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Short communication

Control of *Leveillula taurica* in tomato by *Acremonium alternatum* is by induction of resistance, not hyperparasitism

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Abstract

Spores of the hyperparasite *Acremonium alternatum* reduced powdery mildew infection by *Leveillula taurica* on greenhouse tomato. The effect was slightly increased when spores were applied killed, and therefore not due to direct parasitism. The effect was systemic, protecting untreated leaves above the treated ones. Spores killed by heat had more effect than when killed by UV, so the effect was presumably due to induction of host resistance by substances released when cells were heat killed. The size of the effect depended upon leaf age and level of infection. Effects on primary infection and expansion of successful infections appear to be under independent control.

Powdery mildew caused by *Leveillula taurica* is a serious disease of tomato in the Mediterranean (Diop-Bruckler and Molot, 1987; Palti, 1988) the USA and Canada (Guzman-Plazola, 1997; Cerkauskas et al., 2000). The pathogen propagates asexually on tomato and it affects only the leaves. Hyphae develop endophytically and conidiophores emerge through stomata. Severe infection results in necrotic lesions coalescing and the whole leaf blade collapsing (Palti, 1988).

Because of the threat that resistance to fungicides used will evolve and also to offer a non-chemical alternative to organic growers, research turned towards studying potential biocontrol agents, including hyperparasites. *Acremonium* spp. have been reported to act as hyperparasites on several powdery mildews (Kiss, 2003), although not previously on *L. taurica*. In Crete, an isolate of *Acremonium alternatum* was isolated from a visibly parasitised thallus of *Podosphaera xanthii*, on cucumber (Malathrakis, 1985). The pure isolate was shown to be infectious on *P. xanthii* and was deposited in the American Type Culture Collection (ATCC).

The initial aim of our experiments was to study *L. taurica* epidemics and at the same time test *A. alternatum* as a biocontrol agent on greenhouse tomato. In practice many putative fungal antagonists of powdery mildews are thought to act by induction of resistance rather than parasitism or antibiosis (Belanger and Labbe, 2002). In our experiments, to separate hyperparasitic effects from passive ones we sprayed greenhouse tomatoes with dead as well as living spores of *A. alternatum*. Further experiments with dead *A. alternatum* spores demonstrated that the effect was systemic and affected both lesion expansion and the proportion of successful infections.

For the experiments, tomato seeds F1 hybrid 'Manthos' GC 785, Thiram treated, S&G

Netherlands) were used. Plants were transplanted at the 3–5 true leaf stage. *Acremonium alternatum* (ATCC no. 60645) was cultured on PDA and incubated at 25 °C and 12 hL/12 hD for 7 days before use. To prepare conidial suspensions, spores of *A. alternatum* were washed from the Petri dishes, filtered and adjusted to the required concentration using a haemocytometer. Conidia were killed by: (a) autoclave: at 1 Bar above atmospheric pressure and 121 °C for 15 min (b) heating in an oven: at 60 °C for 30 min or (c) exposure to UV radiation (UVC/HMS/30W/OFR; Osram, Munich) in a 10 cm deep transparent plastic container.

In the greenhouse the experimental design was randomised blocks (replicates). In the first experiment (experiment 1), there were four replicates and treatments were randomly assigned between plots, each of 18 plants. Plants were infected naturally and uniformly (statistics not shown) by developing epidemics of *L. taurica*. The following treatments were sprayed at 7-day intervals starting three weeks after transplanting: Live *A. alternatum*, at 10^7 spores ml^{-1}, live *A. alternatum* at 10^6 spores ml^{-1}, autoclaved *A. alternatum* at 10^6 spores ml^{-1}, and water as control. The proportion of infected area per leaf, denoted y, on leaves 1–12 of the three central plants in each plot was estimated visually at 7-day intervals. As usual in epidemiological work, data were logit transformed assuming an asymptote of 100% and regressed on time by simple linear regression to estimate the intercept $l_0 = (\ln [y_0/(1-y_0)])$ and rate of disease increase (r) on each leaf. A new variate 'T50' was calculated by solving the logit transformed equation for $y = 0.5$ to obtain the formula l_0/r representing the time needed for disease to reach 50% severity. Treatments were compared by using an appropriate set of orthogonal comparison contrasts to subdivide the treatment sum of squares in ANOVA (Mead et al., 2003). Comparisons, showing the coefficients for each treatment mean in the order: *A. alternatum* alive (10^6), *A. alternatum* alive (10^7), *A. alternatum* autoclaved (10^6) and water, were (a) All *A. alternatum* treatments compared to water (1, 1, 1, −3), (b) *A. alternatum* alive at 10^6 ml^{-1} compared to 10^7 ml^{-1} (1, −1, 0, 0), (c) All treatments with *A. alternatum* alive compared to autoclaved (1, 1, −2, 0).

