumbers, however, an analysis comparing total large nymphs collected between 8 and 12 days after initiation of the spray program indicated significant differences among the three fungal treatments (days 8–12 $F_{[2,6]} = 6.19$; $P = 0.035$). However, the difference persisted only briefly and the three fungi were equally effective by the third week (days 16–20 $F_{[2,6]} = 0.45$; $P = 0.657$). Analysis of the large-nymph populations in the three controls (water only, water with 0.01% Silwet, and untreated) over the second and third weeks of the trial indicated no significant differences (days 8–20 $F_{[2,6]} = 1.38$; $P = 0.321$). Compared to the combined controls, a significant 69% reduction in the mean number of large



nymphs was recorded in the fungus-treated plots within 8 days after the first spray (day 8 orthogonal comparisons $F_{[1,15]} = 8.65$; $P < 0.025$). At the end of the trial, average populations of large nymphs in the fungal treatments were 95% lower than in the controls.

With the exception of the anomalous day 16 results, the trends in percentage mortality of all nymphs in the fungus-treated plots were similar (days 8–12 $F_{[2,6]} = 0.12$; $P = 0.892$). Mortality trends recorded for the three control treatments were also similar. Average control mortality remained low over the course of the trial, reaching a maximum of 14.3% on day 12, whereas average mortality in the fungus-treated plots reached 90% by the end of the trial.

Cover-slip conidial samples were collected for each of the six spray applications on the spring cantaloupes (Table 2). The mean conidial deposits did not differ significantly among fungal isolates ($F_{[2,126]} = 1.168$; $P = 0.314$). As observed in cucumbers, interaction between spray date and isolate was not significant ($F_{[10,126]} = 1.467$; $P = 0.159$). The mean ± SE concentrations of all isolates deposited by the initial two applications at the high rate of 1×10^{14} conidia/ha averaged $4.3 \pm 0.37 \times 10^3$ conidia/mm^2 ($n = 48$), while the four applications at 5×10^{13} conidia/ha deposited an average of $1.6 \pm 0.13 \times 10^3$ conidia/mm^2 ($n = 96$). Applications at both rates did not vary significantly over spray dates (high rate $F_{[1,44]} = 0.002$; $P = 0.964$; low rate $F_{[3,90]} = 1.96$; $P = 0.125$).

Cantaloupe melons (fall 1994). As was observed in the spring melon trial, isolates GHA and 657 of *B. bassiana* and 612 of *P. fumosoroseus* were highly efficacious (Fig. 3); in this case, the three fungi caused equal reductions in numbers of large nymphs over the entire course of the trial (days 6–25 $F_{[2,6]} = 1.30$; $P = 0.339$). The mean number of large nymphs in the fungal treatments was a significant 72% lower than that in the carrier control within 13 days after the first application (day 13 orthogonal comparison $F_{[1,12]} = 10.05$; $P < 0.010$). Control increased to 90% over the next 6 days.

As seen in the spring trial, the trends in percentage mortality were similar for the three fungi (day 13 $F_{[2,6]} = 1.12$; $P = 0.386$). Average mortality due to all fungus treatments increased steadily to a maximum of

FIG. 1. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of unformulated conidia of *Paecilomyces fumosoroseus* isolate 612 (□) and *Beauveria bassiana* isolates GHA (▲), 657 (◆), 648 (▼), and MLC8 (○) suspended with 0.01% Silwet in a spring cucumber field with moisture and temperature data (daily means, maxima, and minima). Each application was made at a per-hectare rate of 5×10^{13} conidia in 280 liters of water. Treatments included a spray carrier control of water with 0.01% Silwet (●). The initial application was made on 29 March 1994. Vertical lines represent standard errors of means ($n = 4$); for clarity, some means are offset on the x axis.

TABLE 2

Quantification of Spores Deposited on Coverslips Attached to the Lower Surfaces of Cucumber and Melon Leaves during Spray Applications of *Beauveria bassiana* (Bb) and *Paecilomyces fumosoroseus* (Pfr) Conidia in Three Field Trials

Application date and rate[a]	Plant age (days)	Conidia ($\times 10^3$)/mm^2 ± SE (n) of fungus isolate[b]				
		Pfr 612	Bb GHA	Bb 657	Bb 648	Bb MLC8
Spring 1994 cucumbers: hand-held electrostatic air-assist sprayer						
7 April-5 × 10^{13}	52	2.5 ± 0.31 (12)	2.8 ± 0.34 (16)	1.6 ± 0.22 (16)	1.6 ± 0.26 (16)	1.5 ± 0.29 (16)
11 April-5 × 10^{13}	56	1.4 ± 0.30 (8)	2.6 ± 0.63 (8)	1.7 ± 0.26 (8)	1.4 ± 0.50 (8)	1.3 ± 0.35 (8)
15 April-5 × 10^{13}	60	2.1 ± 0.62 (8)	2.2 ± 0.31 (8)	1.1 ± 0.50 (8)	2.7 ± 0.46 (8)	1.6 ± 0.34 (7)
	Means	2.1 ± 0.25 (28)	2.6 ± 0.24 (32)	1.5 ± 0.18 (32)	1.8 ± 0.23 (32)	1.5 ± 0.19 (32)
Spring 1994 cantaloupe melons: hand-held electrostatic air-assist sprayer						
13 April-1 × 10^{14}	58	4.0 ± 0.78 (8)	4.8 ± 1.12 (8)	4.2 ± 0.96 (8)	—	—
17 April-1 × 10^{14}	62	3.2 ± 0.48 (8)	3.9 ± 0.74 (8)	5.8 ± 1.20 (8)	—	—
	Means	3.6 ± 0.46 (16)	4.4 ± 0.66 (16)	5.0 ± 0.77 (16)		
21 April-5 × 10^{13}	66	0.81 ± 0.27 (8)	1.7 ± 0.56 (8)	1.1 ± 0.41 (8)	—	—
25 April-5 × 10^{13}	70	2.4 ± 0.53 (8)	2.1 ± 0.50 (8)	0.6 ± 0.20 (8)	—	—
29 April-5 × 10^{13}	74	2.0 ± 0.43 (8)	2.0 ± 0.58 (8)	1.8 ± 0.29 (8)	—	—
3 May-5 × 10^{13}	78	1.0 ± 0.34 (8)	1.9 ± 0.35 (8)	1.4 ± 0.40 (8)	—	—
	Means	1.6 ± 0.23 (32)	1.9 ± 0.24 (32)	1.2 ± 0.18 (32)		
Fall 1994 cucumbers: motorized back-pack mist blower						
4 Nov.-5 × 10^{13}	50	—	2.0 ± 0.26 (24)	—	—	—
9 Nov.-5 × 10^{13}	55	—	1.5 ± 0.23 (24)	—	—	—
	Mean		1.8 ± 0.18 (48)			

[a] Application rate expressed as conidia per hectare.
[b] Mean conidia ($\times 10^3$)/mm^2 ± standard error (number of cover slips).

