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Selection of a Highly Virulent *Verticillium lecanii* Strain Against *Trialeurodes vaporariorum* at Various Temperatures

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Abstract The virulence of five *Verticillium lecanii* strains against greenhouse whiteflies, *Trialeurodes vaporariorum*, was tested at various temperatures as a major environmental factor. Strain CS-626 was found to be the most durable strain at a broad temperature range, and highly virulent against greenhouse whiteflies in a detached-leaf bioassay. In a tomato plant pot experiment, the LT_{50} and LC_{50} of the CS-626 strain were 6.2 days and 2.3×10^6 conidia/ml, respectively. The optimal concentration of CS-626 for successful infection was 1×10^8 conidia/ml. These results indicate that the CS-626 strain of *Verticillium lecanii* has a strong potential for effectively controlling greenhouse whiteflies.

Key words: *Verticillium lecanii*, *Trialeurodes vaporariorum*, entomopathogenic fungi, greenhouse whitefly, biological control, virulence

Verticillium lecanii (Zimmermann) Viégas, an entomopathogenic fungus, has been successfully commercialized and applied in Europe for years to control aphids and whiteflies in greenhouses [11, 7]. This fungus is assigned to a nonsexual stage of *Cordyceps*, used as medicine or functional food in China, Japan, and Korea [8]. *V. lecanii* is known to have diverse biological characteristics, including virulence, morphology, and physiology, depending on the strain [6, 3]. A strain successful in one country may not always be successful in other countries due to the different greenhouse environments or the climate of the country [6]. Therefore, the examination of the biological characteristics of various strains is a prerequisite when selecting a highly virulent strain for an indigenous greenhouse environment.

The goal of the current study was to select a strain that is suitable for the Korean greenhouse environment and highly virulent to greenhouse whiteflies. Accordingly, various strains of *V. lecanii* from Korea and other countries were compared for their biological characteristics, including their conidial germination rate and infectivity against greenhouse whiteflies at various temperatures. Furthermore, an economic optimal inoculum concentration of a selected strain was determined by examining the actual dose-response reaction at various concentrations of a conidial suspension in a pot experiment.

Five-week-old tomato plants were infested with adult greenhouse whiteflies for 1–2 days in a glasshouse, and then the plants were maintained until the second instar whitefly scales appeared. Five strains of *V. lecanii* were used for the present study; three strains from Korea, one from the USA, and one isolated from Mycotal (Koppert, London, U.K.). The length and width of the >50 conidia/strains, except for the Mycotal strain, were measured using scanning electron microscopy (SEM) and their means calculated.

The conidial germination rates of the five strains were compared at various temperatures to select a strain that was durable under a broad temperature range. The conidia of each strain were harvested with a sterilized 0.01% Tween 20 solution after 5-day-old culture in PDA, and filtered through cheesecloth to remove any conidial clumps and hyphal debris. A drop of the conidial suspension of each strain, adjusted to 1×10^6 conidia/ml, was spread on a glass slide layered with PDA, and incubated at 15, 20, 25, 30, and 35°C. After 12 h-incubation, the conidial germination was stopped by adding a drop of lactophenol containing aniline blue, and the number of germinated conidia was counted by examining all the conidia observed in three 1,000× microscopic fields per slide and three slides per treatment. Germination was defined as the stage when a germ-tube was clearly observed.

The virulence of the five strains was estimated using the detached-leaf method at 15, 25, and 35°C. All conidial

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suspensions, adjusted to 1×10^8 conidia/ml, were prepared simultaneously to avoid any possibility of bias. For each treatment, 20 ml of the conidial suspension from each strain was sprayed on three detached-tomato leaves, each infested with >75 second instar *T. vaporariorum*. The control treatment was subjected to a spray of 20 ml of 0.01% Tween 20 solution omitting conidia. All exuvia and eggshells on the leaves were removed before the application. The treated leaves were air-dried, placed individually in plastic petri-dishes, and incubated at 15, 25, and 35°C, under >75% R.H., and a photoperiod of 12:12 (L:D). The mortality was assessed daily for 7 days after treatment. Whitefly scales completely enveloped in white *V. lecanii* mycelium were considered to be 'dead' [3].