Infection by *L. taurica* occurred simultaneously and fairly uniformly across the experimental area. There were no trends in the effects on different leaves. R^2 of the fit of epidemic curves was on average 92%, with an inter-quartile range from 85 to 95%. *Acremonium alternatum* on average increased T50 by 5 d (1 df contrast, $P < 0.001$, Figure 1A), a roughly 10% increase over the control value of 44 days. There were no significant differences in T50 between the two doses of live *A. alternatum* spores (1 df contrast, $P = 0.16$) but the autoclaved suspension delayed T50 by 5 days more than the living suspensions (1 df contrast, $P < 0.001$; Figure.1A). Rates were not detectably affected by the treatments (Figure 1B).

In view of these results, in a second greenhouse experiment (experiment 2) the following treatments were applied every 7 days, starting 5 days after transplanting: live *A. alternatum*, *A. alternatum*

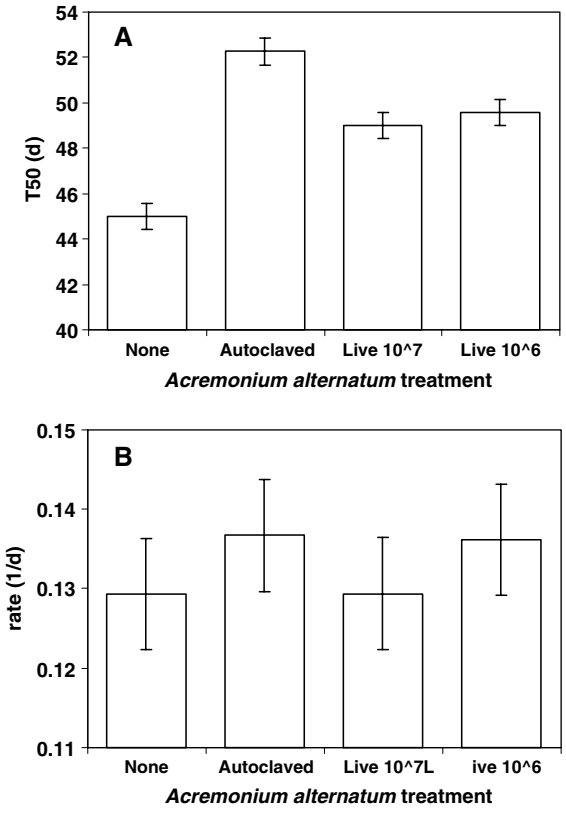

Figure 1. Average values of time to reach 50% severity (T50) (A) and logistic rate of change (B) of *Leveillula taurica* severity on leaf layers 1–12 of greenhouse tomatoes treated with either autoclaved spore suspension of *Acremonium alternatum* or one of two doses of live spore suspension. Error bars represent ±2 SEM based on the between leaf component of variance.

autoclave-killed, *A. alternatum* oven-killed, *A. alternatum* UV-killed and water as control. All spore suspensions contained 10^6 spores ml^{-1}. There were six replicates and treatments were randomly assigned between single plants in each block. The contrast coefficients used to subdivide the ANOVA treatment sum of squares, in the order: *A. alternatum* autoclaved, *A. alternatum* oven, *A. alternatum* UV, *A. alternatum* alive, water, were (a) All *A. alternatum* treatments compared to water (1, 1, 1, 1, −4), (b) *A. alternatum*-killed compared to alive (1, 1, 1, −3, 0), (c) *A. alternatum* heat-killed compared to UV-killed (1, 1, −2, 0, 0), (d) *A. alternatum* autoclaved compared to oven-killed (1, −1, 0 ,0, 0).