82% on day 19. Conidial deposits were not measured during this trial.

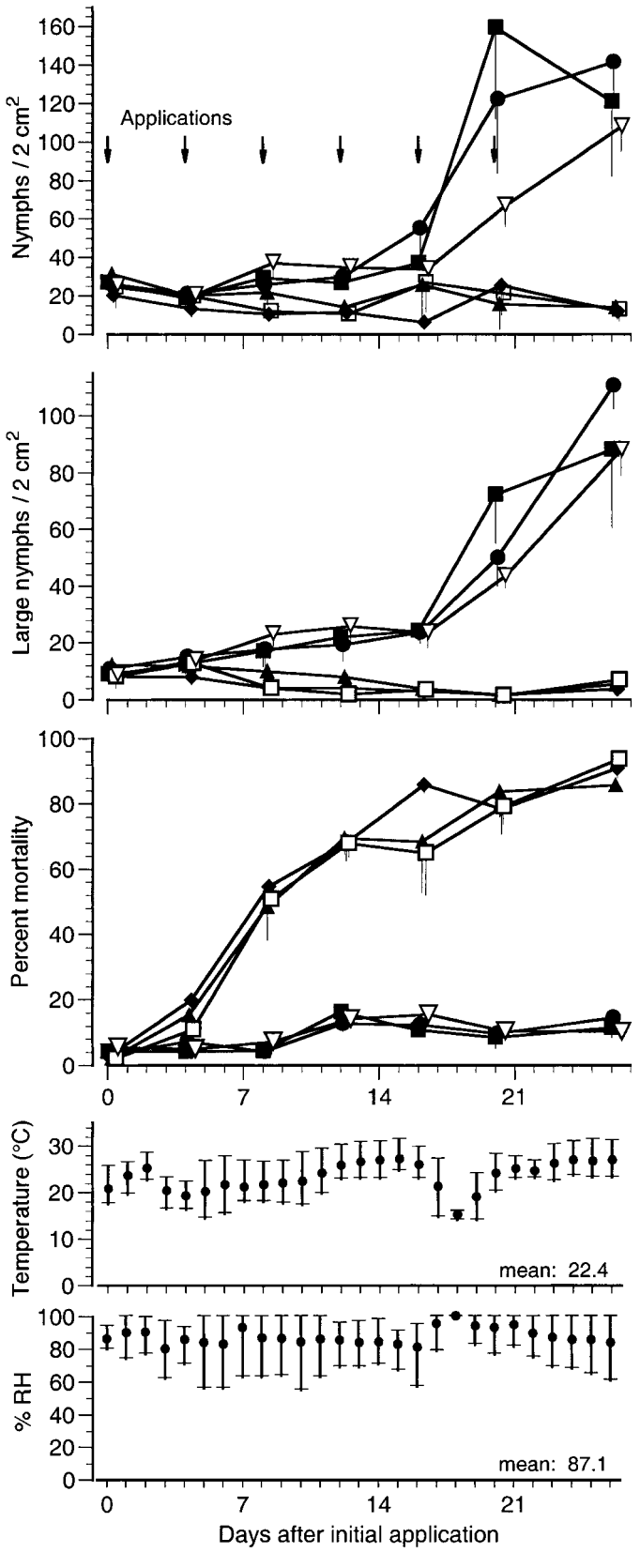
A direct comparison of total large nymphs in the carrier and untreated controls (Fig. 3) revealed a highly significant difference (days 6–25 $F_{[1,3]} = 50.49$; $P = 0.006$), with nymph numbers an average of 49% lower in the Silwet-treated plots. This may have been due, in part, to factors unrelated to the treatments; total nymph populations were lower in the Silwet carrier-control plots than in the untreated control plots at the beginning of the trial. However, the mortality data also indicated a substantial carrier spray effect. Percentage mortality in the two controls was significantly different within 2 weeks after the initial spray (day 13 $F_{[1,3]} = 25.42$; $P = 0.015$), and this difference increased over the next 12 days. In the final sample, mortality in the carrier control was 35% compared to only 11% in the untreated control.

Cucumbers (fall 1994). The formulation of *B. bassiana* GHA as a clay-based wettable powder had no effect on efficacy (Fig. 4). Total numbers of large nymphs collected over the course of the trial from the wettable versus technical powder treatments were not significantly different (days 7–40 $F_{[1,3]} = 0.08$; $P = 0.790$). Significant control of large nymphs was realized within 7 days after the initial application; the mean number of large nymphs in the two treatments was 80% lower than in the carrier control (day 7 orthogonal comparison $F_{[1,9]} = 29.50$; $P < 0.001$). Control reached a maximum of 92% within 14 days.

Percentage mortality of all nymphs in the two fungus treatments was similar on all sample dates (Fig. 4). Mean mortality in the fungus treatments increased rapidly to 77% during the first week of the trial, decreased to 57% during the next week, and then slowly increased to >90%.

During much of the trial, population trends in the control plots were similar to those observed in the fall melon trial, in that fewer large nymphs were recorded in the spray carrier plots than in the untreated plots. In this case, however, the difference was not statistically significant (days 20–35 $F_{[1,3]} = 3.38$; $P = 0.163$). During the first 3 weeks of the trial, percentage mortality in the untreated control plots remained low (≤7%) but then increased gradually to 34% by the end of the test. This mortality resulted primarily from unusually high activity of various predators and parasites. As was also observed in the fall melon trial, mortality of nymphs collected from the untreated and carrier controls became significantly different within 2 weeks after the initial spray (day 14 $F_{[1,3]} = 97.78$; $P = 0.002$). This difference (greater mortality in the carrier control) persisted during the next 3 weeks.

A limited sampling of conidial deposits was conducted during this trial (Table 2). A mean of 1.8 ±



0.18×10^3 conidia/mm^2 ($n = 48$) was deposited on two application dates.

