

Pathogenicity of *Verticillium lecanii* to Different Developmental Stages of the Silverleaf Whitefly, *Bemisia argentifolii*

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Thirty-five strains of *Verticillium lecanii* which originated from different hosts and geographical locations were tested as potential biocontrol agents against silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring. All strains were tested for their pathogenicity to third-instar nymphs. Several isolates which exhibited high pathogenicity to nymphs were also tested against eggs, pupae and adults of *B. argentifolii*. Eggs were found to be immune to infection, but mortality of hatching nymphs reached 95–98%. The rate of hatching nymphs' infection depended on the age at which the eggs were inoculated and the strain's virulence. Mortality of nymphs recorded on day 4 after inoculation varied from $0.5 \pm 0.3\%$ to $83 \pm 2.4\%$; that of the control ranged from 2.5% to 10.2%. The most virulent strains, with LT_{50} ranging between 3.2 and 3.8 days, were isolated from aphids in Israel and probably have a similar origin. The pathogenicity of *V. lecanii* strains to pupae 6 days after inoculation varied between $59 \pm 12.1\%$ and $72.5 \pm 13.1\%$, as compared with natural mortality of $13.5 \pm 4\%$. The maximum adult mortality caused by *V. lecanii* strains was between $34.1 \pm 5.1\%$ and $52.6 \pm 3.8\%$.

KEY WORDS: *Verticillium lecanii*; *Bemisia argentifolii*; biocontrol; entomopathogenic fungi; whitefly.

INTRODUCTION

The silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring, previously known as b-strain sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (18), is a major pest of a wide range of wild and cultivated plants in warm climates worldwide (17). It affects crops grown in greenhouse all year round (*e.g.* cucumbers, peppers, tomatoes, and many cut flowers). It is also known as a principal pest of open field crops such as vegetables, cotton and ornamentals. *B. argentifolii* causes direct damage by feeding on leaves and also indirect damage by vectoring economically important plant viruses. The biology of this insect has been reviewed elsewhere (3). The large populations reached by this insect are attributed, in large measure, to its polyphagous nature, short developmental cycles (less than a month during summer) and high fecundity. Although mainly chemical control has been used against *B. argentifolii*, it is not always successful, possibly due to the waxy nature of the cuticle (3) and the rapid development of resistance (22). The reduced efficiency of insecticidal control and ecological awareness revived the interest in biological control.

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In this regard, *B. argentifolii* is attacked by parasitoids, predators and entomopathogenic fungi.

Strains of *V. lecanii* have been isolated from aphids, scales, whiteflies and other insects in various regions of the world (7). The pathogenicity and potential of *V. lecanii* as a microbial agent for biocontrol was demonstrated for some whiteflies, aphids and thrips (4,6,9,10). In certain cases, the efficiency of *V. lecanii* reached that achieved by chemical treatments (13,20). Commercial preparations of *V. lecanii* have been on the market since 1980 and have been used successfully against *Trialeurodes vaporariorum* (Westwood) infesting cucumbers and tomatoes (7,19).

The majority of natural infections of whiteflies with *V. lecanii* occur on *T. vaporariorum* – which predominates in moderate climates, rather than on *B. argentifolii* – which predominates in tropical and subtropical regions. In Israel's warm climate, *V. lecanii* appears mainly as an aphid and scale pathogen during the winter (2). Nevertheless, *V. lecanii* has been isolated occasionally from *B. tabaci* (= *B. argentifolii*) in other countries with relatively warm climates, such as Spain and Mexico (1,12,16).

In contrast to the voluminous information on the biological control of *T. vaporariorum* by *V. lecanii* (5,8,17,19), the knowledge on the efficacy of the biological control of *B. argentifolii* by *V. lecanii* is limited and the potential of *V. lecanii* as an effective biological control agent against *B. argentifolii* is not entirely clear. There are only a few reports dealing with assays of the virulence of *V. lecanii* on *B. tabaci* (= *B. argentifolii*) under controlled laboratory conditions (14,23). It was found that *V. lecanii* was effective against subimaginal instars of *B. tabaci* as well as some instars of *T. vaporariorum*. *B. tabaci* nymph mortality reached a rate of 89% to 96% (14). In the greenhouse, the population density of *B. tabaci* on melons and tomatoes was maintained at low values over a long period after *V. lecanii* treatment (20,21); however, effective control could not be achieved under field conditions (1).

