In vitro antagonism of *Acremonium mucronatum* against *Diplodia mutila*

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1. Introduction

Among the fungi that are of interest in relation to the problem of oak decline in Italy, two species, *Diplodia mutila* and *Acremonium mucronatum*, seem particularly important, though for different reasons.

D. mutila is frequently found in declining oaks, and its pathogenicity has been experimentally demonstrated (2,12 and 16). It can therefore be considered one of the contributing factors to the decline and death of oaks (7).

By contrast, *A. mucronatum*, as well as other *Acremonium* species, are significantly more common on healthy than on declining trees, and where these species are found, *Diplodia mutila* is much less frequent (10).

The genus *Acremonium* is known to include a number of species that have antagonistic or hyperparasitic effects on pathogens of woody and herbaceous plants. Such effects have been observed *in vitro* as well as *in vivo*.

In view of these data, the present *in vitro* study was undertaken to verify the antagonism of *A. mucronatum* against *D. mutila*.

2. Materials and methods

Antagonism tests were carried out with *A. mucronatum* mycelium and with conidial suspensions. Both types of inoculation were made on PDA at 20°C and pH 6.5, conditions that have been found to be optimal (13).

To test the antagonism potential of *A. mucronatum* mycelium, plugs of mycelium from both fungi were grown in dual cultures.

Five mother colonies from each fungus were cultured to supply mycelium. Forty 9-cm diameter Petri dishes, containing 20 ml of PDA culture medium each, were inoculated in dual culture with 0.5 cm diameter plugs of mycelium, one from *A. mucronatum* and one from *D. mutila*, placed at opposite sides of each dish along a diameter.

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Since *A. mucronatum* grows more slowly than *D. mutila*, it was inoculated seven days earlier to give it the time to develop a fair sized colony by the time of contact.

In 20 of the dishes an increment core of oak wood was placed between the two mycelia to test fungal behaviour in the presence of oak woody tissue. The oak cores (diameter 0.5 cm, length 3 cm), were extracted with an increment borer and autoclaved before use.

The control dishes with PDA as above, were inoculated only with *D. mutila*; to five of these an oak core was also added. The progress of *D. mutila* growth along the diameter toward *A. mucronatum* was measured in the ten days after inoculation.

To test antagonism of conidial suspensions, mycelium was prepared in mother colonies as above. After six days, 52 Petri dishes containing 20 ml PDA each were inoculated with *D. mutila* inoculum. At the same time conidial suspensions of *A. mucronatum* mother colonies were prepared at the following seven concentrations: 1 600 000, 800 000, 400 000, 200 000, 100 000, 50 000 and 25 000 conidial/ml.

Inoculation with conidia followed immediately in two ways. Twenty one of the Petri dishes received a drop of conidial suspension directly on the *D. mutila* mycelium (3 dishes per concentration), and in 21 dishes 0.5 cm wells were made in the culture medium at the four compass points using a sterilized needle, and these wells were filled with enough conidial suspension to permit absorption into the surrounding culture medium. Also with this approach, three dishes were inoculated per concentration.

The remaining nine dishes were the controls: six received distilled (and sterilized) water instead of the conidial suspensions (placed on the *D. mutila* fragment in three dishes; poured in wells on the other three), and in the last three *D. mutila* was allowed to grow without any manipulation. All tests were carried out in duplicate.

3. Results

In the mycelium tests, *D. mutila* was inoculated some time after *A. mucronatum* to compensate for its faster growth; in spite of that, by the time the Petri dishes were almost entirely covered by mycelium, *D. mutila* occupied 60-70% of the dish surface. It was noted that from the start *D. mutila* growth was always somewhat slower in the presence of *A. mucronatum* than when it grew unchecked in the control dishes. After about seven days, when the mycelia of the two fungi made contact, an advancing front formed in the shape of a series of halos in the area colonized by *D. mutila*, signalling the continuing advance of *A. mucronatum*. As soon as contact was made between the two mycelia, *D. mutila* ceased all growth; by contrast *A. mucronatum* continued to advance until it entirely covered the whole dish, undergrowing the *D. mutila* colony. The oak core clearly stimulated *D. mutila* growth with or without the presence of *A. mucronatum*, but did not affect *A. mucronatum* antagonism (Fig. 1).

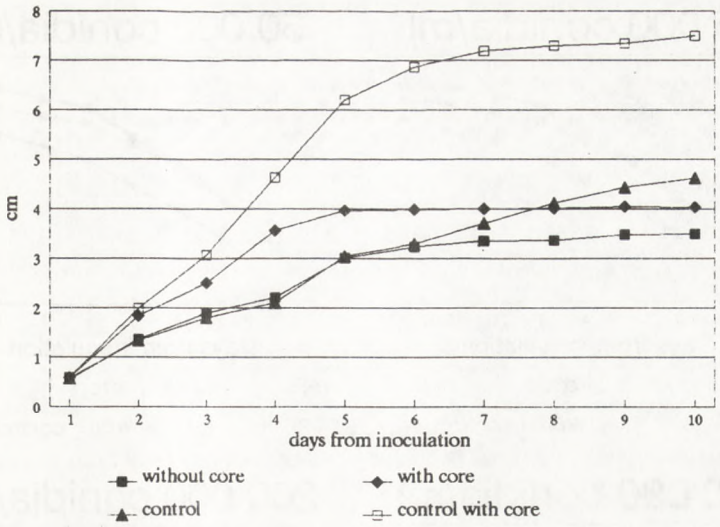


Fig. 1. Growth of *Diplodia mutila* with *Acremonium mucronatum*.