Honeydew melons (spring 1995). Samples were collected only on days 6 and 17 posttreatment (and from only three replicate plots). The data revealed, nevertheless, that the wettable powder formulation of *B. bassiana* GHA was also effective against whiteflies infesting a second variety of melons (Fig. 5). In the second posttreatment sample (day 17), numbers of large nymphs were a significant 76% lower in the fungus treatment than in the carrier control ($F_{[1,2]} = 29.78$; $P = 0.032$). On the same day, percentage mortality in the fungus-treated plots was 74% compared to 26% in the carrier control plots.

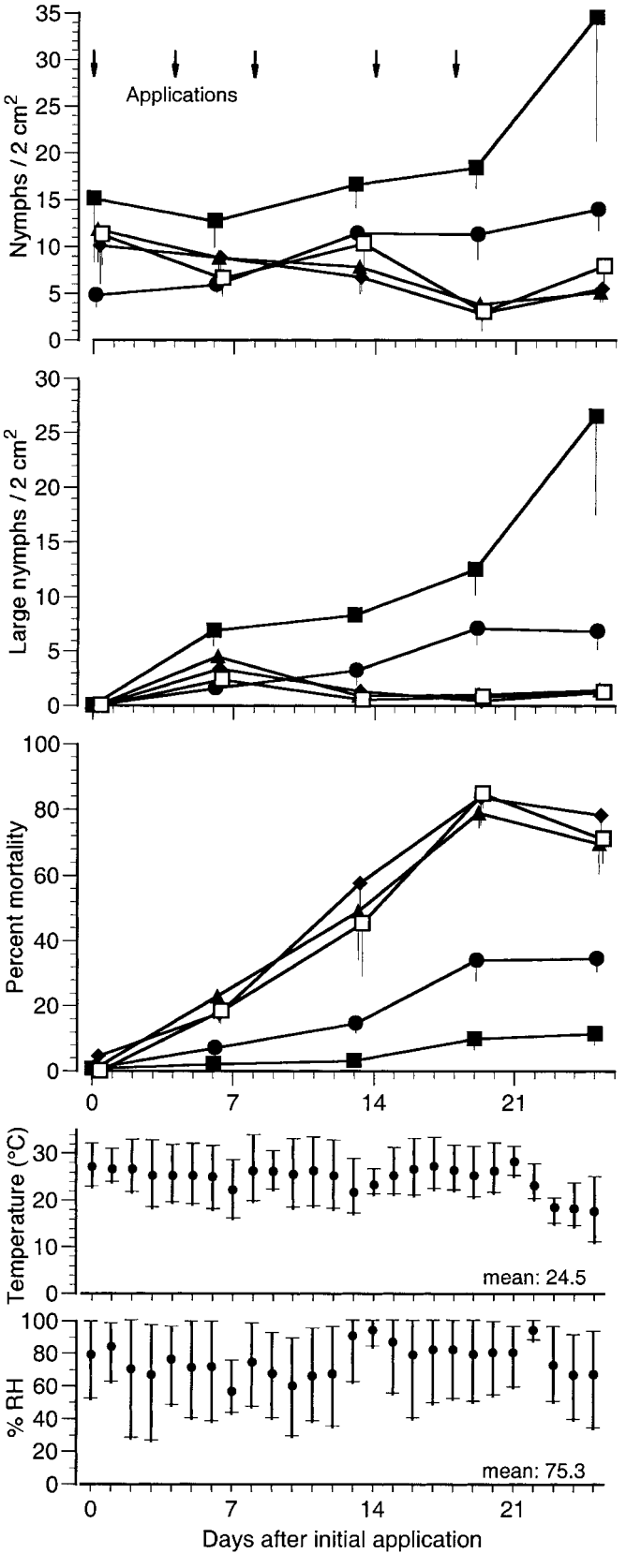
The large-nymph population in the carrier control was 40% lower than that in the untreated control on day 17, and percentage mortality in the respective controls was 26.1 versus 14.7%. These differences were not significant ($F_{[1,2]} = 12.86$; $P = 0.070$, and $F_{[1,2]} = 9.68$; $P = 0.089$, respectively); however, significance would likely have been detected if an additional replicate plot had been treated.

Zucchini squash (spring 1996). Whitefly populations increased very slowly in this crop, apparently due to the cool early-spring weather conditions; the mean temperature during the trial was only 18.5°C (Fig. 6). Significant numbers of large nymphs were not detected in the field until 15 days after the initial application. Because of the lack of random replication, the trial results are not subject to rigorous ANOVA. Nevertheless, 91% control of large nymphs (compared to the carrier control) was recorded within 20 days after their first appearance (28 days after the first spray). Numbers of large nymphs never increased thereafter in the fungus-treated plots, and control remained greater than 90%.

Percentage mortality was numerically greater in the carrier control than in the untreated control during the first 3 weeks of the trial; however, mortality in the two controls became equal on day 28 and remained so until the end of the trial.

Adult susceptibility. In contrast to the high efficacy of the fungus applications against nymphs, effects

FIG. 2. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of unformulated conidia of *Paecilomyces fumosoroseus* isolate 612 (□) and *Beauveria bassiana* isolates GHA (▲) and 657 (◆) suspended with 0.01% Silwet in a spring cantaloupe melon field with moisture and temperature data (daily means, maxima, and minima). Two applications were made at a per-hectare rate of 1×10^{14} conidia, followed by four applications at 5×10^{13} conidia, each in 280 liters of water. Treatments included a water control (▽), a spray carrier control of water with 0.01% Silwet (●), and an untreated control (■). The initial application was made on 13 April 1994. Vertical lines represent standard errors of means ($n = 4$); for clarity, some means are offset on the x axis.