The conidial suspension of the most virulent strain selected from the detached leaf test was adjusted to 1×10^3, 1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8, and 1×10^9 conidia/ml to determine the optimal concentration for the inoculum. Tomato plants, in which each leaf was infested with >75 second instar *T. vaporariorum*, were used for the experiment. Sixty ml of the conidial suspension at each concentration was sprayed on a plant. The controls were treated with just a 0.01% Tween 20 solution. The treated plants were air-dried, placed in plastic cages, and incubated at 75±10% R.H., 25°C. The mortality was assessed daily for 10 days on three leaves at each concentration. To examine the actual dose response, 20 scales of similar size were randomly sampled from the leaves treated at each concentration after air-drying, and homogenized together in a 1.5-ml microcentrifuge tube containing 0.5 ml of 0.01 M buffer-Tween [5], using a sterile glass rod. The homogenized solution was diluted, and then four 0.2 ml aliquots from the solution were individually spread on PDA plates. The number of colonies on each 4 plate/concentration was counted after three days of incubation at 25°C. All experiments were repeated three times. A corrected mortality, LT_{50}, and LC_{50} were calculated for all bioassays using Abbott's formula [1] and the probit analysis program [9]. The statistical analysis was subjected to ANOVA on the basis of the data generated from these experiments.

As shown in Table 1, the strains from Korea and USA belong to the small conidial type. All strains examined in this study showed the highest germination rate at 20–25°C, yet the temperature preferences for their conidial germination differed from each other (Fig. 1). In particular, CS-626 and CS-625 germinated more rapidly than the other strains at most temperatures. Among the five strains examined, the CS-626 strain showed the highest virulence at all temperatures (Table 2). The mortality of the CS-626 strain reached almost 100% at 25°C and 80% at 15 and 35°C on the 7th day. Comparisons of the germination rate and virulence among the strains clearly indicated that the CS-626 strain was the most durable and highly virulent at a broad temperature range. This result indicated that the CS-626 strain would be most suitable for Korean greenhouses, where the temperature during the spring and fall seasons fluctuates from 20°C to 35°C [12].

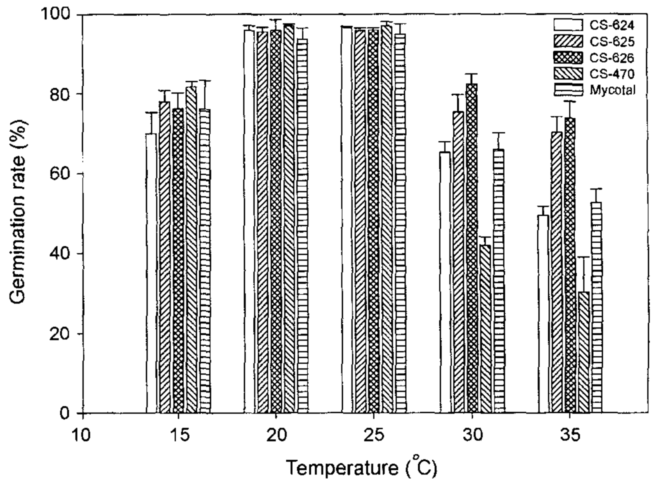

Fig. 1. Effect of temperature on conidia germination of *V. lecanii* strains after 12 h of incubation. Bar=SD.

An economic optimal conidial concentration of a candidate strain needs to be determined, as the virulence of the strain will change depending on the conidial concentration as well as the environmental conditions [2, 4]. Table 3 shows the effects of different concentrations of the CS-626 conidial suspension on the mortality of the whitefly scales. The LC_{50} was estimated as 2.3×10^6 conidia/ml. Treatments at $1\times10^3-10^7$ conidia/ml showed a relatively low mortality (PROC ANOVA; df=16; F=495.13; Pr>F=0.0001). However, approximately 100% mortality was induced at the concentrations of 1×10^9 and 10^8 conidia/ml after 7 and 9 days, respectively. The sharp increase in the mortality at

Table 1. Original hosts and geographical location of *V. lecanii* strains and their conidial size.