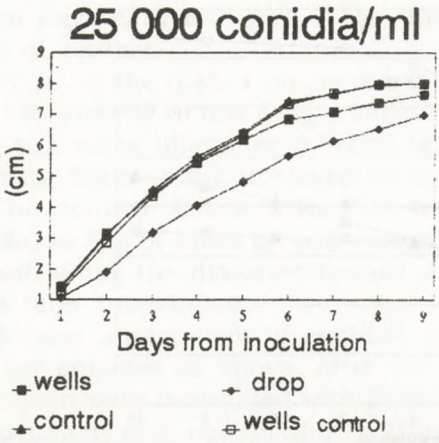
Also with the conidial tests *D. mutila* initially grew and produced its usual copies mycelium. The presence of *A. mucronatum* conidial suspensions, whether introduced directly on *D. mutila*, or into wells, made no difference at any concentration for the first three days, that is until the conidia germinated. At the point, however, fronts were formed and *A. mucronatum* advanced against *D. mutila* in all 42 Petri dishes, in the same way as noted in the mycelium tests above.

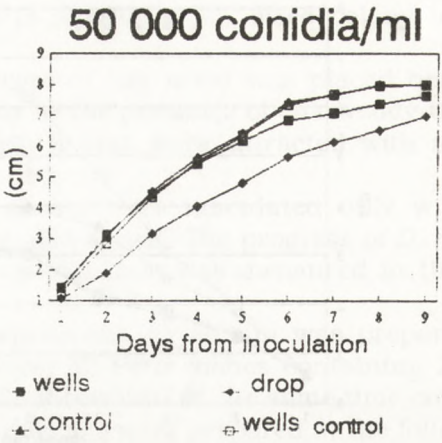
D. mutila growth was slower in the presence of *A. mucronatum* conidia than when it grew alone in the controls. The strongest inhibitory effect was achieved with a concentration of 200 000 conidia/ml placed directly on the *D. mutila* mycelium (rather than in wells) (Fig. 2).

4. Discussion and conclusions

The fact that *D. mutila* growth was much faster than *A. mucronatum* meant that even though *A. mucronatum* had been given a head start, *D. mutila* still managed to occupy considerably more than a half of the available medium before contact was made.

Fast growth is not the only factor that makes a pathogen aggressive, but it conferes an advantage in host colonization in that it may enable the pathogen to forestall or elude the defence mechanisms of the host. Though *D. mutila* initially had the advantage of faster growth, this advantage was nullified since *A. mucronatum* stopped *D. mutila* growth at the moment of contact and then proceeded to undergrow the other fungus completely.


25 000 conidia/ml
(cm)
Days from inoculation
wells
drop
control
wells control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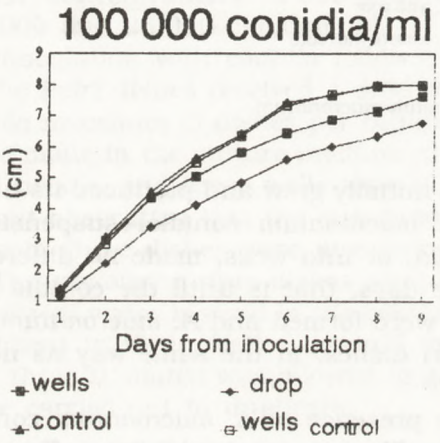
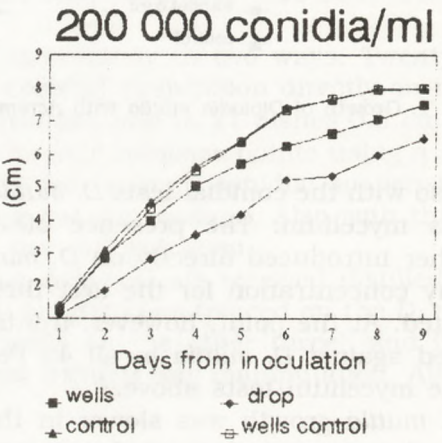
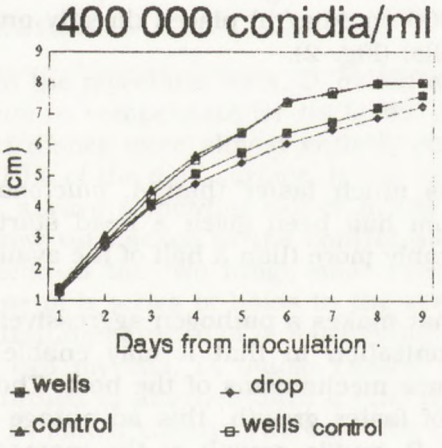
(cm)
Days from inoculation
wells
drop
control
wells control