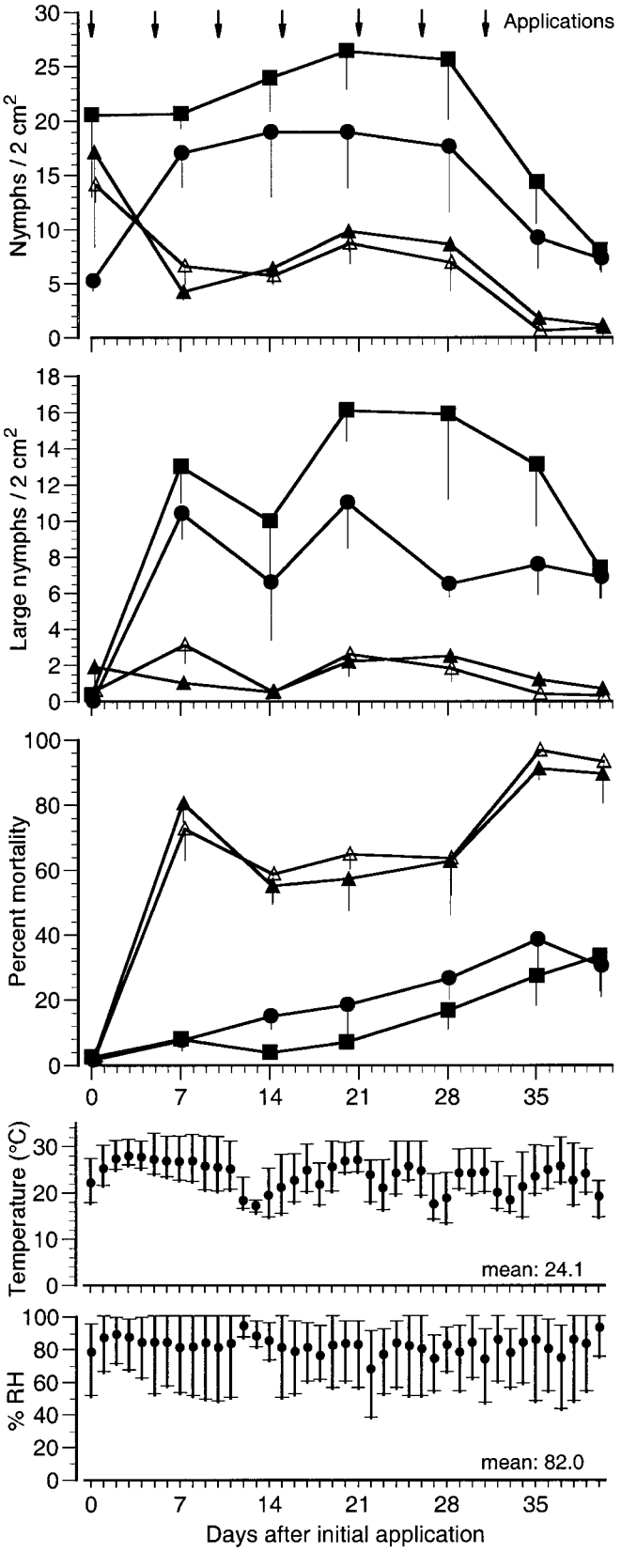
against adult whiteflies were minimal (data not shown). Over some time intervals in some of the trials, fewer adults were counted in the fungal treatments than in the untreated controls; however, few significant differences were observed when comparisons were made to the spray carrier controls. The only consistent effect of the spray treatments was observed at the ends of the trials. In each of the four trials, the final sample revealed greater numbers of adults in the carrier or untreated controls than in the fungal treatments. This effect was most evident in the spring melon trial in which a mean of 67 adults was recorded in the fungus-treated plots versus 133.4 in the carrier control (day 26 orthogonal comparison $F_{[1,12]} = 7.43$; $P < 0.025$) and in the fall cucumber trial in which the mean number of adults in the two fungal treatments was 5.3 compared to 15.2 in the carrier control (day 35 orthogonal comparison $F_{[1,9]} = 25.36$; $P < 0.001$).

DISCUSSION

The results presented here are in accord with our earlier observations of equal pathogenicity of *P. fumosoroseus* and *B. bassiana* against nymphs of *B. argentifolii* under laboratory conditions (Wraight *et al.*, 1998). All of the isolates tested provided >90% control of large-nymph populations. We attribute this high efficacy to a number of factors, including: (1) the ability of both pathogens to infect whitefly nymphs under low-ambient-humidity conditions, (2) the high-ambient-humidity conditions recorded in the field, (3) the moderate temperatures that prevailed during the trials, and (4) the high-density conidial deposits produced by the hand-directed sprayers.

Although seemingly a contradiction to the first, the second factor listed above cannot be dismissed. The laboratory data indicate an ability of both pathogens to exploit the moist conditions of the leaf or insect boundary layer for germination and host infection; however, simple laboratory experiments cannot simulate the field environment. The effects of constantly changing temperature, humidity, wind speed, and even soil moisture levels on conditions at the leaf cuticle–air interface are poorly understood. It cannot, therefore, be assumed that the success in these trials will translate to equal

FIG. 3. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of unformulated conidia of *Paecilomyces fumosoroseus* isolate 612 (□) and *Beauveria bassiana* isolates GHA (▲) and 657 (◆) suspended with 0.04% Silwet in a fall cantaloupe melon field with moisture and temperature data (daily means, maxima, and minima). Each application was made at a per-hectare rate of 1.25×10^{13} conidia in 280 liters of water. Treatments included a spray carrier control of water with 0.04% Silwet (●) and an untreated control (■). The initial application was made on 16 September 1994. Vertical lines represent standard errors of means ($n = 4$); for clarity, some means are offset on the x axis.

