Biocontrol Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713409232

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To cite this Article Miller, T. C., Gubler, W. D., Laemmlen, F. F., Geng, S. and Rizzo, D. M.(2004) 'Potential for using *Lecanicillium lecanii* for suppression of strawberry powdery mildew', Biocontrol Science and Technology, 14: 2, 215 — 220
To link to this Article: DOI: 10.1080/09583150310001639204
URL: http://dx.doi.org/10.1080/09583150310001639204

Biocontrol Science and Technology (March 2004), Vol. 14, No. 2, 215–220

Taylor & Francis
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SHORT COMMUNICATION

Potential for Using *Lecanicillium lecanii* for Suppression of Strawberry Powdery Mildew

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(*Received 27 August 2002; returned 5 December 2002; accepted 30 May 2003*)

Augmenting native populations of the hyperparasite Lecanicillium lecanii *suppressed powdery mildew of strawberry, caused by* Sphaerotheca macularis *f. sp.* fragariae *in California field trials. Repeated sprays significantly reduced disease compared to the untreated controls for periods of the fruit production season, suggesting possible use as a partial, but not total disease control strategy.*

Keywords: Lecanicillium lecanii, Verticillium lecanii, Sphaerotheca macularis *f. sp.* fragariae, Sphaerotheca fuliginea, *strawberry, powdery mildew*

The fungus *Lecanicillium lecanii* (Zimmerm.) Zare & Gams, comb. nov. (= *Verticillium lecanii* (Zimmerm.) Viégas, Gams and Zare, 2001) is a known mycoparasite of powdery mildews (Verhaar *et al.*, 1996, 1997, 1998, 1999a,b; Askary *et al.*, 1998 (and references therein); Miller & Gubler, 1998; Miller *et al.*, 1999). Successful use as a biological control agent has been limited to greenhouse uses (Verhaar *et al*., 1996), most likely due to a requirement for high relative humidity (RH) for growth and sporulation of the fungus (Barson, 1976; Hall, 1980; Mendgen, 1981; Verhaar *et al.*, 1998, 1999a,b).

L. lecanii is parasitic on *Sphaerotheca macularis* (Wallr. ex Frier) Cooke f. sp. *fragariae*, the strawberry powdery mildew pathogen. Mycoparasitism has been observed in growth chambers and greenhouses under controlled conditions as well as in the field under natural conditions. Results from this previous study have been published (Miller & Gubler, 1998; Miller *et al.*, 1999), but length limits precluded presentation of complete disease progress curves. Additionally, field data has been included from a more recent trial. California strawberry fruit production is located along the Pacific coast, where RH is high during the

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ISSN 0958-3157 (print)/ISSN 1360-0478 (online)/04/020215-6
DOI: 10.1080/09583150310001639204