The potential use of a fungus as a biological control agent is greatly influenced by the susceptibility of the various developmental stages of the insect to the pathogen as well as its ability to initiate infection in early stages of the whitefly's development. *V. lecanii* was shown to be effective against different developmental stages of *T. vaporariorum* including the first instar crawlers and adults, but not eggs (5,8,11). However, information on the susceptibility of various developmental stages of *B. argentifolii* to *V. lecanii* is not available. The present study provides information on the pathogenicity of *V. lecanii* against various stages of *B. argentifolii* under laboratory conditions.

MATERIALS AND METHODS

Insects A colony of *B. argentifolii* originally collected from cotton (*Gossypium hirsutum* L.) in Israel was maintained on cotton plants at a temperature of 25–27°C with a 16L:8D photoperiod. In order to obtain a uniform age population of *B. argentifolii*, 50 to 100 adults were placed on small cotton plants for 24–36 h followed by the removal of all adults. Plants with eggs were transferred to environmental growth chambers for further development of homogeneous populations.

Fungal strains The fungal strains used in this study were (a) isolated in Israel, (b) obtained from the collections of R. Kenneth (The Hebrew University of Jerusalem, Faculty

of Agricultural, Food and Environmental Quality Sciences, Rehovot), or (c) obtained from different countries. Strains from Russia and Kazakhstan were provided by either V.A. Pavluschin (All Russian Institute of Plant Protection, St. Petersburg) or N.U. Geschtovt (Kazakh Research Institute of Plant Protection, Almaty). The location and the insect from which various strains were isolated are presented in Table 1.

Fungal maintenance and conidial preparations Cultures were maintained on Sabouraud Dextrose Agar (SDA). All isolates were passed periodically (every 12–18 months) through a pathogenic cycle on *B. argentifolii* and re-isolated on SDA. In order to obtain conidial preparations, fungal strains were cultivated on malt agar with 0.1% yeast extract for 10 days at 25°C. Spores were harvested using an aqueous solution of 0.005% Triton ×100. The spore suspension was filtered through several layers of cheesecloth to remove mycelial mats. The concentration of spores in the final suspension was determined by haemocytometry. The spore preparation used for bioassays was adjusted by diluting concentrated spores with 0.005% Triton ×100 to a final concentration of 10⁷ spores/ml.

Bioassay procedure for nymphs and eggs of *B. argentifolii* Third instar *B. argentifolii* nymphs were used for all screening bioassay procedures. Individual cotton leaves with more or less uniformly distributed insects (5 to 10 insects/cm²) were selected prior to fungal inoculation. Leaf sectors with approximately 50 to 100 insects were used. These leaf pieces bearing nymphs were immersed in a spore suspension for 10 sec; control leaves were immersed in 0.005% Triton ×100 for the same length of time. To prevent development of saprophytic fungi, treated leaves were placed for 20–30 min on filter paper to remove excess moisture. The leaves were then placed in petri dishes which were incubated in a growth chamber at alternating temperatures of 25°C (14 h in light) and 20°C (10 h in the dark). Relative humidity close to 100% was reached by placing a moist filter paper in each petri dish. For aeration purposes, each petri dish was opened daily for 25–30 min. This procedure was necessary to avoid development of saprophytic fungi on whitefly honeydew. The number of insects per leaf sector was counted prior to inoculation. The percentage of dead insects following inoculation and infection was recorded daily. All strains were tested twice using 10 to 12 replications per strain. The natural mortality in control leaves was usually lower than 10% and was not subtracted from the obtained mortality percentage.

Eggs of uniform age were obtained as described earlier. Pathogenicity was determined by calculating the percentage of infected larvae among the total number of emerged larvae.