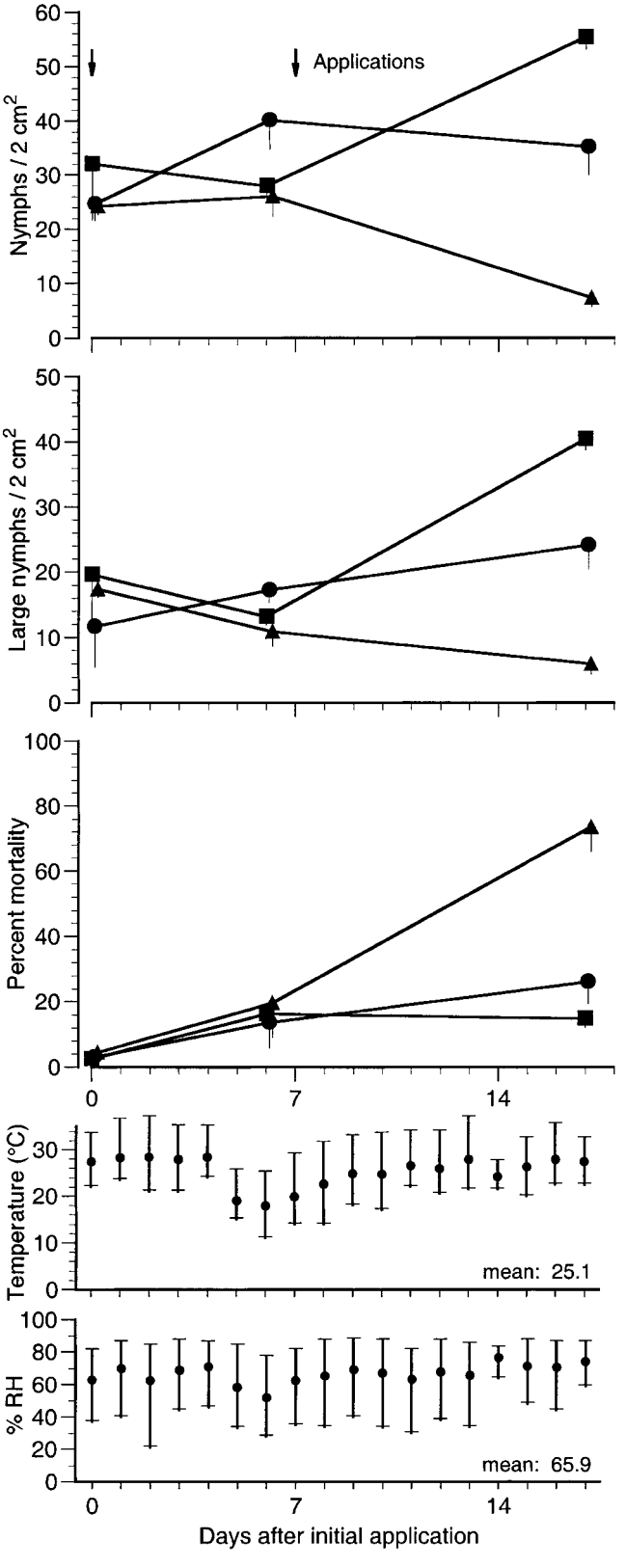


success in other regions with more severe climates. Accepting this, however, it is noteworthy that *B. bassiana* was effective (76% control) even under the drought conditions of the spring 1995 field season. Mean relative humidity during the honeydew melon trial was only 65.9%; nighttime humidity never rose above 85%, and there was virtually no dew formation (Table 3). Also, Jaronski and Lord (1996) reported that multiple, weekly applications of strain GHA at a rate of 2.5×10^{13} conidia/ha gave >70% control of *B. argentifolii* nymphs infesting irrigated cantaloupe melons in the Imperial Valley of southern California, a desert region characterized by severe (hot, dry) weather conditions.

Our report of significant fungal infection at low RH contrasts with the findings of Landa *et al.* (1994), who observed no growth of *P. fumosoroseus* on *B. argentifolii* nymphs incubated at 80% RH. This is likely explained by the substantial differences between assay protocols. In the cited study, early fourth-instar nymphs were removed from foliage and placed on droplets of conidial suspension on glass slides (where they remained for daily observations); in our assays, third-instar nymphs were treated *in situ* on a living leaf. The results from these different assays suggest that the moisture required for fungal development was present in the boundary layer created by the leaf rather than the insect. On the other hand, the nymphs on the glass slides were unable to feed and almost certainly were not in a normal state of hydration [although Landa *et al.* (1994) reported that this treatment of the nymphs had little impact on their development]. It is also possible that the nymph and leaf together contribute to the creation of a zone of high humidity that supports fungal infection. A humid zone established between the nymph and the leaf substrate, for example, might be of great significance following a molt, when the nymph expands and settles back onto the conidia-contaminated substrate. The copious amounts of honeydew secreted by feeding nymphs might also influence humidity in the phyllosphere.

The third efficacy factor identified at the beginning of this section is temperature. Most isolates of *B. bassiana* and *P. fumosoroseus* are inhibited by temperatures greater than 32–35°C (Fargues *et al.*, 1997a; Vidal *et al.*, 1997). However, the trials reported here were

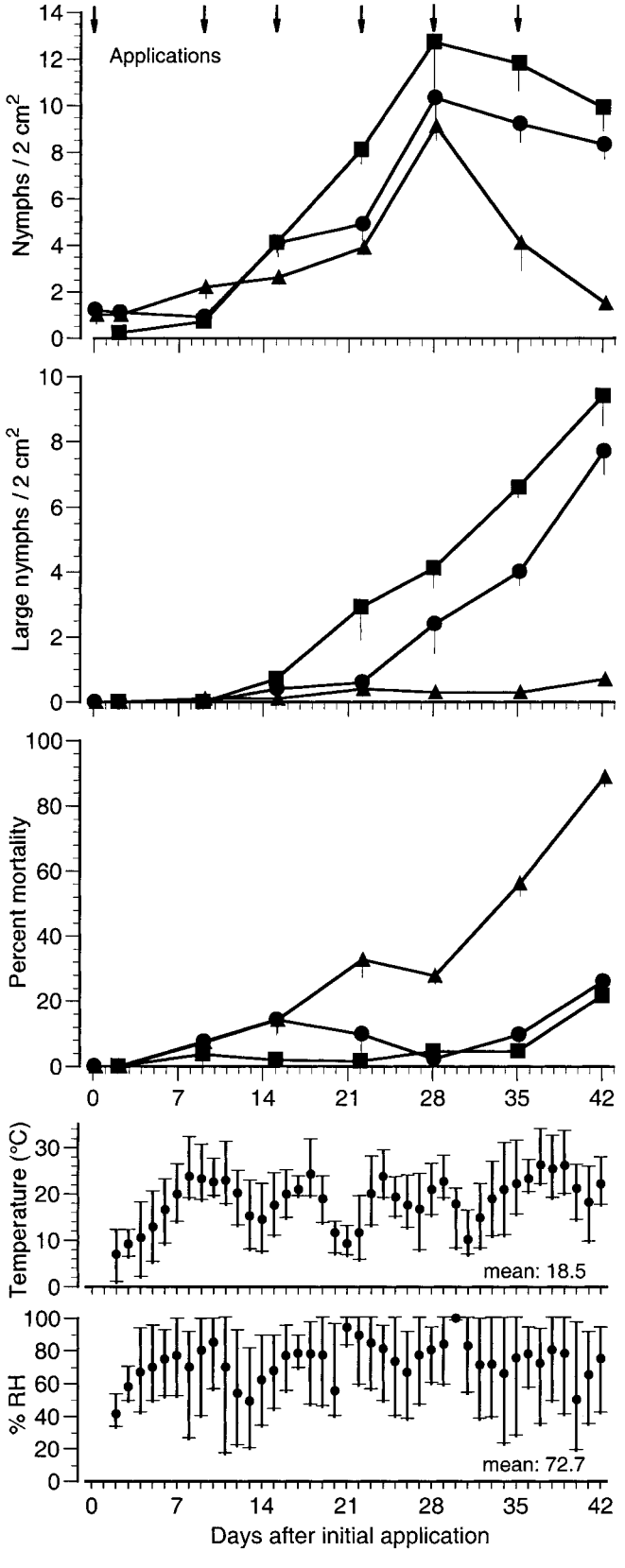
FIG. 4. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of an unformulated technical powder (▲) and wettable powder formulation (△) of *Beauveria bassiana* isolate GHA suspended with 0.04% Silwet in a fall cucumber field with moisture and temperature data (daily means, maxima, and minima). Each application was made at a per-hectare rate of 5×10^{13} conidia in 280 liters of water. Treatments included a spray carrier control of water with 0.04% Silwet (●) and an untreated control (■). The initial application was made on 14 October 1994. Vertical lines represent standard errors of means ($n = 4$); for clarity, some means are offset on the x axis.



