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# *Verticillium lecanii* as a control agent of *Bemisia tabaci* on mist propagated *Euphorbia pulcherima*

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# VERTICILLIUM LECANII AS A CONTROL AGENT OF BEMISIA TABACI ON MIST PROPAGATED EUPHORBIA PULCHERIMA

by

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## ABSTRACT

The fungus Verticillium lecanii was assessed for entomopathogenicity against nymphal Bemisia tabaci in an environmental chamber and in a greenhouse mist chamber on the host Euphorbia pulcherima. Proportional mortality of Bemisia nymphs due to fungal exposure were; in the environmental chamber 89-96%, and in the mist chamber 61-89%. Two fungal characteristics, hyphae growth and sporulation, were used as parameters for augmentation experiments. Response to particular honeydew constituents, glutamine, glutamic acid, serine and sucrose in concentrations ranging 500-40,000 ppm were measured. Hyphae growth, in comparison to water agar (1.26-1.52 mm/24 h), was inhibited by all substrates at all concentrations (0.82-1.47 mm/24 h). Sporulation was enhanced at higher concentrations, 17.0-117.0 ($x10^4$) spores produced, in comparison with water agar, 10.2-15.5 ($x10^4$) spores produced.

# INTRODUCTION

The sweet potato whitefly, Bemisia tabaci (Gennadius), is a highly polyphagous insect that has become a significant pest of many agricultural and greenhouse crops (Byrne et al., 1990). Known also as the tobacco and the cotton whitefly, B. tabaci has been reported on approximately 315 host species worldwide (Butler and Henneberry, 1985); yet it was considered merely a secondary pest on many cultivated crops until the late 1970's (Dittrich et al., 1985; Butler et al., 1985). This insect is capable of causing severe crop damage (Byrne et al., 1990) primarily because of the following: B. tabaci populations can develop high levels of resistance to chemical insecticides within a few generations (Prabhaker et al., 1985), and the physiological traits of high fecundity and short generation time (Bethke et al., 1991). When these traits are combined as in B. tabaci the result is an insect capable of overcoming most chemical control measures in a relatively short time period.

A highly profitable floriculture crop which is jeopardized by this pest are greenhouse-produced poinsettias, Euphorbia pulcherima Willd. (Hamon and Salguero, 1987). In 1986 the sweet potato whitefly was found on this $108 million host (USDA, 1986) and was subsequently shipped nationwide (Lindquist and Tayama, 1987). In the five years since, the

sweet potato whitefly has become a serious insect pest and is now considered to be a primary pest of poinsettia (Ecke et al., 1990).

Another whitefly, the greenhouse whitefly, Trialeurodes vaporariorum (Westwood), has long been a pest of many greenhouse crops such as; chrysanthemum (Chrysanthemum spp.), cucumbers (Cucumis spp.), tomatoes (Lycopersicon spp.), and poinsettia (Euphorbia spp.), (Nelson, 1981). Successful control of this insect on some crops has been attained using the entomogenous fungus Verticillium lecanii Viegas (Hall, 1982; Kanagaratnam et al., 1982).

Many of the life history traits of both whitefly species are similar. Because of the similarities a project was undertaken to determine if V. lecanii could be used as an effective fungal control agent of B. tabaci on poinsettias.

# LITERATURE REVIEW

## Bemisia tabaci As A Pest:

Bemisia tabaci was first reported on Grecian tobacco in 1889 and was assigned the binomen Aleurodes tabaci (Gennadius), (Mound, 1983). The insect was subsequently assigned 23 different scientific names and reported on a wide variety of hosts (Mound, 1983). Examples of recorded hosts are; vegetables in the Solanaceae and Compositae families, woody perennial members of the Fagaceae family, ornamentals in Rosaceae and Compositae, and fiber crops in the family Malvaceae (Mound and Halsey, 1978). It was considered only a secondary pest on several of these host crops until early 1980. In 1981 populations began reaching economic infestation levels on cotton, Gossypium hirsutum L. (Butler and Henneberry, 1985).

Bemisia tabaci was first identified as a pest of economic importance on southwestern United States cotton in 1981 (Butler and Henneberry, 1985), and on Sudanese cotton from 1980 to 1983 (Dittrich et al., 1985). In these areas pesticides were considered to be the major factor in elevating B. tabaci to primary pest status (Butler and Henneberry, 1985).

Insecticides affected B. tabaci populations in two ways. First, the routine use of the same class of chemicals selected

for highly resistant biotypes of B. tabaci (Dittrich et al., 1985; Butler and Henneberry, 1985). These biotypes expressed varying degrees of resistance to various classes of insecticides (Dittrich et al., 1985; Butler and Henneberry, 1985). Secondly, insecticide use eliminated existing populations of natural enemies (Butler and Henneberry, 1985). As a result, a natural and effective method of B. tabaci population suppression was lost.

In both the southwestern United States and the Sudan large tracts of land are planted with cotton. These acted as reservoirs for the resistant populations of B. tabaci (Butler and Henneberry, 1985). From this source the insects eventually moved to a variety of other crops.

This situation has occurred in other areas of the world and previously to 1981 (Horowitz, 1986). The situations in the United States and Sudan are probably the most well documented, but earlier reports are found from around the world. The earliest report is during the late 1920's and early 1930's from India, while later reports are from the various locations of Iran, El Salvador, Mexico, Brazil, Turkey, and Israel (Horowitz, 1986). All these countries have reported B. tabaci as a serious pest of cotton (Horowitz, 1986).

Bemisia tabaci Resistance:

Evidence of resistance is supported by two separate studies. Prabhaker et al. (1985) observed organophosphate (OP) resistance at the 20- to 54- fold level in several field populations from southern California. High OP resistance at the 150- to 311- fold level was also observed in 1983 on Sudanese cotton (Dittrich et al., 1985). Bemisia tabaci has also demonstrated resistance to permethrin (12- 29 fold), fenthion, malathion, and parathion (Prabhaker et al., 1985). In the Sudan, Dittrich et al. (1985) noted additional resistance to dimethoate, monocrotophos, and DDT. More recently, Dittrich et al. (1990) has listed recent resistance in B. tabaci to 15 different insecticides. Research clearly shows the need for judicious use of pesticides when controlling this insect. In some situations pesticides have not provided effective control of B. tabaci, as a consequence alternative methods of management need to be investigated.

Whitefly Damage:

As far as is known, whiteflies are leaf phloem feeders (Janssen et al., 1989). Plant damage can be the result of several activities; direct damage from feeding, indirect damage from plant viruses transmitted by whiteflies, and indirect damage associated with whitefly excreta.

Direct damage from feeding is a result of sap extraction from the phloem tissue. Using their stylet bundles, whiteflies penetrate leaf tissues intercellularly (Janssen et al., 1989) and feed from phloem tissues. Feeding occupies the highest proportion of the whitefly's time (van Lentern and Noldus, 1990). Constant feeding combined with large insect infestations can directly affect plant yields and/or marketability (Johnson et al., 1989).

Indirect damage results from plant viruses transmitted by the salivary secretions of feeding whiteflies. Whiteflies can vector a total of greater than 70 viral pathogens (Duffus, 1987). Bemisia tabaci is considered the most important whitefly vector as it alone can transmit more than 25 different viral pathogens (Costa, 1976). Duffus (1987) records that serious losses can occur on a wide variety of crops in many plant families. Examples of affected families of crops are; Solanaceae, Cucurbitaceae, and Leguminosae (Duffus, 1987). Bemisia tabaci is also responsible for transmitting viruses to families of ornamentals such as: Rosaceae, Compositae, Labiatae, and Oleaceae (Duffus, 1987). In the plant genus Euphorbia spp. several species are affected by whitefly transmitted viruses. These include cassava, castor bean, (Duffus, 1987) and a Brazilian weed, amendoim bravo (Euphorbia prunifolia Jacq.), (Costa and Bennett, 1949). All have shown a relationship between the presence of B.

tabaci and disease outbreak (Duffus, 1987).

The excreta of whiteflies can cause indirect damage to plants by providing a nutrient source for 'sooty mold' fungus, a complex of Capnodium spp. (Johnson et al., 1989). The accumulated excreta or honeydew creates a sticky layer on which Capnodium spp. can grow, forming black hyphae on the upper surfaces of leaves. It is believed that this layer of hyphae results in decreased photosynthetic activity in plants (Byrne and Miller, 1990). This condition can be widespread on plants and within fields, and can result in decreased plant growth which affects yields or marketability (Johnson et al., 1989).