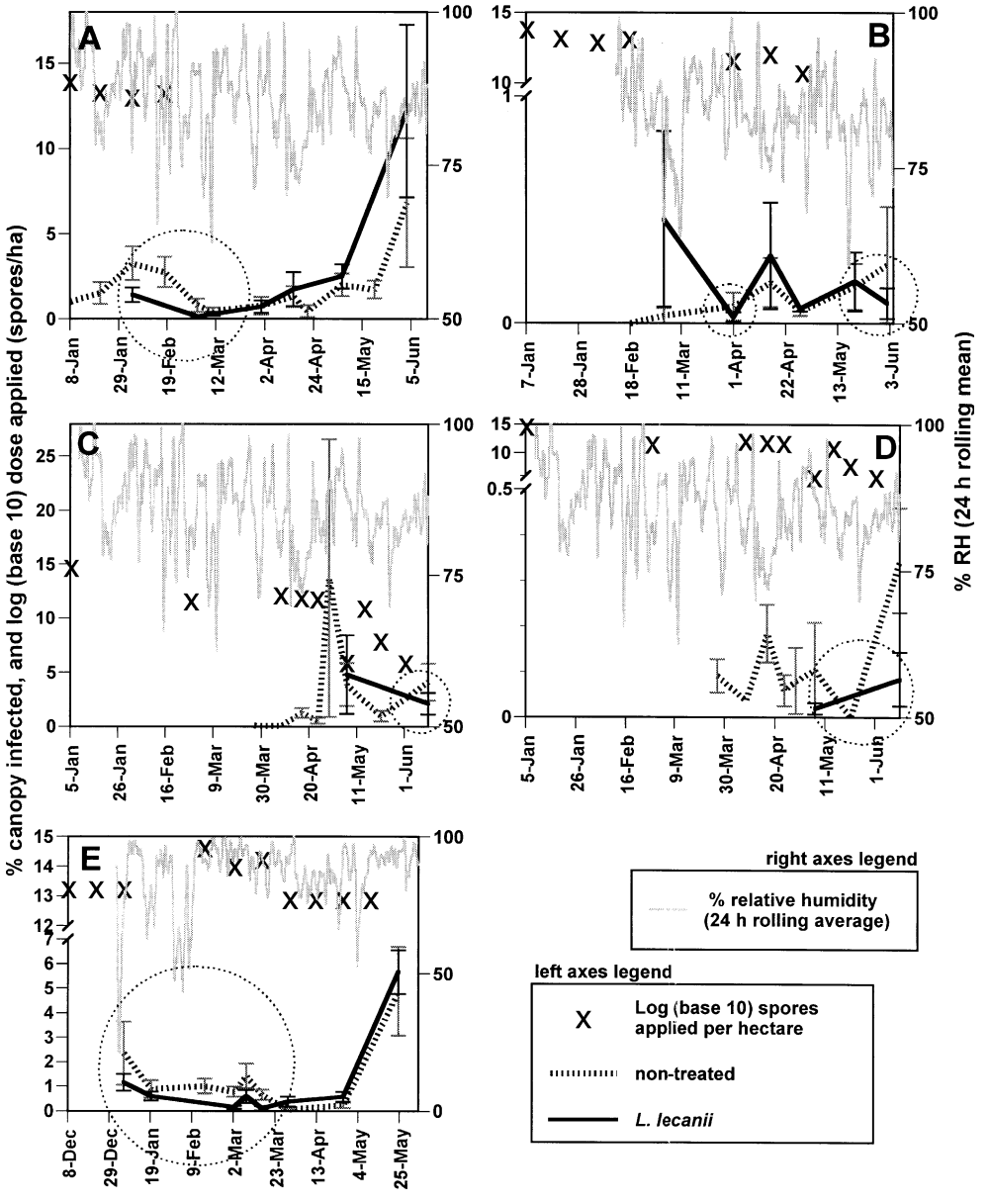

FIGURE 1. Disease progress curves comparing strawberry powdery mildew severity levels with and without application of *L. lecanii*. Circles highlight periods where *L. lecanii*-treated strawberries had less powdery mildew than untreated controls. Bars are mean ± SE. (A) Oxnard 1998, cv. Camarosa; (B) Santa Maria 1998, cv. Camarosa; (C) Watsonville 1998, cv. Pacific; (D) Watsonville 1998, cv.

growing season (Figure 1), which is conducive to growth of *L. lecanii*. The objective was to study the application methods of living spore suspensions of *L. lecanii,* and the effects of the applications on powdery mildew disease levels of strawberries grown in California fields.

Experiments used isolates of *L. lecanii* collected from plants maintained under saturated RH in both growth chambers and greenhouses at Davis, CA. Isolates used in field studies were taken from powdery mildew infected tissues collected in the summer at low-elevation nurseries in Manteca, CA. These isolates were maintained by periodic re-inoculation of pure cultures from potato dextrose agar (PDA, Sigma Chemical Co., St Louis, MO) to freshly established powdery mildew colonies maintained on strawberry plants in growth chambers. From these infected powdery mildew colonies, pure cultures of *L. lecanii* were re-established on PDA and the cycle was repeated. This periodic inoculation and re-isolation from the mildew host was to ensure that isolates retained mycoparasitic abilities.

All spore suspensions for field inoculations in 1998 were prepared as follows. Subcultures of *L. lecanii* were grown on PDA for 2–3 weeks at ambient laboratory conditions (23–25°C, diurnal lighting), after which spores were washed onto water agar (WA, Difco Laboratories, Detroit MI) and incubated for another 2–3 weeks. The latter media favoured abundant spore production with sparse mycelial growth. Spores were then washed from the agar surface, filtered through cheesecloth, quantified by means of a haemacytometer (Reichert Scientific Instruments, Buffalo, NY), and spore density was adjusted to desired concentrations for field use.

Spores used in 2001 were recovered and quantified for field use as described before, but growth was on hulled barley. Twice autoclaved (121°C, 101 kPa, 60 min, 6–12 h separation between cycles) pearl barley (distributed under various grocery labels by Fleming Companies, Inc., Oklahoma City, OK) was inoculated, incubated for 2–3 weeks, and then air-dried for an additional 1–2 weeks under aseptic conditions. Spores were washed from dried infested grain.