conducted during the spring and fall planting seasons, when temperatures were generally moderate and highly favorable for these fungi (Table 3). The highest temperatures were recorded in the spring of 1995 during the honeydew melon trial, but these averaged only 26.2°C (the mean daily maximum was 32.6°C, and temperatures exceeded 35°C on only 3 of 18 days). Temperature is therefore not considered to have been an important negative factor, especially considering that leaf surface temperatures were undoubtedly lower than ambient (Willmer, 1986). The lower efficacy observed in this trial was most likely due to the limited number of applications (two) and the fact that large nymphs already comprised a substantial proportion of the population at the time of the initial spray (Fig. 5).

The significance of the fourth factor (efficient application) cannot be overstated. In our laboratory bioassays, the most virulent isolates of *B. bassiana* and *P. fumosoroseus* killed 50% of third-instar nymphs when applied at densities of ca. 1×10^2 conidia/mm^2, and probit regression slopes averaged 1.13 (Wraight *et al.*, 1998). Assuming a similar dose-response relationship in the field, the dose required to kill 90% of the nymphal population would be 1.4×10^3 conidia/mm^2. Given that concentrations of this magnitude were repeatedly applied in the field (Table 2), the high levels of control achieved in most trials must be considered the expected result. On the other hand, a rate only one-fourth that used in the other trials gave excellent control of nymphs in the fall melons. Conidial deposits were, unfortunately, not sampled in that test, but based on the data from the spring melon trial (Table 2), the concentration was, theoretically, no more than 4–5 $\times$ 10^2 conidia/mm^2. The recording of >85% control in this trial suggests that the effective concentrations were achieved through the multiple, frequent applications with the highly efficient portable sprayers. In parallel and subsequent field trials, achieving effective concentrations on the lower surfaces of crop foliage using conventional tractor-driven sprayers and economically acceptable spray intervals became a difficult challenge. This was especially true in the extremely dense crop canopies of hybrid melon varieties (unpublished data). Use of the plastic cover slips to sample conidial deposits has proven to be an effective technique for assessing effi-

FIG. 5. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of unformulated conidia of *Beauveria bassiana* isolate GHA (▲) suspended with 0.02% Silwet in a spring honeydew melon field with moisture and temperature data (daily means, maxima, and minima). Each application was made at a per-hectare rate of 5×10^{13} conidia in 280 liters of water. Treatments included a spray carrier control of water with 0.02% Silwet (●) and an untreated control (■). The initial application was made on 18 April 1995. Vertical lines represent standard errors of means ($n = 3$); for clarity, some means are offset on the x axis.



ciency of spray applications and correlating dose (on an area basis) with control.

The lack of efficacy against adult whiteflies is another important challenge with respect to commercial success. The only consistent, significant reductions in adult whitefly populations were detected at the ends of the trials, and this reduction was apparently the result of the aging plants becoming less attractive to migrating adult populations to the degree that the treatment effects on nymph survival and adult emergence became evident. It must be acknowledged that monitoring adult populations in small research fields with randomized plots is a questionable protocol. The highly active adults fly freely from plot to plot, and the extensive border areas associated with small-plot trials and areas neighboring the controls are especially vulnerable to population changes unrelated to the applied treatments. Nevertheless, in studies of natural *P. fumosoroseus* epizootics in broccoli (Carruthers *et al.*, 1993) and soybeans (unpublished observations) in the Lower Rio Grande Valley we observed that many adult whiteflies remained attached to the host plants after succumbing to fungal infection. Yet, in the numerous trials that we have conducted on cucurbits, broccoli, tomatoes, and cotton in southern Texas, few fungus-killed adults have been observed on the plants, and patent mycosis has never exceeded 10–15% of the total number of live and dead adults; mycosis due to *B. bassiana* has never exceeded 1% (unpublished data). Even the humid conditions that prevailed during the spring 1994 trials (Table 3) did not support *P. fumosoroseus* epizootics. These results, even accounting for large rates of migration into and out of the small research plots, confirm low susceptibility of adult whiteflies to the sprayed fungus. The reasons for this are not known; the covering of the body by membranous wings and waxy scales may be an important factor; the adults are also much less intimately associated with the leaf boundary layer than the highly susceptible nymphs and may spend sufficient time exposed to low humidity and solar radiation to escape infection.

It is well known that many surfactants possess insecticidal properties. Silwet L-77 at a concentration of 0.1% is extremely toxic to aphids when applied at high volumes (to runoff) under conditions of high humidity (Imai *et al.*, 1995). The results reported

FIG. 6. Trends in nymphal whitefly populations and mortality during a program of multiple spray applications of unformulated conidia of *Beauveria bassiana* isolate GHA (▲) suspended with 0.03% Silwet in a spring zucchini squash field with moisture and temperature data (daily means, maxima, and minima). Each application was made at a per-hectare rate of 5×10^{13} conidia in 234 liters of water. Treatments included a spray carrier control of water with 0.03% Silwet (●) and an untreated control (■). The initial application was made on 6 March 1996. Vertical lines represent standard errors of means ($n = 4$); for clarity, some means are offset on the x axis.