Species Similarities:

Bemisia tabaci and T. vaporariorum are two diverse yet similar species of whiteflies. B. tabaci is of Old World origin, primarily Asia (Mound and Halsey, 1978), and T. vaporariorum is believed to be of New World origin, possibly Mexico (Mound, 1983). Now found worldwide, both species are polyphagous with broad, overlapping host ranges (Mound, 1983). The two species are similar in life cycle, feeding activity and behavior, and the damage that they cause on crops.

The length of the life cycle of both species is approximately 17 d at optimal temperatures of 27°C (Byrne and Bellows, 1991). The development of B. tabaci and T.

vaporariorum consists of six stages which are the egg, four nymphal stages, and the adult (Hill, 1969). In both species first instars are referred to as "crawlers" (Gill, 1990). Crawlers are actively mobile while remaining nymphal instars are sessile and lack movement capability (Gill, 1990). Additionally, during the extended fourth nymphal instar extensive tissue reorganization into the adult form occurs (Hill, 1969). The general appearance of B. tabaci and T. vaporariorum is somewhat similar at each stage yet there are many variations such as dorsal setae and size which differentiate the two (Hill, 1969).

Euphorbia pulcherima, Poinsettia:

At present, poinsettias are a crop requiring high pesticide use. This is primarily due to industry standards which require low numbers of observable insects; marketable plants must have less than three adults per plant (W.B. Miller, personal comm.). Additionally, only a limited number of registered pesticides are available for use on poinsettias, thus these pesticides are used frequently in order to meet the industry's standards. The majority of these registered pesticides are in four chemical families, three of the families are synthetics, and the last is naturally occurring. The chemical families are: organophosphates, carbamates, pyrethrums and pyrethroids (Ecke et al., 1990), and a

chlorinated hydrocarbon (Anonymous, 1988). Bemisia tabaci has demonstrated resistance to two of these chemical families, organophosphates and certain pyrethroids (Dittrich et al., 1985). This factor of resistance, combined with high pesticide use could create an environment in which B. tabaci may emerge as the most unmanageable insect pest of the poinsettia industry.

Verticillium lecanii Viegas, Fungus:

Normally a saprophyte, the fungus Verticillium lecanii Viegas is found world-wide on many substrates such as soil, wood, and plastic (Hall, 1981), yet it is also capable of killing many living hosts of differing biology. Spencer and Atkey (1981) report hyperparasitism of the carnation rust Uromyces dianthi, and the brown wheat rust Puccina recondita (Spencer and Atkey, 1981). Hyperparasitism of the powdery mildews, Euascomycetidae spp., has also been reported (Hall, 1981). A filarial host, the sugar beet nematode, Heterodera schachtii, was observed by Hanssler and Hermanns (1981). The principal hosts are insects however, with members of Homoptera predominating (Hall, 1981). Bemisia tabaci has never been reported as a host for V. lecanii. Hosts have also been reported in other insect orders, Coleoptera, Collembola, and Diptera (Hall, 1981). Other insect hosts are Hymenoptera (on a single Ichneumonid), and an acarine host, eriophyid mites

(Hall, 1981). Verticillium lecanii presents little risk to vertebrates as it cannot grow at 37°C (Hall, 1981).

Usually found infecting isolated insects, the fungus rarely initiates epidemics in natural situations except among aphid and scale insects (Hall, 1981). When introduced into homopteran populations the fungus appears to be highly successful. The earliest examples of successful insect control by V. lecanii were 13 reports of its use against aphids and scales from 1910 to 1941 which were summarized by Baird (1958). More recently, the fungus has had greater success in greenhouses, to the extent that commercial preparations of two fungus biotypes have been developed and marketed.

Verticillium lecanii has been marketed in commercial preparations as Vertelac$^{R}$ and Mycotal$^{R}$ (Microbial Research Ltd. U.K.). Vertelac and Mycotal are two fungal biotypes that have exceptional virulence against their specific hosts (respectively, aphids and whiteflies). These hosts are; various greenhouse aphid species, Myzus sanborni, Myzus persicae, and Brachycaudus helichrysi (Hall, 1981), and the whitefly, T. vaporariorum (Drummond et al., 1987). In Europe successful control of these pests by the commercially produced fungus has been attained on two crops, cucumbers, Cucumis spp., and chrysanthemums, Chrysanthemum spp. (Hall, 1981). Neither product is registered in the United States (Drummond

et al., 1987), and is still not at this time.

Attributes Of V. lecanii:

The fungus has many attributes which enhance its appeal as a possible microbial control agent. It is easily and quickly produced (Hall, 1981), does not infect commercial parasitoids and predators (Hall, 1981), and is somewhat tolerant of certain pesticides (Khalil et al.,1985).

Production of V. lecanii is eased by the fact that it can be grown on many of the available conventional solid media; Sabouraud, Potato Dextrose, Czapeck-Dox (Hall, 1981) and V-8, (Meade, unpublished observations). The fungus can also be grown on liquid media, however; the fungus assumes a semi-yeast morphology and produces fragile blastospores rather than conidia on these media (Hall, 1981). Other factors important in production of the fungus are, germination, growth, and sporulation. These can be rapid at the optimal conditions of 22°C and 95% humidity (Hall, 1981). Under these conditions, 100% germination of spores will occur in only 15 h (Hall, 1981), colony growth rate averages 2-3 mm per day (Hall, 1981), and sporulation can be profuse within 5 d (Meade, unpublished observations).

When introduced into existing biological control schemes it is reasonable to assume the fungus will not negatively

affect introduced commercial parasitoids, (Hall, 1981) and that it can be used as an additional control measure. Both Encarsia formosa, an aphelinid, and Phytoseiulus persimilis, a predatory mite, were not infected when active in crops where the fungus naturally occurred (Hall, 1981).

Assessment of fungus compatibility with pesticides is far from thorough, yet laboratory experiments shows some compatibility with several broad spectrum fungicides and some insecticides (Hall, 1981). Compatibility was defined as reduced inhibition of; radial hyphae growth and the number of spores produced (Khalil et al., 1985). Spore germination and mycelial growth were insignificantly affected by the recommended rates of cypermethrin, fenbutatin-oxide, formothion, mevinphos, copper-oxychloride, oxamyl, permethrin, pirimicarb, thiophanate-methyl, and triadimefon (Khalil et al., 1985). However, the fungicides fenithrothion, mancozeb, and the insecticide methomyl were found to be only partially compatible with V. lecanii while incompatible pesticides were the fungicides metiram, bitertanol, and dichlofluanid (Khalil et al., 1985). Time of exposure was also found to be an important factor affecting compatibility in one instance. Gardner et al. (1984) found application scheduling to be an important factor when using V. lecanii with the fungicide benomyl.

Limitations Of V. lecanii:

Verticillium lecanii has one biological characteristic that limits its usefulness, especially for field crops. It has a strict requirement for high relative humidity, greater than 95%, in order to initiate an invasive infection within an insect or population (Drummond et al., 1987). Milner and Lutton (1987) found humidity to be a significant factor in transmission of the pathogen to the green peach aphid, Myzus persicae Sulzer, with the greatest rate of infection occurring at 100% relative humidity. Lower humidities gave correspondingly lower infection rates, with 80% relative humidity yielding completely uninfected insects (Milner and Lutton, 1987). Drummond et al. (1987) also found high humidity to be a critical factor in the establishment of infection on T. vaporariorum.

Another limitation of V. lecanii is its poor spore dispersal (Hall, 1981). Conidia from V. lecanii adhere to the mycelium when dry and do not disperse (Hall, 1981). This prevents the fungus from initiating new infections in other areas (Hall, 1981). In order to maintain a high level of infection within crops the fungus must be periodically reintroduced.

## PROJECT RATIONALE

Poinsettia production includes a 6 wk period that requires frequent misting (3 s every 6 min) for asexual propagation of the plants (Ecke et al., 1990). It is believed the frequent misting would fulfill the high humidity requirement of V. lecanii and allow it to develop an epizootic in the whitefly populations. By purposely introducing the fungus at this time an alternative whitefly control strategy could be developed.

Protection of the cuttings during propagation is very difficult as insecticide applications are inappropriate because they can be damaging to cuttings and are ineffective.