Bioassay procedure for adults of *B. argentifolii* Spraying adults with spore suspension generally resulted in high mortality. The sensitivity of this procedure did not allow differentiation in degree of virulence among the various strains. In order to overcome this limitation, pupae were inoculated for a brief period prior to emergence and the infection rate was determined when adults exited from their pupae cases. Small plants with two to four leaves were exposed to *B. argentifolii* adults for 24–30 h. The adults were then removed and the plants were transferred to growth chambers for 20–22 days. A leaf with 50–100 pupae nearing emergence was retained on each plant, whereas the other leaves were removed. The leaf was sprayed with a conidial suspension (10⁷ spores/ml) and after water evaporation the plants were covered with a plastic cage with an outlet in the upper side. The

opening was covered with several layers of cheesecloth. To maintain high moisture, the cheesecloth was wetted daily. The covered plants were transferred to a growth chamber at alternating temperatures of 25°C (14 h in light) and 20°C (10 h in the dark). Virulence was determined by recording the percentage of infected pupae and the percentage of infected adults emerging from treated pupae.

RESULTS AND DISCUSSION

Pathogenicity of *V. lecanii* strains on nymphs Infection of insects by mycoinsecticides is affected by several parameters, including temperature, humidity, age, and physiological condition of the host and the pathogen. A critical factor that appears to influence fungal pathogenicity is the population density of the insect. Uneven population density on different leaves may modify the microenvironment in which the interaction between the host and pathogen takes place. For example, high population densities may enhance pathogen fitness by facilitating its development and dissemination among insects due to the increased abundance of honeydew. Therefore, for standardization of the bioassay procedure, it was necessary to determine the effect of nymphal density on the variation of the rate of infection. Leaf sectors (approximately 8 cm²) with population densities ranging from 2.5 to 25 insects/cm² were inoculated with *V. lecanii* strain Is-5 as described in Materials and Methods. The data in Figure 1 demonstrate that population densities of nymphs on leaves influenced the rate of their infection. Thus, mortality of nymphs caused by *V. lecanii* increased from 34.4% at the lowest density to 100% at the highest density. However, the lowest variation among different nymph densities was obtained at 5–10 insects/cm² (Fig.1). Consequently, the population density 5–10 insects/cm² was used for all further experiments.

Successful biological control is dependent on fungal strains with a high rate of penetration and infection. Therefore, the mortality rate was recorded daily after inoculation. Thirty-five strains of *V. lecanii* isolated from various insects and different geographic regions were tested for pathogenicity on nymphs of *B. argentifolii* (Fig. 2, Table 1). The earliest signs of infection were observed within 3 days following inoculation and were expressed as fungal colonization of nymphs. Several virulent isolates (Is-1, Is-2, Is-5, R-1) reached mortality of 12.3±4.6% to 26.5±8.9% within 3 days; however, 15 of the 35 strains tested exhibited only 1.5% mortality during this period.

Mortality of nymphs recorded on the fourth day varied from 0.5% to 83%, as compared with mortality of uninoculated whiteflies, which ranged between 2.5% and 10.2% (Fig. 2). Significant mortality was recorded on the fourth day by 18 isolates, ranging from 16.3±3.3% to 83±2.4%. The mortality caused by the rest of the isolates on the fourth day did not differ significantly from the control. A sharp increase in mortality was recorded for many strains 6–7 days after inoculation, reaching 95–100% (results not shown). Certain strains that exhibited low rates of infection after 4 days became highly pathogenic after 7 days. Thus, strains Is-2668 and Is-3457, which caused only 0.5% to 2.2% mortality after 4 days, reached 73.5±9.7% and 48.3±8.3%, respectively, after 7 days. Conversely, nymphs inoculated with strain Is-2614 reached 24.5±3.4% mortality on the fourth day but only 38.5±6.2% mortality on the seventh day. Five strains were nonpathogenic and did not cause significant mortality as compared with the control even after 7 days.

Thirty-five *V. lecanii* strains were classified for degree of virulence (Table 2) according