TABLE 3

Efficacy of *Beauveria bassiana* Isolate GHA (Bb) and *Paecilomyces fumosoroseus* Isolate 612 (Pfr) against *Bemisia argentifolii:* Summary of Field Trial Results and Weather Data

Trial	Fungus	Treatment/spray interval[a]	Max. control (sprays)[b]	Time to significant control[c]	Mean temperature[d]	Mean relative humidity[d]	Mean vapor pressure deficit[d]	Mean max. vapor pressure deficit[e]
Spring 1994 Cucumber	Bb	5×10^{13}/4 days	96.3 (5)	13 days	20.9 (16.5–26.1)	79.1 (58.4–94.9)	5.03	12.20
	Pfr		98.8 (5)	13 days				
Spring 1994 Cantaloupe melons	Bb	5–10 $\times 10^{13}$/4 days	96.6 (5)	8 days	22.4 (19.3–26.3)	87.1 (69.3–98.1)	3.00	8.34
	Pfr		97.1 (5)	8 days				
Fall 1994 Cantaloupe melons	Bb	1.25 $\times 10^{13}$/4 days	85.9 (4)	13 days	25.3 (20.0–31.5)	74.9 (45.9–96.7)	8.27	20.43
	Pfr		88.7 (4)	13 days				
Fall 1994 Cucumber	Bb	5×10^{13}/5 days	92.4 (3)	7 days	26.2 (22.0–27.8)	84.6 (77.8–88.8)	4.71	13.41
Spring 1995 Honeydew melons	Bb	5×10^{13}/7 days	75.5 (2)	17 days	25.1 (19.5–32.6)	65.9 (39.3–84.6)	9.41	22.28
Spring 1996 Zucchini squash	Bb	5×10^{13}/7 days	92.5 (5)	28 days	18.5 (12.9–24.9)	72.7 (45.1–94.4)	4.92	12.63

[a] Viable conidia per hectare/spray interval in days.

[b] Maximum percentage reduction in large-nymph populations compared to the spray carrier control. Values in parentheses are the number of spray applications made prior to achievement of maximum control.

[c] Time (days after initial application) until first observation of statistically significant reduction in large-nymph populations compared to spray carrier control.

[d] Mean of temperature (°C), relative humidity (%), or vapor pressure deficit (mm of Hg) values recorded at hourly intervals from the day of initial treatment until day of maximum recorded level of control. Values in parentheses are mean daily minima and maxima.

[e] Mean of daily maximum values.

herein suggest that 0.02–0.04% may be the approximate threshold concentration of this material for causing significant mortality of whitefly nymphs under the conditions of these trials. No difference in percentage mortality of nymphs in untreated versus carrier controls was detected in the spring melon trial when the Silwet carrier was applied at 0.01% (Fig. 2). Increasing the concentration to 0.02 and 0.03% produced maximum differences, respectively, of 11.5% between the untreated and the carrier controls of the honeydew melon trial and 12.5% between these controls during the first half of the zucchini trial (Figs. 5 and 6). Increasing concentration to 0.04% produced significant maximum differences of 11.4 and 24.12% between the untreated and the carrier controls in the fall cucumber and melon trials, respectively (Figs. 3 and 4).

Among the different isolates of fungi tested, it was difficult to identify any one as being clearly more efficacious than the others; all of the fungi tested effectively controlled large-nymph populations. However, during the spring cucumber trial, in which all the selected isolates were compared, *B. bassiana* isolates GHA and 657 produced the most rapid mortality of nymphs (Fig. 1). Efficacy of isolate 657 was equal to that of GHA, even though the conidia sampling data (Table 2) indicate that the actual concentration of 657 applied was lower than that of GHA.

Despite the documented greater epizootic potential of *P. fumosoroseus*, the difficulties experienced in mass production eliminated this pathogen from immediate consideration for commercial pest control in field crops (see Wraight *et al.*, 1998). There, nevertheless, remains considerable interest in continued development of this important natural enemy of whiteflies. Liquid fermentation methods and media for high-yield production of desiccation-tolerant blastospores have been developed (Jackson *et al.*, 1997), and blastospore-based products (PFR 97 and PreFeRal) have been developed for greenhouse applications (Eyal *et al.*, 1994b; Bolckmans *et al.*, 1995; K. Bolckmans and R. Georgis, personal communications).

Following the promising results of the spring 1994 field trials, Mycotech Corp. developed a clay-based wettable powder formulation of *B. bassiana* strain GHA containing 4.41×10^{13} conidia/kg for control of whiteflies and other homopteran pests. After performing as well as the GHA technical powder in its initial trial against whiteflies in the fall of 1994 (Fig. 4), this product (trade named Mycotrol WP) was submitted to the US Environmental Protection Agency for registration. Registration was granted in March 1995 for use on various fruit and vegetable crops.

The presented results indicate considerable potential for use of entomopathogenic fungi to control nymphal whiteflies on cucurbit crops, especially in greenhouse production systems and in low-technology regions where manual spray equipment is commonly used on an operational scale. Extensive testing with conventional tractor-driven hydraulic and air-assist sprayers is in progress to determine if this potential can be translated

into broader commercial success. In any case, additional testing with yield assessments and economic analyses must be conducted before ultimate conclusions are drawn.

ACKNOWLEDGMENTS

The laboratory and field studies in Weslaco, Texas would not have been possible without the excellent technical work of many individuals. The research team included Frank De La Garza, Andy Sattler, Mike Bustamante, Mari DeAnda, Joe Riveira, Anselmo Rosales, Nancy Underwood, Jim Britton, and Michael Becerra. Production and formulation of the fungus preparations in Butte, Montana were carried out by Pauline Wood, Nancy Underwood, and Jim Britton. The *B. bassiana* isolate MLC8 was provided by Michael Brownbridge of the University of Vermont. Isolates 252 and 2882 of *B. bassiana* and 3594 of *P. fumosoroseus* were provided from the Agricultural Research Service Collection of Entomopathogenic Fungus Cultures (ARSEF) by Richard Humber. We gratefully acknowledge Leeda Wood for providing whiteflies from the Mission Biological Control Laboratory and thank Jeff Lord and Mark Jackson for their critical reviews of the manuscript.

REFERENCES

Bolckmans, K., Sterk, G., Eyal, J., Sels, B., and Stepman, W. 1995. PreFeRal, (*Paecilomyces fumosoroseus* strain Apopka 97), a new microbial insecticide for the biological control of whiteflies in greenhouses. *Med. Fac. Landbouww. Univ. Gent.* **60,** 713–717.

Bradley, C. A., Black, W. E., Kearns, R., and Wood, P. 1992. Role of production technology in mycoinsecticide development. *In* "Frontiers in Industrial Mycology" (G. F. Leatham, Ed.), pp. 160–173. Chapman & Hall, New York.

Carruthers, R. I., Wraight, S. P., and Jones, W. A. 1993. An overview of biological control of the sweetpotato whitefly, *Bemisia tabaci. In* "Proceedings Beltwide Cotton Conferences" (D. J. Herber and D. A. Richter, Eds.), Vol. 2, pp. 680–685. National Cotton Council of America, Memphis, TN.