The field trials were conducted at one research and two commercial sites in California's central coast production fields where powdery mildew disease pressure is normally high. At the Watsonville research site, cultivars (cv.) Gaviota and Pacific (dug from high elevation, high latitude nurseries, Lassen Canyon Nursery, McDoel, CA) were transplanted on 21 October and 15 November, respectively, into raised beds spaced 132 cm from the centre with two plant rows/bed. Space between plants was 36 cm. At each of the two commercial sites, cv. Camarosa (Lassen Canyon Nursery, McArthur, CA) was planted into four rows/bed planting systems. For Oxnard, strawberries were dug on 13 October and transplanted into beds 173 cm from the centre, with plants spaced at 38 cm on 17 October. For Santa Maria, plants were dug on 17 October and transplanted into beds 163 cm from the centre, with plants spaced at 45 cm on 24 October. Irrigation was done by placing two drip tapes beneath the black (or clear for Oxnard) plastic mulch bed cover at all sites. Foliar *L. lecanii* treatments were applied at specific dose rates (see Table 1). Each treatment was replicated four times in plots (1.78×1.32 m) of 10 plants for each of the two cultivars in Watsonville. The same design was used at Santa Maria and Oxnard, but the plot sizes were 2.25×1.63 and 1.91×1.73 m, respectively, with 20 plants/variety for each replication. In 2001, another trial was conducted at Oxnard using cv. Camarosa. In this trial, 16 replicate plants were used at 43 cm in-row spacing (plot size 1.73×1.73 m).

Spore suspensions of *L. lecanii* were applied from a two-stroke backpack sprayer (Echo, Lake Zurich, IL) or a CO_2-powered (R&D, Opelousas, LA) applicator at a rate of 1870 L ha^{-1} using T-Jet® (Spraying Systems Co., Wheaton, IL) nozzles customized to planting practice. Application pressures ranged from 202 to 404 kPa across all sites. Amounts applied are shown in Table 1, and the concentrations ranged from approximately 5.0×10^3 to 5.0×10^7 spores mL^{-1}. Controls were untreated.

On site visits, trifoliate strawberry leaflets were selected randomly from within the plant canopy at a sampling density of one leaflet/plant. Samples were collected throughout the season at intervals shown (Figure 1). Microscopic evaluation followed sampling. Tissues were either rated or stained immediately, or cold-stored at 5°C for 1–10 days, a temperature found to halt lesion expansion effectively (Miller *et al.*, 2003). Leaves were immersed in

TABLE 1. Application doses and timing for field augmentation of the biocontrol agent *L. lecanii*

	Dose applied (spores ha^{-1})[a]				Dose applied (spores ha^{-1})[a]
Date of application (1998)	Oxnard (Camarosa)	Santa Maria (Camarosa)	Watsonville (Pacific and Gaviota)	Date of application (2001)	Oxnard (Camarosa)
				Dec 8 (2000)	1.9×10^{13}
				Dec 22 (2000)	1.9×10^{13}
Jan 5			6.4×10^{14}	Jan 5 (2001)	1.9×10^{13}
Jan 7		8.2×10^{13}			
Jan 8	9.1×10^{13}				
Jan 21	2.1×10^{13}	1.9×10^{13}			
Feb 4	1.1×10^{13}				
Feb 5		1.0×10^{13}			
				Feb 15	4.8×10^{14}
Feb 18	1.9×10^{13}	1.7×10^{13}			
Feb 27			5.3×10^{11}		
				Mar 2	1.1×10^{14}
				Mar 16	1.9×10^{14}
				Mar 30	8.4×10^{12}
Apr 1		5.1×10^{11}			
Apr 8			1.9×10^{12}		
				Apr 12	8.4×10^{12}
Apr 16		1.5×10^{12}			
Apr 17			1.1×10^{12}		
Apr 24			8.6×10^{11}		
				Apr 26	8.4×10^{12}
Apr 29		6.8×10^{10}			
May 7			1.1×10^{6}		
				May 10	8.4×10^{12}
May 15			1.1×10^{11}		
May 22			1.0×10^{8}		
June 2			1.0×10^{6}		

[a]Spray volumes ranged from 205 to 1870 L ha^{-1} depending on the method of application and inoculum availability. Lower volumes were used to apply *L. lecanii* at the highest concentrations possible when material was limited. For quick comparison, subtract 8 from the exponent to obtain spores cm^{-1}; example: 1.9×10^{13}spores ha$^{-1} = 1.9 \times 10^{5}$spores cm^{-1}.

aqueous cotton blue (methylene blue water-soluble, 1 g L^{-1}, Sigma Chemical Co.) for three or more days, and then washed under running tap water. The stained leaves were dried in an oven at 38–41°C. This was followed by microscopic evaluation, and marking of infection using either a dark permanent marker or white typewriter correction fluid. These images were scanned into a Macintosh computer and analyzed by the NIH Image Program (versions 1.60–1.61, http://rsb.info.nih.gov/nih-image). One-way analysis of variance (ANOVA) was used to compare the level of disease in the canopy for each field site.

Monitoring of on-site weather (RH, temperature, leaf wetness, and rainfall taken throughout the season at 15-min intervals) was provided by an Adcon® radio telemetry system (licensed through Western Farm Services, CA).