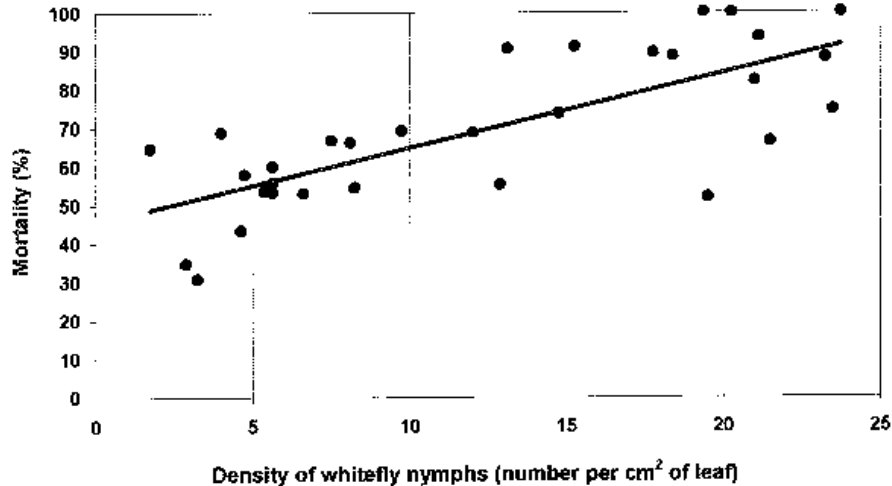


Fig. 1. Pathogenicity of *Verticillium lecanii* at different densities of *Bemisia argentifolii* nymphs. Relationship between density of *B. argentifolii* nymphs per cm² of leaf and mortality of *B. argentifolii* nymphs (%) 4 days after inoculation by strain *V. lecanii* Is-5 (10⁷ spores per ml). Treated leaves with nymphs were incubated at temperatures alternating between 25°C (14 h in light) and 20°C (10 h in the dark), at 100% r.h.

to the time in days required to achieve 50% mortality (LT₅₀). The most virulent strains of *V. lecanii* (Is-5, Is-2 and Is-6, with LT₅₀ ranging between 3.2 and 3.8 days) were isolated from aphids in Israel. In a previous study (15) in which the RAPD profiles of different *V. lecanii* strains were compared, we found that these three strains are grouped in the same cluster and may have a similar origin. They were superior to the strain from the commercial *V. lecanii* product Mycotal[®] (produced by the now defunct Microbial Resources Ltd.; LT₅₀ = 3.9), which caused 54% mortality of *B. argentifolii* nymphs within 4 days after inoculation. The most virulent strains were isolated from aphids rather than from whiteflies (Fig. 2), indicating lack of fungal specificity towards the host. Surprisingly, the strain isolated from *B. argentifolii* (Is-11) had relatively low pathogenicity against *B. argentifolii* nymphs (43.5% mortality 4 days after treatment). Another eight strains isolated from whitefly (*T. vaporariorum*) caused mortality ranging between 16.3±1.6% and 56.3±4.1% within 4 days after inoculation. However, there were strains isolated from aphids, whiteflies and other insects or rust fungi that were avirulent to *B. argentifolii*. These results are in agreement with the broad host specificity of *V. lecanii* as an insect pathogen.

Pathogenicity of *V. lecanii* on eggs and hatched nymphs Eggs of *B. argentifolii* are immune to infection by *V. lecanii*. However, in preliminary studies we have noticed that when there is a population consisting of different stages, and eggs are found in the vicinity of infected nymphs or adults, the eggs may become covered with fungal hyphae. Although the chorion of these eggs was not invaded by any of the strains tested, the eggs covered with hyphae either did not hatch or hatched with a delay of 3–4 days. In addition, the hyphae present on the eggs were found to infect the nymphs immediately upon hatching.