Eyal, J., Mabud, M. D. A., Fischbein, K. L., Walter, J. F., Osborne, L. S., and Landa, Z. 1994a. Assessment of *Beauveria* bassiana Nov. EO-1 strain, which produces a red pigment for microbial control. *Appl. Biochem. Biotechnol.* **44,** 65–80.

Eyal, J., Walter, J. F., Osborne, L., and Landa, Z. 1994b. Method for production and use of pathogenic fungal preparation for pest control. United States Patent Number 5,360,607.

Fang, Q. X., Gong, Y. X., Zhou, Y. Y., Hu, Y. M., and Yang, S. F. 1983. *Paecilomyces fumosoroseus* var. *beijingensis* n. var. *Acta Mycol. Sinica* **2,** 168–172.

Fargues, J., Goettel, M. S., Smits, N., Ouedraogo, A., and Rougier, M. 1997a. Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia* **89,** 383–392.

Fargues, J., Oedraogo, A., Goettel, M. S., and Lomer, C. J. 1997b. Effects of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviride. Biocontr. Sci. Technol.* **7,** 345–356.

Feng, M. G., Poprawski, T. J., and Khachatourians, G. G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: Current status. *Biocontr. Sci. Technol.* **4,** 3–34.

Ferron, P. 1977. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* (Fungi Imperfecti, Moniliales) in imagines of *Acanthoscelides obtectus* (Col.: Bruchidae). *Entomophaga* **22,** 393–396.

Goettel, M. S., Jaronski, S. T., and Prior, C. 1997. Safety and registration of microbial agents for control of grasshoppers and locusts. *Mem. Entomol. Soc. Can.* **171,** 83–99.

Jackson, M. A., McGuire, M. R., Lacey, L. A., and Wraight, S. P. 1997. Liquid culture production of desiccation tolerant blastospores of the bioinsecticide fungus *Paecilomyces fumosoroseus. Mycol. Res.* **101,** 35–41.

Jaronski, S. T. 1997. New paradigms in formulating mycoinsecticides. *In* "Pesticide Formulations and Application Systems: 17th Volume, ASTM STP 1328" (G. R. Goss, M. J. Hopkinson, and H. M. Collins, Eds.), pp. 99–113. American Society for Testing and Materials, Philadelphia, PA.

Jaronski, S. T., and Lord, J. C. 1996. Evaluation of *Beauveria bassiana* (Mycotrol WP) for control of whitefly in spring cantaloupes, 1995. *Arthropod Manage. Tests* **21,** 103.

Imai, T., Tsuchiya, S., and Fujimori, T. 1995. Aphicidal effects of Silwet L-77, organosilicone nonionic surfactant. *Appl. Entomol. Zool.* **30,** 380–382.

Lacey, L. A., Fransen, J. J., and Carruthers, R. I. 1996. Global distribution of naturally occurring fungi of *Bemisia*, their biologies and use as biological control agents. *In* "*Bemisia* 1995: Taxonomy, Biology, Damage, Control and Management" (D. Gerling and R. T. Mayer, Eds.), pp. 401–433. Intercept Limited, Andover, UK.

Landa, Z., Osborne, L., Lopez, F., and Eyal, J. 1994. A bioassay for determining pathogenicity of entomogenous fungi on whiteflies. *Biol. Contr.* **4,** 341–50.

Marcandier, S., and Khachatourians, G. G. 1987. Susceptibility of the migratory grasshopper, *Melanoplus sanguinipes* (Fab.) (Orthoptera: acrididae), to *Beauveria bassiana* (Bals.) Vuillemin (Hyphomycete): Influence of relative humidity. *Can. Entomol.* **119,** 901–907.

Osborne, L. S., and Landa, Z. 1992. Biological control of whiteflies with entomopathogenic fungi. *Fla. Entomol.* **75,** 456–471.

Ramoska, W. A. 1984. The influence of relative humidity on *Beauveria bassiana* infectivity and replication in the chinch bug, *Blissus leucopterus. J. Invertebr. Pathol.* **43,** 389–394.

Riba, G., and Marcandier, S. 1984. Influence de l'humidité relative sur l'agressivite et la viabilité des souches de *Beauveria bassiana* (Bals.) Vuillemin et de *Metarhizium anisopliae* (Metsch.) Sorokin, hyphomycètes pathogenes de la pyrale du maïs, *Ostrinia nubilalis* Hubn. *Agronomie* **4,** 189–194.

SAS Institute. 1995. "JMP Statistics and Graphics Guide," SAS Institute, Inc., Cary, NC.

Torres, S. E., and Cárdenas, C. H. M. 1996. Produccion y comercializacion de hongos entomopatogenicos en Sinaloa. *In* "XIX Congreso Nacional de Control Biológico: Memorias," pp. 21–22. Sociedad Mexicana de Contol Biológico.

Vidal, C., Fargues, J., and Lacey, L. A. 1997. Intraspecific variability of *Paecilomyces fumosoroseus:* Effect of temperature on vegetative growth. *J. Invertebr. Pathol.* **70,** 18–26.

Willmer, P. 1986. Microclimatic effects on insects at the plant surface. *In* "Insects and the Plant Surface" (B. Juniper and R. Southwood, Eds.), pp. 65–80. Arnold, London.

Wraight, S. P., and Bradley, C. A. 1996. Production, formulation, and application technologies for use of entomopathogenic fungi to control field crop pests. *In* "V SICONBIOL, Simpósio de Controle Biológico, Anais: Conferências e Palestras," pp. 170–177. Foz do Iguaçu, Brazil.

Wraight, S. P., and Carruthers, R. I. 1999. Production, delivery, and use of mycoinsecticides for control of insect pests of field crops. *In* "Methods in Biotechnology, Vol. 5: Biopesticides: Use and Delivery" (F. R. Hall and J. J. Menn, Eds.), pp. 233–270. Humana Press, Totowa, NJ.

Wraight, S. P., Carruthers, R. I., Bradley, C. A., Jaronski, S. T., Lacey, L. A., Wood, P., and Galaini-Wraight, S. 1998. Pathogenicity of the entomopathogenic fungi *Paecilomyces* spp. and *Beauveria bassiana* against the silverleaf whitefly, *Bemisia argentifolii. J. Invertebr. Pathol.* **71,** 217–226.

Wright, J. E., and Chandler, L. D. 1995. Biopesticide composition and process for controlling insect pests. United States Patent No. 5,413,784.