Damage can result because the stressed and rootless condition of the plant cuttings creates a low tolerance for insects and pesticides (Anonymous, 1988: Ecke, 1990). Systemic pesticides are particularly damaging because they may delay root initiation (Anonymous, 1988).

Whitefly honeydew excreta is utilized as a food source by V. lecanii (D.N. Byrne and M. E. Stanghellini, personal observations). It was reasoned that this honeydew would augment fungal growth and sporulation and thereby enhance the fungus' potential as a biological control agent in the propagation environment. By adding a honeydew-like substrate to the inoculum in which the cuttings are immersed, introduced fungal spores would complete their life cycle without a

whitefly host. Similar results were observed when commercial formulations of V. lecanii were used to control T. vaporariorum and Aphis gossypii (Hall, 1982). When a commercial formulation containing a nutritional substrate was used, spread of the epizootic through the whitefly populations was enhanced (Hall, 1982).

Theoretically, addition of a nutritional substrate increases V. lecanii's effectiveness in a two-fold manner. Those spores not immediately contacting a nymphal host can still grow hyphae, thereby contacting a greater percentage of hosts. Secondly, since spores that do not infect whiteflies can complete their life cycle and sporulate, the number of viable spores available to attack subsequent whiteflies increases. This would allow the fungus to act as a prophylactic.

Because the fungus may reduce dependence on pesticides it was felt that its use may slow resistance development in the poinsettia industry.

Project Summary And Outline:

The project had two general objectives. The first objective was to determine if V. lecanii could initiate epizootics within nymphal populations and effectively control B. tabaci during mist propagation of E. pulcherima. The second objective was to conduct pilot studies which would

determine whether augmentation of the fungus using the most prevalent amino acids and sucrose found in honeydew and characterized by Byrne and Miller (1990) would significantly increase infection rates and/or allow V.lecanii to act prophylactically.

The project consisted of three studies designed to complement each other.

Study I. The first study consisted of an assessment of V. lecanii virulence against B. tabaci. This study was conducted in the laboratory. The objectives were to determine if virulence existed, and to quantify that virulence.

Study II. The second study was a nutritional assessment. Using the three most prevalent amino acids and a carbohydrate found in honeydew by Byrne and Miller (1990); the objective of this study was to quantify the effect of these compounds on V. lecanii growth and sporulation. This information was then used to create a synthetic honeydew to be used in the propagation environment. This study was also conducted within the laboratory.

Study III. The third study was an assessment of the fungus as a control agent in the propagation environment. This study had several objectives; 1) to determine if the fungus was effective in a mist chamber, 2) to quantify fungal effectiveness, and 3) assess whether addition of synthetic honeydew substrates increased fungal effectiveness on existing

or founding whitefly populations. This study was conducted in a propagation misting chamber.

## MATERIALS AND METHODS

### Study I. Mortality Of *B. tabaci* Associated With *V. lecanii*:

Cultures of *V. lecanii* used in this study were obtained from the University of Florida. These were maintained on 20% V-8 agar (Tuite, 1969) in 60 x 15 mm Petri plates at 21 ± 1°C in an environmental chamber (Revco Scientific Inc., Asheville, NC). These cultures were from spores found in the commercial formulation Mycotal$^{R}$.

Two species of whitefly were cultured on *E. pulcherima*, cv. "Glory", in greenhouses maintained by the University of Arizona in Tucson, AZ. *Bemisia tabaci* had been in culture on the host plant since the fall of 1988 while *T. vaporariorum* had been cultured on *E. pulcherima* for two generations.

In this study age specific cohorts were used. They were obtained by placing adult *B. tabaci* and *T. vaporariorum* in separate clip cages (Costa et al., 1991) on the undersides of *E. pulcherima* leaves and allowing oviposition for 24 - 48 h. Adults were removed by hand held aspirators and the plants maintained in the laboratory until nymphs had reached the required instar. At appropriate ages, leaves bearing cohorts were subjected to the fungus.

Seven-day-old cultures of the fungus were selected for use and assessed for viability using spore germination as a criterion. Germination tests consisted of inoculating three

100 x 20 mm Petri plates containing 20% V-8 agar (Tuite, 1969) with a spore suspension of 3.7 to 4.1 x $10^5$ spores per ml of sterile distilled water ($SDH_2O$). After 20 - 24 h, germination percentage was determined using a microscope count.

To harvest spores for leaf inoculum, $SDH_2O$ was rinsed across lightly rubbed seven-day-old culture plates. Suspensions were decanted into a 50 ml glass beaker that had a double layer cheesecloth filter stretched across the opening to remove hyphae fragments. Spore counts at final concentration ranged from 3.7 to 4.1 x $10^5$ spores per ml $SDH_2O$. A surfactant, Triton X-100 (Rohm & Haas, Philadelphia, PA), was added at a concentration of 0.05% to the final spore solution to insure wetting of leaf surfaces.

Application of the final spore solution to leaves bearing either B. tabaci or T. vaporariorum consisted of misting leaves until runoff. Afterward, leaves were placed on paper toweling until dry.

Four stadia (eggs, first, second, and third instar nymphs) of both whitefly species were subjected to three treatments; $SDH_2O$ alone, $SDH_2O$ plus surfactant, and $SDH_2O$ plus surfactant and spores. Adults were not included in this experiment. Treatments were replicated three times for each stadia of the two species. Each leaf was examined immediately after drying, and the number of nymphal whiteflies per leaf

recorded. Leaves were then placed upright in 250 ml french square bottles which contained 50 ml of $SDH_20$ together with 250 ppm of fertilizer (Peters General Purpose 20-20-20, W.R. Grace & Co., Fogelsville, PA). Bottles were placed horizontally to maximize photosynthesis in an environmental chamber under 21 $\pm$ 1°C, and 12:12 LD. Relative humidity within bottles ranged from 89-95% and was determined by placing an DataLogger$^R$ probe (OmniData Inter., Logan, UT) within a duplicate bottle.

After 7 d, microscopy counts of the number of dead and live individuals per leaf were recorded. Whiteflies were recorded as dead if a mycelial mass was present or their bodies had collapsed. Counts of the number of live and dead (both from fungal infection and natural causes) individuals were then converted to percentages. Percentages were used for statistical analysis.

Percentages were first transformed by arcsine transformation in order to normalize data and reduce error variance. This was followed by statistical analysis using a three-way ANOVA (CoStat Software, Berkeley, CA) to determine significant differences due to treatment, species, and instar. To further identify significant differences in the means of the three factors, a Student-Newman-Kuels test (CoStat Software, Berkeley, CA) was conducted.

Study II. Nutritional Assessment Study:

A total of seven experiments were conducted. Three of the experiments measured radial hyphae growth as a response to the presence and concentration of a substrate, and four of the experiments measured sporulation as a response. Four substrates were used, they were; glutamic acid, glutamine, serine, and sucrose. Many of the methods and materials used in these experiments were duplicated. Similar methods and materials are presented first and are followed by the dissimilar methods and materials in the sections subtitled Growth Experiments, and Sporulation Experiments.

All cultures of V. lecanii used in these experiments were obtained from subcultures of fungus used in the mortality study. Cultures had been maintained as before, in 100 x 50 mm Petri plates on either 20% V-8 agar (Tuite, 1969) or on Sabouraud agar in an environmental chamber at 21 $\pm$ 1°C. All cultures were approximately 2 - 3 wks in age. Spore viability of these cultures was again determined using percent germination as a criteria. Viability tests consisted of placing spores on Sabouraud agar in 100 x 50 mm Petri plates and examination by microscopic count 20 - 24 h later.

Cultures were again lightly rubbed, and filtered through cheesecloth to remove larger hyphae fragments. These suspensions were then washed to remove any culture media residue.

To wash spores, suspensions were vacuum filtered in a Millipore$^{R}$ filter unit (Millipore Corp., San Francisco, CA) equipped with a 0.22 $\mu$m membrane filter. This deposited a layer of unwashed spores on the membrane filter. Two hundred ml of $SDH_2O$ was then added to the filtration unit for the wash. Afterward, membrane filters bearing the layer of clean, washed spores were removed and placed in 50 ml of $SDH_2O$. Membrane filters were first agitated to resuspend the spores and then removed. This yielded a suspension of clean spores in $SDH_2O$ which was used for inoculum.