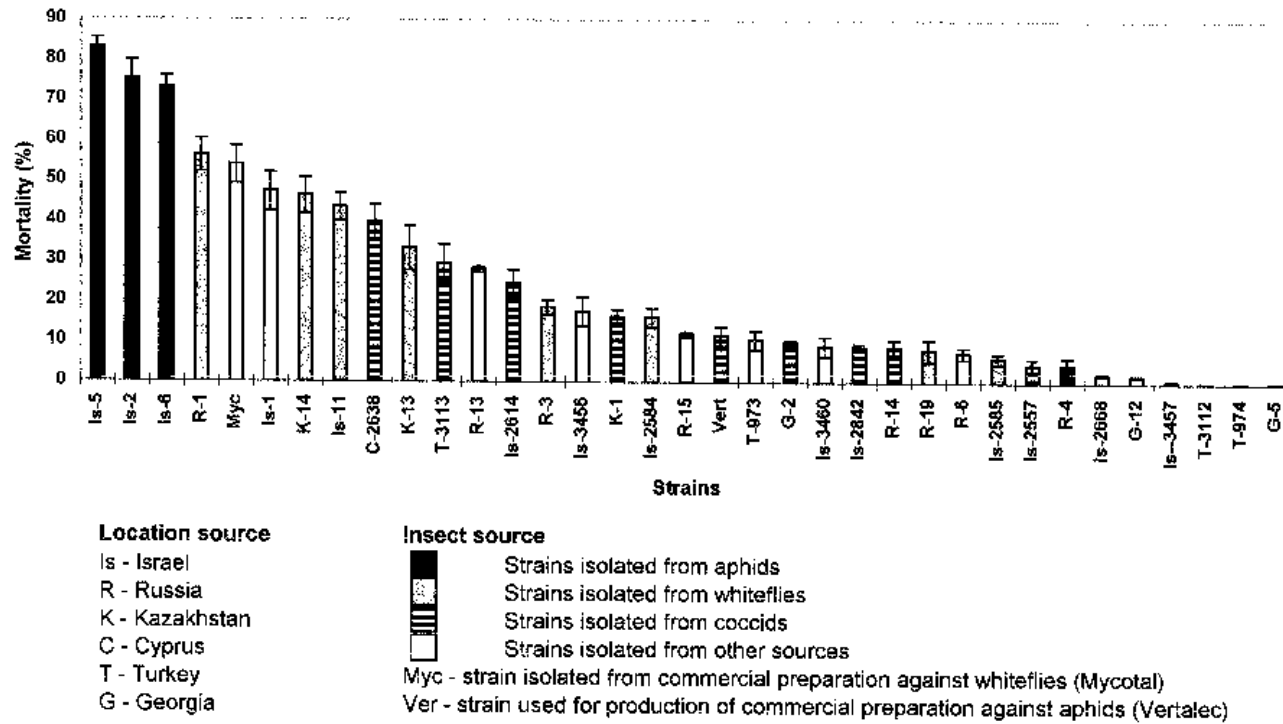


Fig. 2: Pathogenicity of *Verticillium lecanii* strains for *Bemisia argentifolii* nymphs. Mean percent mortality of *B. argentifolii* nymphs 4 days after inoculation by spore suspensions of *V. lecanii* strains (10^7 spore per ml). Treated leaves with nymphs were incubated at temperatures alternating between 25°C (14 h in light) and 20°C (10 h in the dark), at 100% r.h. Vertical lines represent standard deviation of means (n = 20–24 replications per strain).

TABLE 1. Host source and location of isolates of *Verticillium lecanii* used in the study

<i>Verticillium lecanii</i> isolates	Host source	Location
Is-1	<i>Oxycarenus hyalinipennis</i> (Homoptera: Lygaeidae)	Israel
Is-2	<i>Myzus persicae</i> (Homoptera: Aphididae)	Israel
Is-5	<i>Myzus persicae</i> (Homoptera: Aphididae)	Israel
Is-6	<i>Acyrtosiphon pisum</i> (Homoptera: Aphididae)	Israel
Is-11	<i>Bemisia argentifolii</i> (Homoptera: Aleyrodidae)	Israel
Is-2557	<i>Aphis gossypii</i> (Homoptera: Aphididae)	Israel
Is-2584	Unknown	Israel
Is-2585	Unidentified coccid (Homoptera: Coccidae)	Israel
Is-2614	<i>Coccus hesperidus</i> (Homoptera: Coccidae)	Israel
Is-2668	Unknown	Israel
Is-2842	<i>Saissetia oleae</i> (Homoptera: Coccidae)	Israel
Is-3456	Plant leaf	Israel
Is-3457	Unidentified coccid (Homoptera: Coccidae)	Israel
Is-3460	<i>Protopulvinaria pyriformis</i> (Homoptera: Coccidae)	Israel
R-1	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Russia
R-2	Unidentified rust fungus	Georgia
R-3	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Russia
R-4	Unidentified <i>Aleyrodidae</i> sp. (Homoptera: Aleyrodidae)	Russia
R-5	<i>Ceroplastes japonicus</i> (Homoptera: Coccidae)	Georgia
R-6	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Russia
R-12	<i>Planococcus citri</i> (Homoptera: Pseudococcidae)	Georgia
R-13	Unknown	Russia
R-14	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Russia
R-15	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Russia
R-19	<i>Puccinia graminis</i> (rust fungus)	Russia
K-1	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Kazakhstan
K-13	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Kazakhstan
K-14	<i>Trialeurodes vaporariorum</i> (Homoptera: Aleyrodidae)	Kazakhstan
Vert	Vertalec ^z	—
Myc	Mycotal ^y	—
C-2638	<i>Coccus hesperidus</i> (Homoptera: Coccidae)	Cyprus
T-973	<i>Cossus cossus</i> (Lepidoptera: Cossidae)	Turkey
T-974	<i>Pulvinaria floccifera</i> (Homoptera: Coccidae)	Turkey
T-3112	<i>Pulvinaria floccifera</i> (Homoptera: Coccidae)	Turkey
T-3113	<i>Parthenolecanium cornii</i> (Homoptera: Coccidae)	Turkey
T-3113	<i>Parthenolecanium cornii</i> (Homoptera: Coccidae)	Turkey