Infection by *L. taurica* was reasonably uniform. There were no obvious trends in effects across leaves. Average R^2 for the logistic fits was about 93%. *Acremonium alternatum* treatments increased T50 by up to 100% (Figure 2A). Autoclave killed *A. alternatum* had more effect than live spores (and was also more consistent over leaves). Autoclaved and oven-killed spores increased T50 by approximately 20 days more than UV-killed (1 df contrast, $P < 0.001$), and autoclaved increased T50 by about 20 days more than oven-killed. Effects on rates were moderate, but the overall effect of all *A. alternatum* treatments was a reduction in rate of about 20% (1 df contrast, $P = 0.01$); again autoclaved spores had the largest effect (Figure 2B).

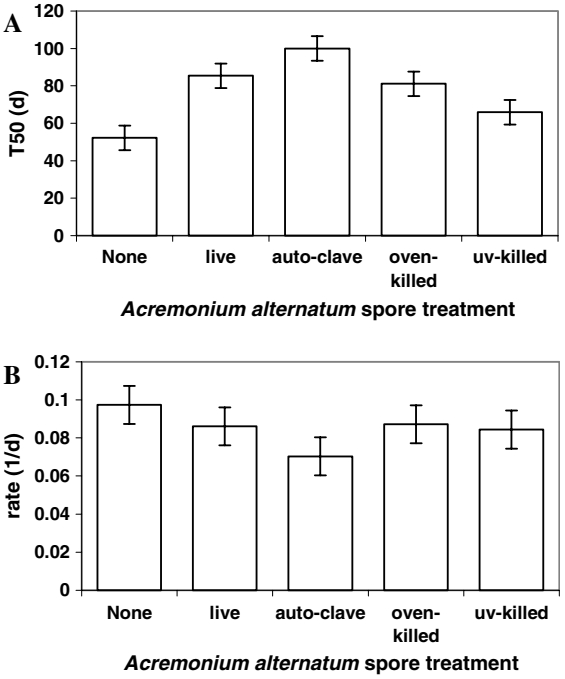

Figure 2. Average values of time to reach 50% severity (T50) (A) and logistic rate of change (B) of *Leveillula taurica* severity on leaf layers 1–12 of greenhouse tomatoes treated with *Acremonium alternatum* spore suspensions, either live or killed in various ways. Error bars represent ±2 SEM.

A third experiment (experiment 3) confirmed the systemic effect of *A. alternatum*. Two identical C.E. chambers (Conviron EF7, Canada, 125 × 60 × 110 cm) at 25 °C and 12/12 hL with 4 pairs of 60 W fluorescent lamps and 460 W incandescent bulbs were used. Tomato plants at the 4th true leaf stage were transplanted into plastic pots (10 × 12 cm) and inoculated with *L. taurica* (10^4 spores ml^{-1}) by spraying the leaves to run-off through a spray gun (Humbrol, UK). Plants were placed in three rows of five. Five treatments were applied, each to one plant in a row. The treatments were the factorial combinations of *A. alternatum* suspensions sprayed to run-off 7 and 2 days before artificial inoculation with *L. taurica* to either the 1st leaf or the 1st and the 3rd leaves, plus a water sprayed plant as a control. The number of disease lesions and percentage of infected area, estimated visually, were recorded on each leaf when symptoms were first seen (12 days after inoculation) and 5 days later. A new variate 'size' was calculated by the formula: size = severity/number of lesions, and log transformed for analysis. The number of lesions was analysed untransformed following preliminary tests. Treatments were again compared using orthogonal contrasts in ANOVA. Contrasts, showing the coefficients used for each treatment mean in the order: *A. alternatum* on leaf 1 and 3 at −7 days, leaf 1 and 3 at −2 days, leaf 1 at −7 days, leaf 1 at −2 days and water control were (a) All *A. alternatum* treatments compared to water (1, 1, 1, 1, −4), (b) All day 7 *A. alternatum* treatments compared to day 2 (1, −1, 1, −1, 0), (c) Treatment of leaf 1 compared to treatment of leaves 1 + 3 (1, 1, −1, −1, 0), (d) Interaction between 'day of treatment' and 'leaves treated' (1, −1, −1, 1, 0). Contrasts were expressed as a percentage of the unsprayed control value.