Strain	Original host	Conidial size (μm)		Geographical origin
		Length	Width	
CS-624	*M. persicae*	3.23±0.48	0.97±0.17	Suwon, Korea
CS-625	*M. persicae*	3.31±0.50	1.14±0.22	Suwon, Korea
CS-626*	*M. persicae* and *T. vaporariorum*	3.32±0.44	1.11±0.13	Imsil, Korea
CS-470	*T. vaporariorum*	2.89±0.43	1.09±0.25	U.S.A.

*This strain was observed infecting *M. persicae* and *T. vaporariorum* on the same leaf.

Table 2. Corrected mortality and LT_{50} in greenhouse whitefly scales inoculated with conidia of *V. lecanii* strains and maintained at different temperatures.

Strain	Temperature (°C)					
	15		25		35	
	Mortality[1]	LT_{50}[2]	Mortality	LT_{50}	Mortality	LT_{50}
CS-624	33.6±3.2b	9.7	87.5±5.5ab	4.5	43.4±14.9b	7.5
CS-625	21.3±12.7b	10.0	72.3±1.8bc	5.2	52.0±13.6b	7.1
CS-626	72.5±11.8a	5.6	98.2±1.4a	3.7	81.8±12.0a	5.5
CS-470	22.7±3.5b	12.3	65.0±10.3c	6.2	34.1±7.8b	9.3
M*	33.9±5.5b	8.0	64.2±23.8c	5.4	48.2±9.6b	6.6

[1]Corrected mortality (%) 7 days after inoculation. Means within the same column followed by the same letter are not significantly different at P≤0.05, as determined by the ANOVA procedure and LSD test.
[2]Days.
*The strain isolated from Mycotal, a commercial mycopesticide.

concentrations of 10^8 and 10^9 conidia/ml may have resulted from large numbers of conidia adhering to a scale (Table 3). The LSD test failed to show a difference between the numbers of adhering conidia at 10^8 and 10^7 conidia/ml, because of the extremely large numbers of conidia at 10^9 conidia/ml. However, the difference in mortality between the two concentrations was evident. These results indicated that over 473 conidia of the strain needed to be adhered to a whitefly scale for effective control (Table 3). Even though the number of conidia adhering to a whitefly scale at a concentration of 10^9 conidia/ml was twice as high as that at 10^8 conidia/ml, the difference in the LT_{50} between the two concentrations was only one day (Table 3). Therefore, a spray application of a conidial suspension at a concentration of 10^8 conidia/ml seemed to be the most economical and effective way of controlling this pest. These data may not be directly applicable to a prediction of mortality in greenhouses due to the lack of data on each stage of whitefly scales. Nonetheless, they do serve as possible means to predict the approximate mortality and to investigate the uniformity of the spray application in the greenhouse. Further field studies in greenhouses are still required prior to the commercialization of the CS-626 strain as a microbial insecticide.

Table 3. LT_{50} for *V. lecanii* CS-626 strain tested against greenhouse whitefly scales, and number of conidia adhering to a single scale, with various concentrations of conidial suspension (mean±SD).

Concentrations (conidia/ml)	LT_{50} (days) and 95% confidence limits	No. of conidia adhering to a scale*
10^9	5.1 (3.6–6.7)	991.67±119.62a
10^8	6.2 (6.0–6.5)	473.33±134.91b
10^7	8.1 (7.6–8.6)	118.89±59.54b
10^6	–	56.46±19.59c
10^5	–	48.33±26.53c
10^4	–	38.13±15.95c
10^3	–	34.38±0.88c

*Means within the same column followed by the same letter are not significantly different at P≤0.05, as determined by the ANOVA procedure and LSD test.

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