All experiments utilized 60 x 15 mm Petri plates containing water agar amended with either glutamine, glutamic acid, serine, or sucrose at varying concentrations. A well (5 mm diameter x 3 mm deep) was cut in the agar at the edge of each culture plate. Into each well, 10 $\mu$l of fungal inoculum was introduced. Wells prevented movement of the inoculum. All plates were again placed in an environmental chamber at 21 $\pm$ 1°C.

Media preparation for all experiments consisted of separate preparations of 1.5% agar-agar (Sigma Corp., St. Louis, MO) water agar which was sterilized for 30 min, and allowed to cool before adding the desired amino acid or sucrose. Substrates were filter sterilized using a 60 mm plastic syringe attached to a Millex-GS$^{R}$ 0.22 $\mu$m disposable filter (Millipore Corp., San Francisco, CA).

Growth Experiments:

The three growth experiments used concentrations of the amino acids and sucrose which varied in concentration from 500 to 40,000 ppm. Each experiment was conducted using a completely randomized design with five replications, each petri plate represented one replication. Experiments ran a total of either 14 or 23 d during which either three or four measurements of radial hyphae growth were taken.

All plates were allowed a stabilization period to compensate for differences in hyphae growth rate due to culture media residue. Stabilization periods differed for each experiment. Following stabilization, the amount of subsequent mycelial growth away from the well was marked on the undersides of the plates. Stabilization periods were not included in the 14 or 23 d.

Data collection consisted of measurement in mm of the radial hyphae growth from the previously marked growth line to the edge of the new mycelial growth. The edge of the new growth was also noted on the undersides of plates. Three measurements of the new growth were taken per plate. These were averaged and recorded as mm growth per number of days. The averaging of the three measurements per plate insured that an observed growth rate on a particular plate was not affected by uneven radial growth. An average growth rate per 24 h over

the total time period of either 14 or 23 d was used in the analysis.

The first of the three growth experiments examined the three amino acids at concentrations of 10,000 ppm (0.0396 μg/ml), 20,000 ppm (0.0792 μg/ml), 30,000 ppm (0.1188 μg/ml), 40,000 ppm (0.1584 μg/ml). Spore concentration of the inoculum was $2.6 \times 10^6$ spores per ml $SDH_2O$. This experiment ran a total of 23 d, and four measurements were taken during that period.

The second experiment focused on sucrose at the same concentrations. Spore concentration of the inoculum for this experiment was $1.4 \times 10^5$ spores per ml $SDH_2O$. This experiment also ran 23 d, with measurements being recorded four times.

The third growth experiment utilized each of the amino acids and sucrose at concentrations of: 500 ppm (0.00197 μg/ml), 2,500 ppm (0.00986 μg/ml), 5,000 ppm (0.01972 μg/ml), 10,000 ppm (0.03944 μg/ml), 12,000 ppm (0.04752 μg/ml). Spore concentration of the inoculum was $4.7 \times 10^6$ spores per ml $SDH_2O$. This experiment ran 14 d, with three measurements being taken during that time.

Data from each experiment were analyzed using a two-way ANOVA (CoStat Software, Berkeley, CA) to identify significant differences due to substrate and concentration. This was followed by analysis using polynomial and linear regression in order to identify growth response trends (CoStat Software,

Berkeley, CA).

Sporulation Experiments:

Sporulation experiments consisted of four tests which measured the number of spores produced by V. lecanii when grown on amended water agar. Agar was amended with either glutamine, glutamic acid, serine, or sucrose at varying concentrations. As in the growth experiments, concentrations ranged from 500 - 40,000 ppm. All experiments again used a completely randomized design with five replications, and again, each plate constituted a replicate.

Plates were inoculated in wells with washed spores whose concentrations ranged from 4.2 - 8.8 x $10^6$ spores per ml $SDH_2O$. After inoculation, plates bearing amended water agar and fungus were placed in an environmental chamber (Revco Scientific, Asheville, NC) at 21 $\pm$ 1°C for 29 d. After 29 d, spores were removed from plates, and microscopically counted.

Spores were dislodged by first adding 1000 $\mu$l of $SDH_2O$ to each plate and then by rubbing plates with a small spatula for 1 min. Suspensions from each plate were decanted into plate lids, and hand agitated for more than 3 min. Agitation insured rupture of the slime heads (Hall, 1981), and release of all mature conidia. It also insured even dispersal of the conidia in the $SDH_2O$.

Suspensions were singly loaded into a hemocytometer

(Bausch & Lomb, Rochester, NY) and the number of spores within a grid area of 2,000 $\mu^3$ counted. A total of five observations were recorded per plate to give an average number of spores per replication.

Data was statistically analyzed using a two-way ANOVA (CoStat Software, Berkeley, CA) to identify significant differences in the number of spores produced, and further identify any interaction between the two. To further separate means and identify particular substrates and concentrations which significantly affected spore production a Duncan's Multiple Range test (CoStat Software, Berkeley CA) was conducted.

Study III. Greenhouse Assessment:

Two experiments were conducted in this study. The first experiment was to determine if V. lecanii would initiate an epizootic on cuttings with established whitefly populations in a propagation environment. This experiment was designed to quantify the level of infection and compare infectivity using amended and non-amended inoculum when the host was present. The second experiment was designed to quantify fungal effectiveness when whiteflies were introduced after inoculation. Again comparisons were made between amended and non-amended inoculum.

Both experiments were conducted on a greenhouse misting

table (0.91 m x 2.44 m) equipped with three upright hollow cone spray nozzles (R&D Sprayers, Opelousas, LA), spaced 0.61 m apart. Nozzle misters were approximately 0.46 m in height and nozzle orifice size was 6-X. Misting occurred for 6 s every 3 min for the first 10 d, and for 12 s every 6 min thereafter. Daily maximum and minimum temperatures were recorded from a Taylor$^{R}$ (A.H. Hummert Co., St. Louis, MO) maximum/minimum thermometer.

Spore inoculum used in these experiments were from subcultures of the commercial product Mycotal$^{R}$. Suspensions of harvested spores were again washed using vacuum filtration and a Millipore$^{R}$ filter unit equipped with a 0.22 $\mu$m disposable membrane filter as previously described. Inoculum spore viability was again assessed using germination as a criterion.

Established Whitefly Experiment:

Cuttings were from B. tabaci-infested poinsettia plants, that had been maintained in culture together for greater than 3 months. Cuttings used were 4-7 nodes in length, and prior to treatment had their stems treated with the commercial formulation of indole-3-butyric acid, Hormex No. 1 (Brooker Chemical Co., Hollywood, CA).

Cuttings were immersed into one of the three treatments: 1) $SDH_2O$ alone, 2) spores in $SDH_2O$, and 3) spores in $SDH_2O$

containing glutamic acid at 2,500 ppm and sucrose at 500 ppm. All treatments contained a surfactant, Triton X-100 at 0.05%.

Spores used for experimental treatments were approximately 10 d old. Spore concentration in the final inoculum was $5.0 \times 10^6$ spore per ml.

After immersion, treated plant cuttings were placed into Oasis$^R$ rootcubes, 2.54 cm x 2.54 cm x 3.81 cm (Smithers-Oasis Co., Kent, OH), which had been positioned within a plastic flat. Flats were then placed randomly within the misting chamber. Each flat constituted a replication. Each replication consisted of a single plastic flat containing 30 rootcubes and 15 plant cuttings. Experimental design consisted of a completely randomized design with three replications.

Data was collected by sampling leaves from each flat. At 10 and 19 d after inoculation, 15 leaf samples were taken from each flat. Each leaf was examined microscopically and the number of live and dead nymphal whiteflies per leaf was recorded. Nymphs were considered dead if a hyphae mass was present or their bodies had collapsed and there was no flow of honeydew from the vasiform orifice. These counts were totalled and converted to a percent mortality per leaf. Leaf percentages were totaled and averaged to give an average percent mortality per replicate.

To stabilize variances, proportion killed data were

transformed using an arcsine transformation. Data were statistically analyzed using a one-way ANOVA (CoStat Software, Berkeley, CA) to determine if there was a treatment effect on percent mortality and significant differences in overall percent mortality due to treatment. To identify the treatments responsible for the significant differences, a Student-Newman-Kuels (CoStat Software, Berkeley, CA) was conducted.