^z *V. lecanii* strain used for production of commercial preparation against aphids (Vertalec).

^y *V. lecanii* strain isolated from commercial preparation against whiteflies (Mycotal).

One- and 4-day-old eggs were inoculated with strains Is-1 and Is-5, respectively, and the mortality of hatching nymphs was recorded (Fig. 3). First-instar emergence always began in 7- to 8-day-old eggs, regardless of the time of inoculation, and reached approximately 80%. The rate of infection of hatching nymphs was found to depend on the age at which the eggs were inoculated and the fungal strain's virulence. The first signs of infection for nymphs emerging from inoculated one-day-old eggs were observed after 8 days. At that time nymph mortality reached $5 \pm 1.6\%$ to $7.1 \pm 2.6\%$ for both strains. However, 10 days after treatment the mortality of nymphs differed significantly ($P < 0.05$) and ranged from $14.5 \pm 3.6\%$ for strain Is-1 to $26 \pm 7\%$ for strain Is-5 (Fig. 3).

A significant increase in nymph mortality was obtained with both strains when 4-day-old eggs were inoculated. Mortality was 26% and 75%, respectively, at 8 and 10 days after inoculation of 4-day-old eggs with Is-1 (Fig. 3). The rate of nymph mortality with strain

TABLE 2. Pathogenicity of *Verticillium lecanii* strains for *Bemisia argentifolii* nymphs as expressed by LT₅₀

High virulence, LT ₅₀ =3.2–4.0 days	Moderate virulence, LT ₅₀ =4.0–7.0 days	Low virulence, LT ₅₀ >7 days	Avirulent strains
Is-5	Is-1	R-2	T-3112
Is-2	K-14	R-3	R-5
Is-6	K-1	R-13	R-14
R-1	K-13	R-12	T-974
Mycotal	Is-11	R-15	Is-2557
	R-4	R-6	
	R-19	Is-2585	
	T-973	Is-3457	
	Vertalec	Is-2584	
	C-2638	Is-2614	
	T-3113		
	Is-3456		
	Is-3460		
	Is-2842		
	Is-2668		

Is-5 was significantly higher than with strain Is-1, and 8 days after inoculation of 4-day-old eggs reached $40 \pm 6.3\%$ as compared with $26 \pm 6.3\%$ with strain Is-1. However, final mortality did not differ significantly, and reached 95–98% of the hatching nymphs in both strains.

These results indicate that egg inoculation with *V. lecanii* has no effect on egg hatch rate, but does affect the mortality of hatching nymphs. The rate of infection depends on the time required for nymph emergence after inoculation and the virulence of the fungal inoculum. The inoculation of one- vs 4-day-old eggs caused lower infection of hatching nymphs at the same time after nymph emergence. This fact reflects a decline in efficiency of inoculation with time on the one hand, but, on the other, indicates the survival of the inoculum on leaves for at least 8 days.

Pathogenicity of *V. lecanii* strains against pupae and adults Infection of pupae was recorded 3 to 4 days after inoculation. Pupae mortality on the fourth day varied from $4.9 \pm 2\%$ in strain Is-1 to $22.1 \pm 6\%$ in strain Is-5 (Fig. 4). Mortality of untreated pupae did not exceed 5%. Infection of pupae continued until adult emergence was complete. A substantial increase in pupae mortality was observed between the fourth and seventh days after inoculation (Fig. 4). Thus the proportion of pupae mortality caused by different strains 7 days after inoculation was $59 \pm 12.9\%$ to $72.5 \pm 13.1\%$, as compared with $13.5 \pm 4\%$ mortality of controls. The most virulent strain against pupae was Is-2 ($72.5 \pm 13.1\%$ mortality), which caused slightly higher pupae mortality than Is-5; and both strains showed significantly higher virulence than the strain of *V. lecanii* isolated from the commercial mycoinsecticide Mycotal.