Assessments at 12 and 17 days gave very similar results. On average treatment with *A. alternatum* halved the number of *L. taurica* lesions appearing ($P < 0.001$, Figure 3A). Application 7 days before

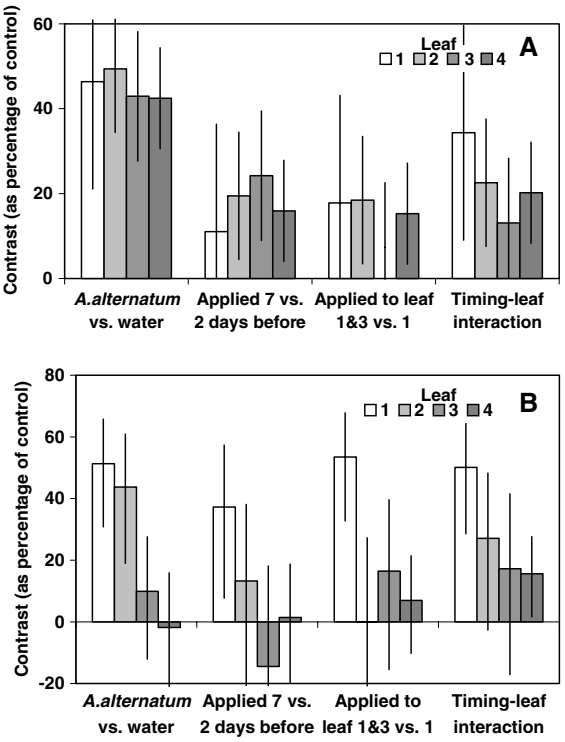

Figure 3. Estimates of contrasts between treatments for the number of *Leveillula taurica* lesions (A) and size of lesion (B), on leaves 1–4 of tomato plants grown at 25 °C and inoculated on leaf 1 or leaves 1 and 3 with autoclaved spores of *Acremonium alternatum*, 2 or 7 days before inoculation with *L. taurica*. Measurements made 17 days after inoculation with *L. taurica*. Contrasts are expressed as percentages of the water control: Error bars ±2 SEM.

inoculation gave a slight further reduction in numbers compared with application 2 days before inoculation ($P < 0.05$, Figure 3A). Numbers were reduced as much on leaves not directly treated as on those directly treated (Figure 3A). Size of *L. taurica* lesions was approximately halved on leaves 1 and 2 sprayed with killed *A. alternatum* spores ($P < 0.01$, Figure 3B). On leaf 1, there was a further effect from treatment of both leaves 1 and 3 at 7 days before inoculation, but on leaf 2 this made no difference. On leaves 3 and 4, neither timing nor leaf sprayed altered the effect on lesion size (Figure 3B).

In experiments 1 and 2, treatment with *A. alternatum* substantially prolonged the time taken by *L. taurica* to reach 50% infection of a leaf layer and often reduced the rate of disease development on leaves. The effect was relatively greater in experiment 2, in which disease development rates were lower (about 0.09 day^{-1} in the control, compared to about 0.13 day^{-1} in the control of experiment 1) which agrees with observations of other authors on induced resistance in solanaceous crops (Ozeretskovskaya, 1995). The effect was greater with killed preparations, showing that it must have been due to induction of resistance rather than parasitism of the powdery mildew. The inducing factor appeared to be heat-stable and perhaps released on breakdown of the cells, because effects were greater with heat-killed (autoclave and oven) than UV-killed preparations.

Few studies have investigated the effect of age on the ability of a plant/plant part to produce pathogenesis-related (PR) proteins or other components of induced systemic resistance (Heil, 2001). In our experiments *A. alternatum* effects and probably intrinsic susceptibility varied substantially with leaf age. On the older leaves 1 and 2, the main reduction was in *L. taurica* lesion expansion, while on the younger leaves 3 and 4, lesion numbers were reduced but expansion was hardly affected (Figure 3).

There have been reports of non-pathogens acting as inducers of resistance against powdery mildews (Reuveni and Reuveni, 2000) but to the best of our knowledge, this is the first report of a hyperparasite effective in biocontrol of powdery mildews acting as an inducer of resistance.

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