Introduced Whitefly Experiment:

Cuttings used in this experiment were from non-infested poinsettia plants, which had been maintained in culture for 6 months. Cuttings were 6-9 nodes in length and were again treated with indole-3-butyric acid.

Cuttings were immersed into one of three treatments: 1) spores in $SDH_20$ alone, 2) spores in $SDH_20$ containing 0.06297 g of naturally occurring honeydew, and 3) spores in $SDH_20$ containing glutamine, glutamic acid, serine, and sucrose, all at 10,000 ppm. All treatments contained a surfactant, Triton X-100 at 0.05%.

Natural honeydew used in this experiment was obtained by fastening pre-weighed, petri plates (50 x 9 mm) to the undersides of poinsettia leaves. To each of these plates approximately 400 adult B. tabaci were added. Leaf tissue covered the open section of the plates and provided a food

source for whiteflies. Petri plates collected their honeydew. Plates were held on leaves using metal clips which were 15 mm in length (Sekine, New York, NY). Whiteflies were allowed to feed and excrete for 48 h, after which plates were removed. Petri plates were dried in a drying oven (National Appliances, Portland, OR) at 26.6 ± 1°C for 2 h to remove all moisture. Plates were then reweighed.

After weighing, 5 ml of distilled water was added to each plate to solubilize honeydew. Honeydew suspensions were then filter sterilized using a 60 cc syringe to which was attached a sterile Millex-GS$^R$ 0.22 $\mu$m disposable filter (Millipore Corp., San Francisco, CA). This sterile honeydew suspension was then added to the spore inoculum.

Cultures used for experimental treatments were 7-10 d in age. Spore concentration of the finished inoculum was 3.1 x $10^5$ spores per ml.

Once immersed, treated plant cuttings were placed into wedge shaped Oasis$^R$ rootcubes, 2.54 x 2.54 x 5.0 cm (Smithers-Oasis Co., Kent, OH), which were contained within plastic flats. Flats were then placed into the misting chamber.

Experimental design consisted of a randomized complete block design, with each flat representing a block. There were three blocks and each block contained one replicate of each of the three treatments. Each treatment within a block consisted of nine plant cuttings.

Ten days after inoculation three poinsettia plants bearing populations of poinsettia-adapted B. tabaci whiteflies were placed on the misting table in order to introduce insects. Additional introduction of insects was by aspiration. Water was withheld from the introduction plants by enclosing their pots in plastic. This prompted wilting and induced adult movement onto the cuttings.

Twenty days after whitefly introduction and 29 d after inoculation, data collection began. Fifteen leaf samples were taken from each of the three treatments within a flat and examined microscopically to determine the number of live and dead nymphal whiteflies per leaf. Counts from each leaf were again converted to percentages and averaged to obtain an mean percent mortality per replicate.

Percent mortality data was again transformed using an arcsine transformation. Statistical analysis in a one-way ANOVA (CoStat Software, Berkeley, CA) was used to determine significant differences in percent mortality due to treatment.

# RESULTS

## Study I. Mortality Of B. tabaci Associated With V. lecanii:

First, second and third instars of both species suffered significant mortality when exposed to the fungus (approximately 90%). This was significantly more than the mortality suffered by nymphs exposed to either $SDH_20$ alone or $SDH_20$ plus surfactant ($P<0.001$) (Table 1). Additionally, there was no significant difference in mortality when comparing instars of the same species, nor when comparing instars of the different species ($P<0.05$) (Table 1). Eggs of both species had extremely low rates of infection (<1%).

Spore viability of the inoculum, as determined using the criterion germination, was found to be 98-99%.

## Study II. Nutritional Assessment:

### Growth Experiments:

In all three growth experiments there were highly significant differences in hyphael growth rates due to substrate and concentration. F-values and P-values from analysis of variance on the effect of substrate for all three growth experiments were; 1) $F=287.16$ & $P<0.001$ for the experiment utilizing high concentrations of amino acids, 2) $F=160.17$ & $P<0.001$ in the sucrose experiment, and 3) $F=28.55$ & $P<0.001$ in the all substrates experiment. Concentration was a significant factor in only two of the experiments. In these

experiments F-values and P-values were; 1) $F=56.25$ & $P<0.001$ for the experiment utilizing amino acids at high concentrations, and 2) $F=35.76$ & $P<0.001$ for the all substrates experiment. In all three growth experiments significant interactions between substrates and concentrations was observed. F-values and P-values for the factor of interaction were; 1) $F=13.69$ & $P<0.001$ for the amino acid experiment, 2) $F=5.75$ & $P=0.002$ in the sucrose experiment, and 3) $F=3.85$ & $P<0.001$ in the all substrates experiment.

Further analysis using a polynomial regression revealed an inhibited response to all amino acids at all concentrations of 10,000-40,000 ppm (Figure 1). All slopes for each of the amino acid substrates were negative; glutamine = -0.2835, serine = -0.3497, glutamic acid = -0.0338.

Hyphae growth rates on sucrose was also poor. As before, an inhibitory response when data were subjected to a polynomial regression, slope for the substrate sucrose = -0.1269, (Figure 2).

Mean growth rates of V. lecanii hyphae in response to decreased concentrations of the four substrates are presented in Figure 3. Analysis by regression, both polynomial and linear, again revealed an inhibition response to all substrates. Slopes of the regression lines were again found to be negative, sucrose = -0.0626, glutamic acid = -1.1564, glutamine = -8.0610, and serine = -1.7287.

Sporulation Experiments:

The number of spores produced by V. lecanii when grown on the amino acids at high concentrations was found to be significantly different than the production observed on water agar ($F=50.62$ & $P<0.001$). Concentration was also a significant factor in this experiment ($F=11.91$ & $P<0.001$). Interaction between substrate and concentration was also significant ($F=10.81$ & $P<0.001$). Further analysis using Duncan's Multiple Range tests identified particular amino acids and their concentrations which were the most significant (Table 2). In comparison with the water agar control, significant differences in spore production were observed with only two treatments, glutamic acid at 30,000 and 40,000 ppm (Table 2).

When grown on sucrose, the number of spores produced by V. lecanii was greater than the production observed on water agar ($F=66.42$ & $P<0.001$). Concentration was also a significant factor ($F=5.46$ & $P=0.003$). Interaction between substrate and concentration was also significant, yet to a lesser degree ($F=4.43$ & $P=0.01$). In mean comparisons using Duncan's Multiple Range tests, none of the concentrations yielded significantly greater spore production, yet the 40,000 ppm level gave the greatest number of spores produced (Table 3).

The above two experiments were partially repeated.

Analysis of variance revealed significant differences due to substrate ($F=31.73$ & $P<0.001$), concentration ($F=30.36$ & $P<0.001$), and interaction between the two ($F=10.10$ & $P<0.001$). Results from the means comparisons of the Duncan's Multiple Range test are given in Table 4. Again, none of the substrates or concentrations yielded significantly greater spore production, however one treatment, sucrose at 30,000 ppm, gave the highest number of spores produced (Table 4).

Spores production in response to decreased concentrations of the various amendments are presented in Table 5. Analysis of variance revealed significant differences were attributable to only one factor, substrate ($F=6.61$ & $P<0.001$). Comparisons of means using Duncan's Multiple Range tests again revealed no significant differences in the numbers of spores produced. One substrate, sucrose at 5,000 ppm gave high numbers of spores produced, however, this was not significantly greater than that observed on water agar (Table 5).

Results of spore viability tests of all inoculum used in the above experiments ranged 96-99%.

Study III. Greenhouse Assessment:

Established Whitefly Experiment:

The proportion of nymphal whiteflies which died due to exposure to V. lecanii in the misting environment show that the fungus had a significant impact on nymph mortality

($F=56.0364$ & $P<0.0001$) (Table 6). However, addition of glutamic acid and sucrose did not significantly increase mortality levels (Table 6). Mortality percentages were cumulative, thus comparisons were made only within a single sampling date (Table 6).

Spore viability of inoculum used in this study as determined by germination was 99%.

Introduced Whitefly Experiment:

No significant differences in percent mortality due to any treatments were observed when whiteflies were introduced ($P>0.05$) (Table 7). Additionally, augmentation of V. lecanii using substrates or honeydew did not significantly increase percent mortality among nymphal whiteflies ($P>0.05$). Spore viability of the inoculum was 97%.