The pathogenicity of various *V. lecanii* strains to adults emerging from treated pupae is shown in Figure 5. Adult emergence from treated and untreated pupae began on the third day and infected adults were recorded on leaves on the sixth day after inoculation. Many adults had already been infected and died prior to or at the time of emergence from the pupae. The proportion of adults emerging from untreated pupae after 7 days reached $85.5 \pm 3.6\%$ and their natural mortality was less than 2%. In contrast, the

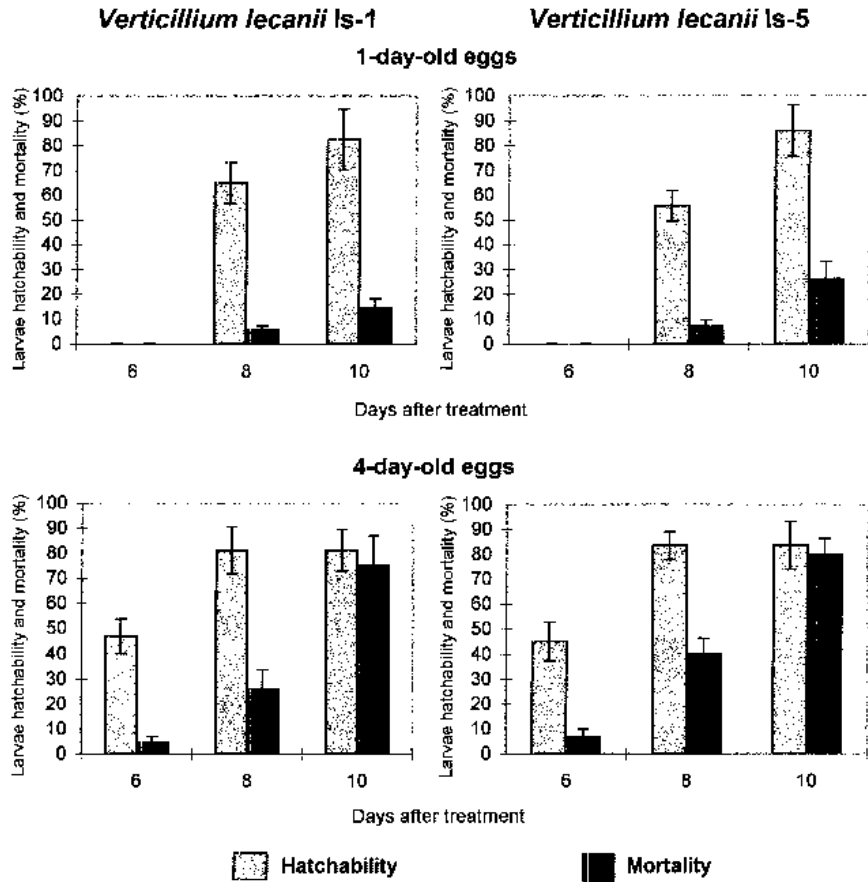


Fig. 3. Pathogenicity of *Verticillium lecanii* to *Bemisia argentifolii* eggs and hatched nymphs. Mean percent hatchability and mortality of *B. argentifolii* nymphs after treatment of 1- and 4-day-old eggs by spore suspension of *V. lecanii* (10^7 spores per ml). Treated leaves with eggs and nymphs incubated at temperatures alternating between 25°C (14 h in light) and 20°C (10 h in the dark), at 100% r.h. Vertical lines represent standard deviation of means (n = 8–10 replications per strain).

proportion of adults emerging from pupae infected by Is-2 was $27.5 \pm 13.1\%$, as compared with $41 \pm 12.1\%$ emerging from pupae infected by the *V. lecanii* strain isolated from the commercial Mycotol preparation (Fig. 5). The proportion of adults showing mycosis was $34.1 \pm 5.1\%$ to $52.6 \pm 3.8\%$ of the total emerged adults. The total whitefly population which survived after fungal treatment of pupae was 15–25%, vs 83–85% in the untreated control.