Each trial resulted in periods of control and lack of control of powdery mildew. The *L. lecanii* treatments reduced powdery mildew at all locations for some, but not all of the fruit production season. One-way ANOVA indicated significant ($\alpha = 0.05$) treatment differences in disease levels by application of the biological control agent on cv. Gaviota at Watsonville on 16 June 1998, and on cv. Camarosa at Oxnard on 2 March 2001. However, late season (April to June) powdery mildew levels were higher in the *L. lecanii*-treated plots at the Oxnard site in both 1998 and 2001.

Comparison of powdery mildew control with weather records showed that temperature was moderate, and ambient RH was usually $>70\%$ throughout the growing season, with drier conditions in mid-winter. RH data is shown (Figure 1) for comparison with control efficacy.

Other reports of RH limiting mycoparasitism of powdery mildew (of cucurbits, i.e., *Sphaerotheca fuliginea*, co-generic pathogen) in the glasshouse have been published (Verhaar *et al.*, 1998, 1999a,b). Tactics to accommodate these limits and strategies on selecting strains to overcome these limits were discussed by these authors.

Noting the high RH requirements for mycoparasitic and entomopathogenic activities by *L. lecanii* (Barson, 1976; Easwaramoorthy & Jayaraj, 1977; Hall, 1980a,b; Mendgen, 1981; Milner & Lutton, 1986), it was felt that the marine climate of coastal California, where commercial strawberry production is largely located, would be a favourable environment for survival of the fungus. This was confirmed by isolation of indigenous strains from these fields before the trials. It was noted that average RH was often $<70\%$ during periods where *L. lecanii*-treated strawberries had less powdery mildew than untreated controls (Figure 1). This might be explained by noting that the RH data presented is a 24-h rolling mean and does not show the range of fluctuation. That is, the graphed measure is the mean value derived from the 12 h before and after the plotted points, and is therefore less in its range compared to actual measured values over that same period. Each point of the graphed line is calculated from the mean of the 96 telemetry readings surrounding and including the plotted value. At all sites, there was near daily ($>95\%$ of days throughout the season) saturation (100% RH) for at least one or more readings, suggesting that 15 min of 100% RH was all that was required for survival of this hydrophilic mycoparasite.

The spores applied did not germinate except in the presence of powdery mildew (or some other fungus). Microscopy revealed that the applied spores were present in ungerminated form, except when they were within immediate (<100 μm) proximity to *S. macularis* f. sp. *fragariae* spores or colonies. This would suggest that re-application would be necessary to replace spores washed and weathered from the strawberry.

Review of the level of disease control with respect to the applied dose of *L. lecanii* is interesting. The data from Oxnard in 1998 (Figure 1A) showed control during the period and for a few weeks following the final application on 18 February. In Santa Maria (Figure 1B), inability to provide higher doses after 18 February might account for the low level of control seen during the latter part of the season. It is possible that levels applied at $<10^{13}$ spores ha^{-1} ($=$ ca. 5×10^6 spores mL^{-1}) are below a threshold for sufficient control, and the two periods of apparent control are within normal pathogen population variations. There was no statistically significant reduction in disease levels at this site alone, which effectively had no visible powdery mildew (detectable only by microscopy). Some significant control was observed at Watsonville in 1998 (Figure 1D) on cv. Gaviota (also with an insignificant field level epidemic), but not on cv. Pacific (Figure 1C), a variety which is more susceptible to powdery mildew. Although infection severity levels were reduced, the difference compared to the untreated control was not significant. Here again, ability to provide sufficient doses of *L. lecanii* in the latter season might account for less than optimal control. Finally, at Oxnard in 2001 (Figure 1E), good control was maintained for the early part of the season, with a significant reduction in disease levels on 2 March. However, as the applied dose level dropped in late March and April, so did control of powdery mildew.

Overall, sufficient evidence is observed in this study that the disease can be successfully suppressed when frequent applications were maintained above a threshold of 10^{13} spores ha^{-1} to warrant further experimentation with *L. lecanii*.

L. lecanii already can be found parasitic on strawberry powdery mildew throughout California fruit production and low elevation nursery production from summer through to autumn. Collection of *S. macularis* f. sp. *fragariae* free of *L. lecanii* after September is difficult. Augmenting these native populations earlier in the year could tip the ecological

balance in favour of the mycoparasite earlier in the year when growers can benefit more from this natural control.

ACKNOWLEDGEMENTS

Appreciation is noted for the generous co-operation of the growers who participated in these studies at Conroy Farms, Camarillo, CA, and also D & B Specialty Farms, Santa Maria, CA. Thanks also to the California Strawberry Commission for generous research support and management of the Watsonville, CA research site. Additionally, appreciation to NatureChem, LLC, Woodland, CA is due for supporting research into dry-substrate production of the mycoparasitic biocontrol.

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