# DISCUSSION AND CONCLUSIONS

## Mortality Of B. tabaci Associated With V. lecanii:

Results show that V. lecanii is highly virulent to B. tabaci (Table 1). Under optimal conditions the fungus can initiate epizootics within a relatively short period of time (>7 d.) that could effectively control whitefly populations (mortality rates range from 79-96%) (Table 1).

## Effects Of Honeydew-Like Substrates On V. lecanii:

Results of this study provide information about the effects of some amino acids and sucrose on the growth and sporulation of V. lecanii.

The most abundant amino acids found in whitefly honeydew, glutamine, serine, and glutamic acid, (Byrne and Miller, 1990), appear to severely inhibit hyphae growth at the higher concentrations of 10,000-40,000 ppm (Figure 1). Sucrose, another constituent of honeydew (Byrne and Miller, 1990), also has a negative effect on growth at high concentrations (Figure 2). At decreased concentrations, hyphae growth is still inhibited on all of the substrates used, yet to a lesser degree when compared to growth observed on water agar (Figure 3).

Higher concentrations of 30,000-40,000 ppm gave an increased, yet not significantly greater, numbers of spores produced on all substrates (Tables 2-5). At decreased

concentrations, sporulation was overall depressed, yet again not significantly so (Table 5).

This data suggests that V. lecanii may selectively invest in vegetative or reproductive growth as a response to nutrient concentrations. At low concentrations of nutrients, the fungus appears to invest in vegetative growth while in the presence of highly concentrated nutrient sources, the fungus begins to reproduce. Such selective activities may reflect optimization of the available resources within its environment. One possible explanation for this apparent trend is that growth may be of the greatest investure in order to escape a poor environment, while in periods of high resource availability, reproduction is enhanced.

Another possible explanation is that enhancement of reproduction is due to an unsuitable or stressful environment. This phenomenon has been observed in plants by many researchers, and certainly may be descriptive of the growth versus sporulation trend observed in these experiments. The possibility of an inappropriate pH level within the media used may have created an unsuitable environment for the fungus.

Lastly, the use of single substrates may be inappropriate for V. lecanii. Combinations of substrates, or the additions of vitamins, mineral salts, and buffers may be necessary in order to significantly enhance the two characteristics of growth and sporulation.

Augmentation Of V. lecanii By Honeydew-Like Substrates:

The data presented here does provide evidence that certain honeydew-like substrates can affect important characteristics which are necessary for epizootics to occur. Augmentation is apparently possible given the existence of commercial formulations (Hall, 1982). Furthermore, correspondence by this author with a European manufacturer, indicate the possibility that augmentation exists in commercial formulations. Yet, from these experiments it is apparent that concentrations of individual constituents are inappropriate for augmentations since most result in decreased growth. This does not suggest that buffers, other constituents, or combinations of the two might give better results. Lastly, exploratory studies of this type do not negate the value of this technology.

Greenhouse Assessment:

Results of the established whitefly study indicate that the environment found in a greenhouse propagation chamber is suitable for the fungus to establish an epizootic. However, results of this greenhouse study may not be entirely indicative of V. lecanii's abilities as a potential biological control agent.

This experiment was conducted in an arid environment and

during the month of June when ambient air temperatures are the highest. Daily maximum and minimum temperatures within the greenhouse were recorded and ranged between 14.4°C-33°C. It is highly probable that high temperatures during this experiment had an effect on the ability of the fungus to establish and attack its insect host. Hall (1981) observed that both germination and growth of V. lecanii declined steeply above 25°C and ceased completely above 30°C. High humidity caused by misting in the propagation chamber may have compensated for adverse temperature conditions although not enough to give infectivity rates comparable to those observed in the virulence study.

Reasons for the poor results of the introduced whitefly experiment are unknown, yet several possibilities present themselves.

The substrates and the concentrations used may be insufficient to significantly affect mortality rates. Again, additions of buffers, other constituents, or combinations of the two may achieve the desired result. Secondly, virulence of the spore inoculum may have been greatly reduced. Virulence reduction may be due to the conditions of culturing or adverse environmental factors within the mist environment. Lastly, the time period between inoculation and introduction may be too lengthy. This may allow the inoculum to succumb to natural mortality and adverse environmental conditions before

insect hosts become available.

One significant observation of this experiment is the persistence of some fungal spores 29 d after inoculation. This persistence allowed V. lecanii to achieve low levels of mortality within the introduced whitefly populations.

Significance Of Results:

The fungus can be useful in a greenhouse propagation chamber, especially since little or no method of insect control is used. At present, control of whitefly pests in production facilities is accomplished by cultural practices, and the use of only clean plants for cuttings. Verticillium lecanii may provide an alternative control when none is available. Additionally, prior research has indicated that excellent control results can be obtained when the fungus is used with parasitoids. Hall, (1982) reports satisfactory control of T. vaporariorum when an 'aphid' isolate of the fungus was used in conjunction with Encarsia formosa.

Furthermore, use of the fungus at this point of poinsettia production will decrease the numbers of whiteflies entering the greenhouse on mist propagated cuttings. With this decrease, it is reasonable to assume that any developing resistance in the industry may be slowed by the elimination of resistant whiteflies by V. lecanii.

Table 1. Percent mortality of various instars of *Bemisia tabaci* and *Trialeurodes vaporariorum* resulting from exposure to *Verticillium lecanii*

| Instar | Species | Treatment | % ± %SE |
|---|---|---|---|
| First | *B. tabaci* | Fungus + Surf. | 94.6 ± 0.09 a[1] |
| | | Water Alone | 20.6 ± 2.54 b |
| | | Surfactant | 15.6 ± 0.19 b |
| | *T. vaporariorum* | Fungus + Surf. | 90.3 ± 0.22 a |
| | | Water Alone | 21.6 ± 0.29 b |
| | | Surfactant | 20.0 ± 1.10 b |
| Second | *B. tabaci* | Fungus + Surf. | 89.3 ± 0.19 a |
| | | Water Alone | 0.3 ± 0.01 b |
| | | Surfactant | 25.0 ± 1.21 b |
| | *T. vaporariorum* | Fungus + Surf. | 79.3 ± 0.18 a |
| | | Water Alone | 27.0 ± 1.30 b |
| | | Surfactant | 35.0 ± 0.02 b |
| Third | *B. tabaci* | Fungus + Surf. | 96.0 ± 0.05 a |
| | | Water Alone | 1.6 ± 0.04 b |
| | | Surfactant | 4.0 ± 0.06 b |
| | *T. vaporariorum* | Fungus + Surf. | 95.6 ± 0.05 a |
| | | Water Alone | 0.0 ± 0.01 b |
| | | Surfactant | 19.6 ± 0.40 b |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Student-Newman-Kuels test).

Figure 1. Hyphae growth response of V. lecanii when grown on agar containing differing amino acids at varying concentrations during the 23 d time period.