The present study indicates high pathogenicity of *V. lecanii* to all developmental stages of *B. argentifolii* except eggs. The susceptibility of different stages of the whitefly to the pathogen and its ability to transmit infection among the various developmental stages and generations, lend support to the economic potential of *V. lecanii* for biological control of *B. argentifolii*.

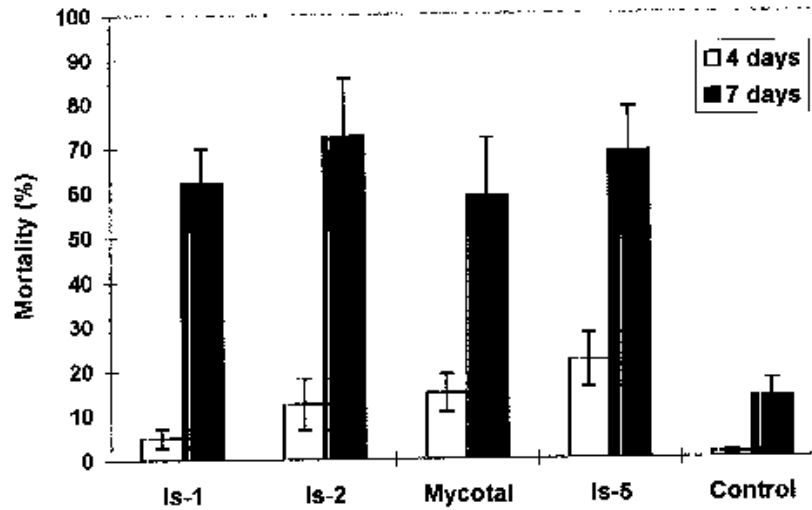


Fig. 4. Pathogenicity of *Verticillium lecanii* strains to *Bemisia argentifolii* pupae. Mean percent mortality of *B. argentifolii* pupae 6 days after inoculation by spore suspensions of *V. lecanii* strains (10^7 spores per ml). Treated plants with pupae incubated at temperatures alternating between 25°C (14 h in light) and 20°C (10 h in the dark), at 100% r.h. Vertical lines represent standard deviation of means ($n = 8-10$ replications per strain).

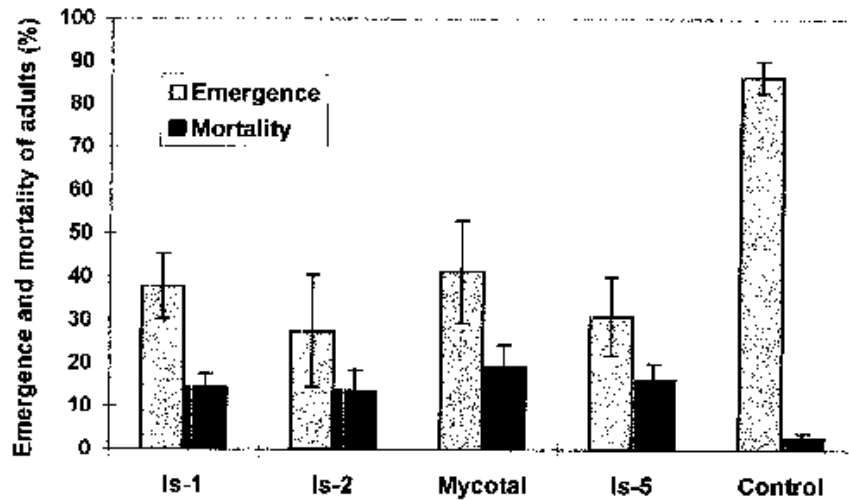


Fig. 5. Effect of *Verticillium lecanii* strains on emergence and mortality of *Bemisia argentifolii* adults after pupae inoculation. Mean percent emergence and mortality of *B. argentifolii* adults 7 days after treatment of pupae by spore suspension of *V. lecanii* (10^7 spores per ml). Treated leaves with pupae incubated at temperatures alternating between 25°C (14 h in light) and 20°C (10 h in the dark), at 100% r.h. Vertical lines represent standard deviation of means ($n = 8-10$ replications per strain).

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