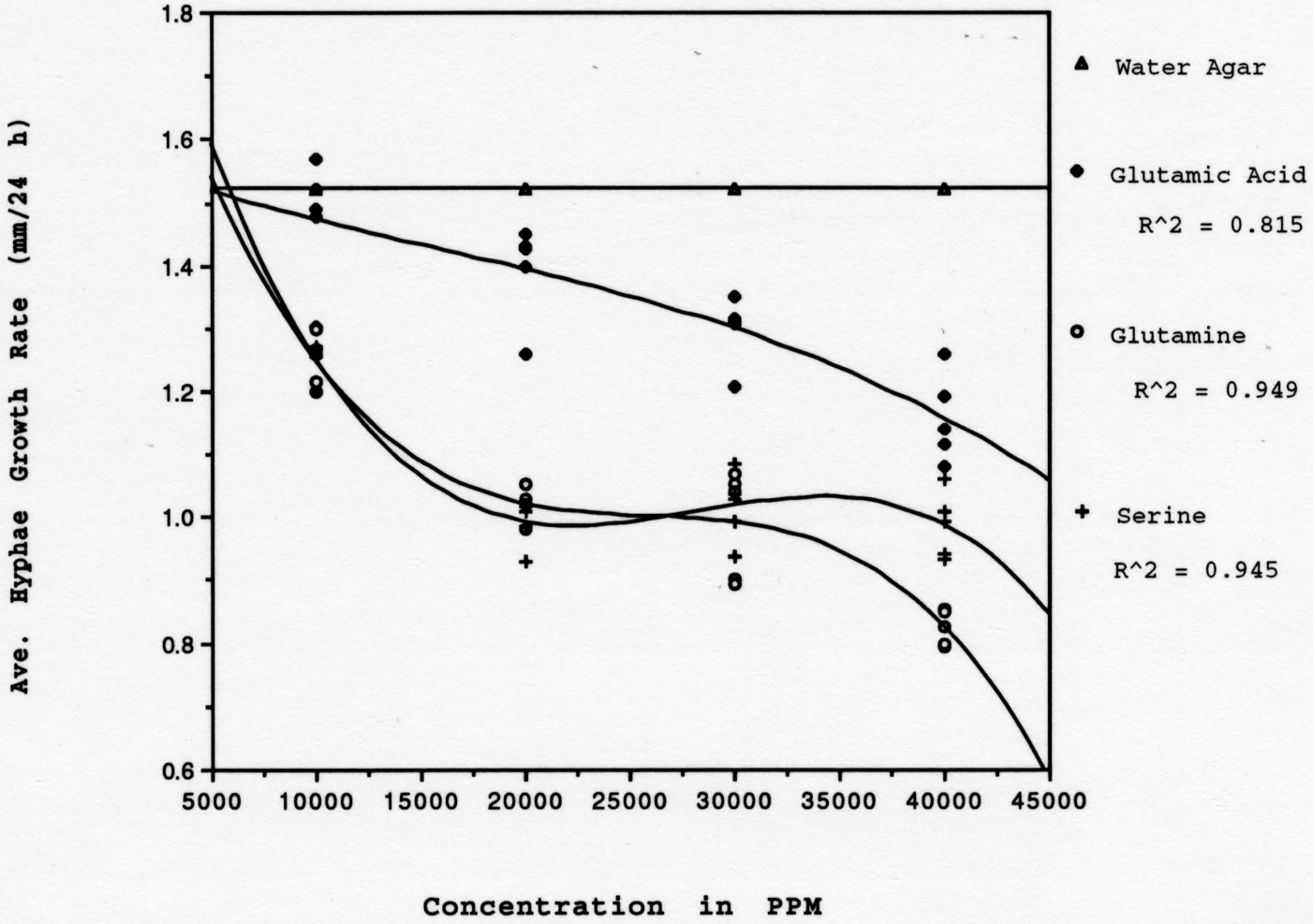
Ave. Hyphae Growth Rate (mm/24 h)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
5000
10000
15000
20000
25000
30000
35000
40000
45000
Concentration in PPM
Water Agar
Glutamic Acid
R^2 = 0.815
Glutamine
R^2 = 0.949
Serine
R^2 = 0.945

Figure 2. Hyphae growth response of V. lecanii when grown on agar containing the carbohydrate sucrose at varying concentrations during the 23 d time period.

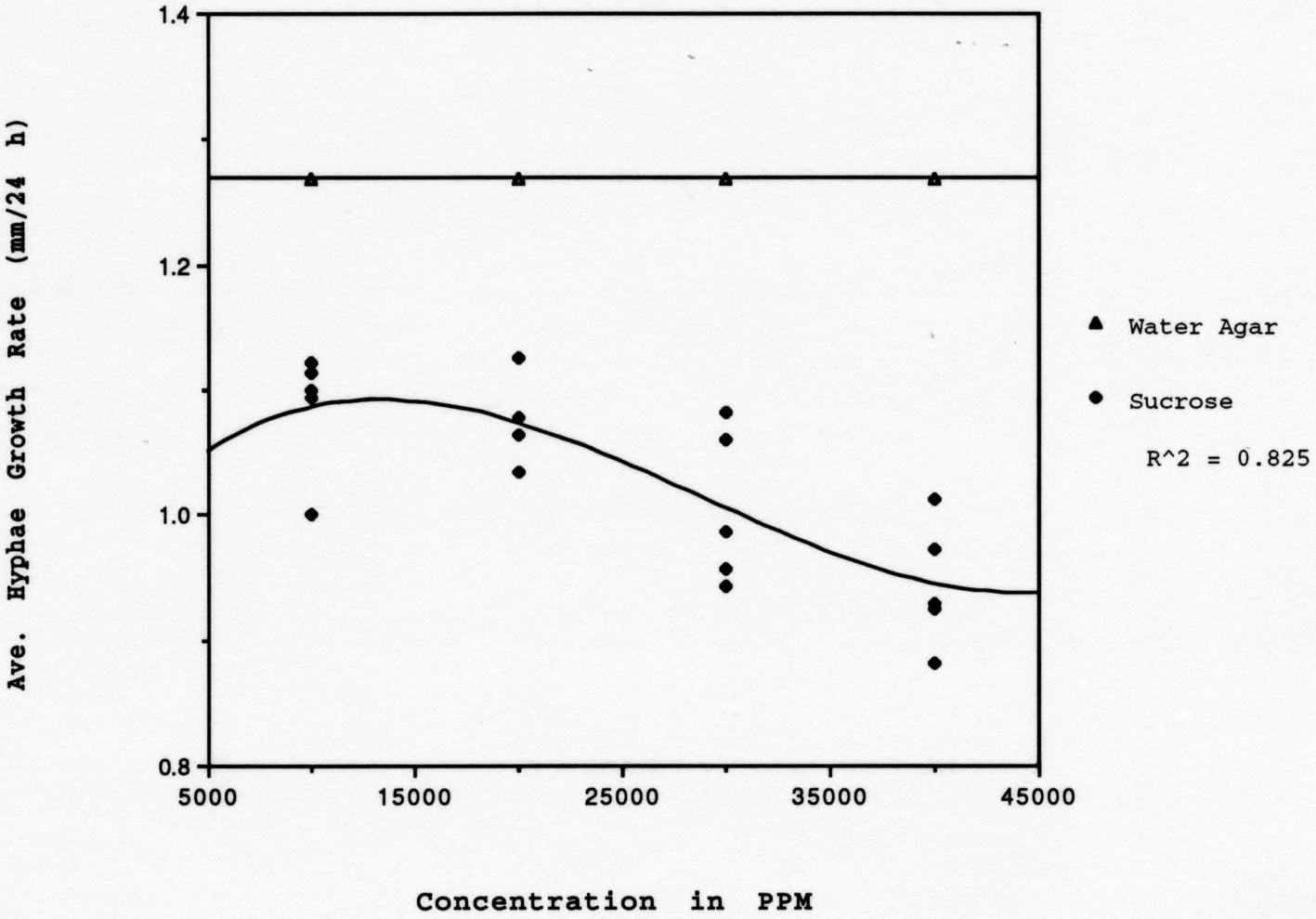
Ave. Hyphae Growth Rate (mm/24 h)
1.4
1.2
1.0
0.8
5000
15000
25000
35000
45000
Concentration in PPM
Water Agar
Sucrose
R^2 = 0.825

Figure 3. Hyphae growth response of V. lecanii when grown on agar containing differing substrates at varying concentrations during the 23 d time period.

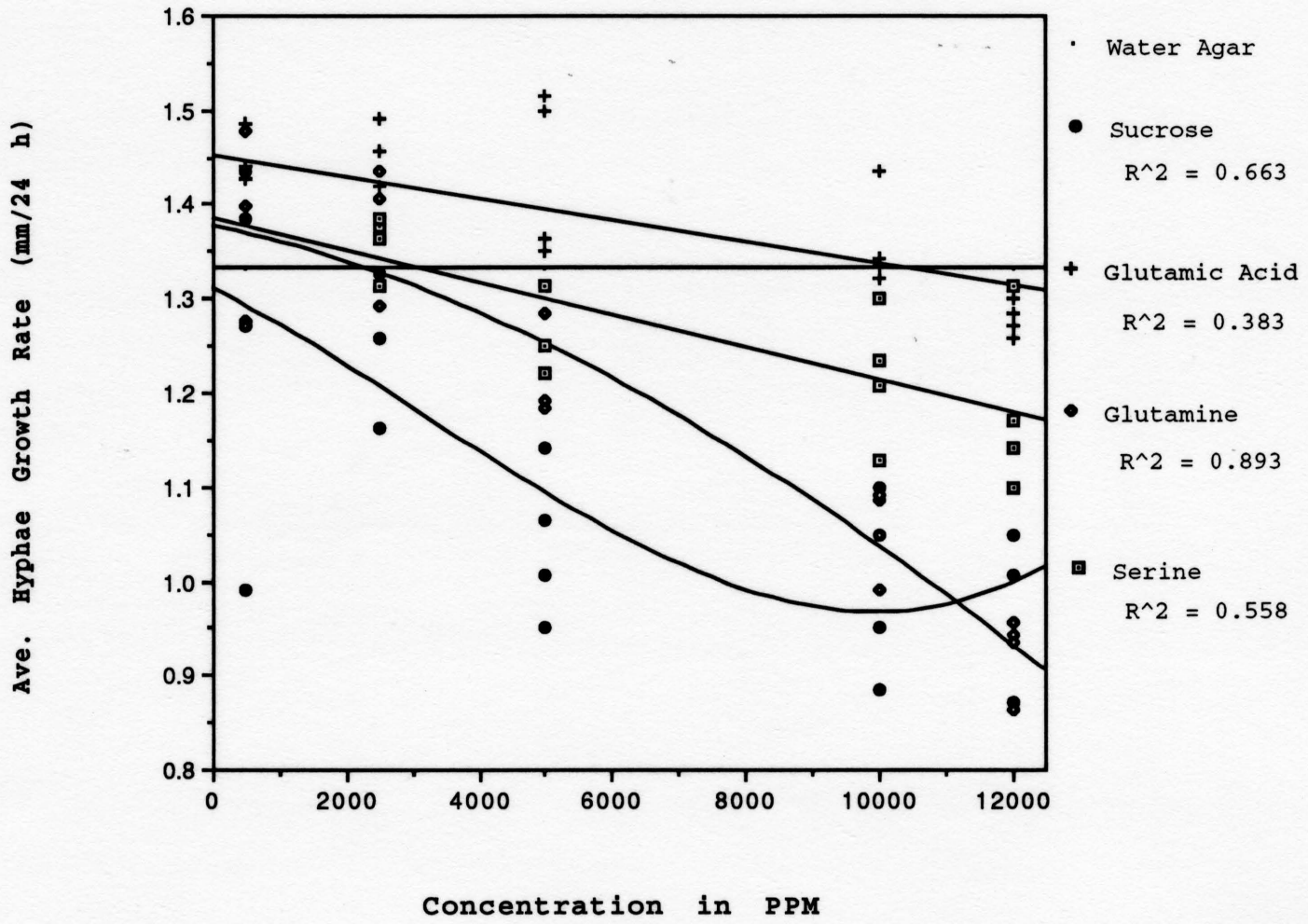
Ave. Hyphae Growth Rate (mm/24 h)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0
2000
4000
6000
8000
10000
12000
Concentration in PPM
Water Agar
Sucrose
R^2 = 0.663
Glutamic Acid
R^2 = 0.383
Glutamine
R^2 = 0.893
Serine
R^2 = 0.558

Table 2. Mean number of spores produced (x $10^4$) by *V. lecanii* when grown on agar containing differing amino acids at varying concentrations.

| Substrate | Conc (ppm) | Mean ± SE | |
|---|---|---|---|
| Glutamic Acid | 10,000 | 25.6 ± 4.03 | bc[1] |
| | 20,000 | 48.8 ± 8.90 | abc |
| | 30,000 | 85.2 ± 9.40 | a |
| | 40,000 | 81.4 ± 7.39 | ab |
| Glutamine | 10,000 | 17.6 ± 1.80 | c |
| | 20,000 | 10.4 ± 2.08 | c |
| | 30,000 | 47.6 ± 1.20 | abc |
| | 40,000 | 14.2 ± 1.31 | c |
| Serine | 10,000 | 60.8 ± 16.84 | abc |
| | 20,000 | 10.6 ± 2.56 | c |
| | 30,000 | 42.8 ± 4.95 | abc |
| | 40,000 | 24.0 ± 1.26 | c |
| Water Agar | 0 | 10.4 ± 3.02 | c |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Duncan's Multiple Range test).

Table 3. Mean number of spores produced (x $10^4$) by V. lecanii when grown on agar containing the carbohydrate sucrose at varying concentrations.

| Substrate | Conc (ppm) | Mean ± SE |
|---|---|---|
| Sucrose | 10,000 | 29.4 ± 4.56 ab[1] |
| | 20,000 | 81.7 ± 16.50 ab |
| | 30,000 | 62.9 ± 10.13 ab |
| | 40,000 | 107.2 ± 21.34 a |
| Water Agar | 0 | 10.2 ± 4.07 b |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Duncan's Multiple Range test).

Table 4. Mean number of spores produced (x $10^4$) by V. lecanii when grown on agar containing differing substrates at three concentrations.

| Substrate | Conc (ppm) | Mean ± SE |
|---|---|---|
| Sucrose | 10,000 | 17.7 ± 2.3 b[1] |
| | 20,000 | 67.5 ± 15.1 ab |
| | 30,000 | 117.0 ± 21.0 a |
| Glutamic Acid | 10,000 | 18.7 ± 7.9 b |
| | 20,000 | 26.0 ± 2.0 ab |
| | 30,000 | 45.7 ± 5.6 ab |
| Glutamine | 10,000 | 14.2 ± 1.6 b |
| | 20,000 | 15.2 ± 2.4 b |
| | 30,000 | 34.7 ± 12.4 ab |
| Serine | 10,000 | 13.7 ± 2.5 b |
| | 20,000 | 24.2 ± 2.7 ab |
| | 30,000 | 17.0 ± 1.8 b |
| Water Agar | 0 | 15.5 ± 3.5 b |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Duncan's Multiple Range test).

Table 5. Mean number of spores produced (x $10^4$) by V. lecanii when grown on agar containing differing substrates at varying concentrations.

| Substrate | Conc (ppm) | Mean ± SE | |
|---|---|---|---|
| Sucrose | 12,000 | 29.7 ± 1.9 | ab[1] |
| | 10,000 | 34.7 ± 4.7 | ab |
| | 5,000 | 61.5 ± 27.8 | a |
| | 2,500 | 26.5 ± 4.4 | b |
| | 500 | 17.5 ± 4.1 | b |
| Glutamic Acid | 12,000 | 29.5 ± 1.5 | ab |
| | 10,000 | 25.7 ± 3.4 | b |
| | 5,000 | 26.0 ± 5.6 | b |
| | 2,500 | 16.0 ± 1.4 | b |
| | 500 | 23.0 ± 5.8 | b |
| Glutamine | 12,000 | 13.2 ± 1.6 | b |
| | 10,000 | 13.7 ± 0.8 | b |
| | 5,000 | 14.0 ± 1.4 | b |
| | 2,500 | 13.0 ± 0.4 | b |
| | 500 | 16.2 ± 0.2 | b |
| Serine | 12,000 | 25.2 ± 8.4 | b |
| | 10,000 | 16.7 ± 1.3 | b |
| | 5,000 | 18.7 ± 4.6 | b |
| | 2,500 | 24.2 ± 4.5 | b |
| | 500 | 19.2 ± 2.0 | b |
| Water Agar | 0 | 22.4 ± 6.8 | b |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Duncan's Multiple Range test).

Table 6. Mean proportion of B. tabaci nymphs killed due to treatments with and without V. lecanii.

| Sample Date (Days after Inoculation) | Treatment | % ± %SE |
|---|---|---|
| 10 | Fungus + Surfactant | 61.6 ± 0.08 a[1] |
| | Fungus + Surf. + Sucrose + Glutamic Acid | 63.0 ± 0.06 a |
| | Surfactant Alone | 17.0 ± 0.06 b |
| 19 | Fungus + Surfactant | 88.7 ± 1.47 a |
| | Fungus + Surf. + Sucrose + Glutamic Acid | 89.2 ± 2.52 a |
| | Surfactant Alone | 23.9 ± 7.52 b |

[1]Means followed by the same letter are not significantly different from one another ($P<0.05$) (Student-Newman-Kuels test). Comparisons are only within a single sampling date.

Table 7. Mean proportion of B. tabaci nymphs killed when exposed to V. lecanii with and without additives.

| Treatment | % ± %SE |
|---|---|
| Fungus Alone | 36.8 ± 0.25 a[1] |
| Fungus + Honeydew | 30.8 ± 0.29 a |
| Fungus + All Substrates at 10,000 ppm | 34.2 ± 0.25 a |

[1]Means followed by the same letter are not significantly different from one another (P<0.05) (ANOVA).

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