100 000 conidia/ml
(cm)
Days from inoculation
wells
drop
control
wells control


200 000 conidia/ml
(cm)
Days from inoculation
wells
drop
control
wells control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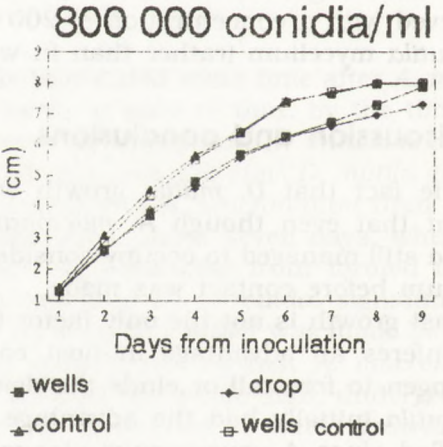
(cm)
Days from inoculation
wells
drop
control
wells control


800 000 conidia/ml
(cm)
Days from inoculation
wells
drop
control
wells control

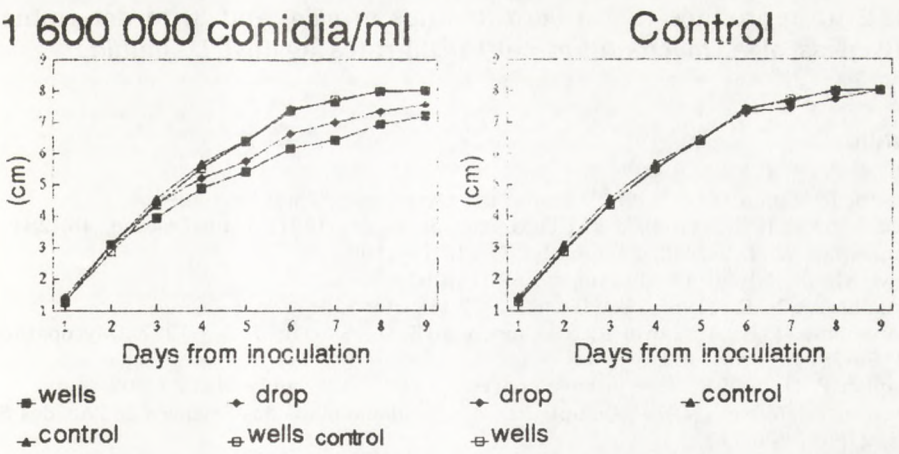


Fig. 2. Growth of *Diplodia mutila* with *Acremonium mucronatum* at the seven different conidial suspensions.

The *in vitro* inhibition of *D. mutila* by *A. mucronatum* is consistent with earlier field studies which indicated that the two fungi are not found together on oak (10), and the data suggest that *A. mucronatum* could well be a factor limiting the spread of *D. mutila* in nature.

The antagonistic activity of many *Acremonium* species is already well established: Morquet and Touvet (8) reported that *A. crotocinigenum* has an antagonistic effect against *Armillaria mellea*; Lewis (4) found that *Cephalosporium* (= *Acremonium*) *diospyri* Crandall inhibited *Botryodiplodia theobromae* Pat., which causes cancer on *Platanus occidentalis*; Bharat and Singh (1) observed that *Acremonium roseogrisum* (Saksena) W. Gams metabolities inhibited *Alternaria brassicae* (Berk.) Sacc. on *Brassica campestris* L.; White and Cole (17) reported that *Acremonium coenophialum* Morgan-Jones & Gams, an endophyte of *Festyca arundinacea* Schreber, conferred resistance against *Alternaria alternata* (Fr.) Keisser, *Cladosporium cladosporioides* (Fres.) de Vries, and *Rizoctonia cerealis* Van der Hoeven; Manandhar et al. (6) noted an antagonist effect of various *Acremonium* spp. against parasites on soybean and Janisewicz (3) reported that *Acremonium breve* (Sukapure & Thirumalacer) W. Gams inhibited *Botrytis cinerea* Pers. ex Fr.

Equally well established is the hyperparasitism of *Acremonium* spp.: *A. aranearum* Pech. on *Puccinia graminis* f.sp. *tritici* (Eriks and Hemm) (9); *A. sordidulum* W. Gams and D. Hawks on *Colletotrichum dematium* (Pers ex Fr.) Grove f. *tunicata* (Schw.) von Arx (14); and *A. alternatum* Lince. Fr. on *Sphaerotheca fuliginea* (Schlecht. ex Fr.) Poll. (5).

Finally, Subraimanian (15) reported that *Acremonium* spp. also produce antibiotics such as cephalosporines and isoprenoids.

In view of our earlier field observations and the findings of the present

study, further research is being undertaken: 1) into the biology of both fungi; 2) to reproduce the *in vitro* findings *in vivo*; and 3) to determine the effectiveness of *A. mucronatum* culture filtrates against *D. mutila*.

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Antagonistyczne reakcje *Acremonium mucrenatum* przeciw *Diplodia mutila* w badaniach *in vitro*

Streszczenie

Obecność grzyba *Acremonium mucronatum* na dębach jest często powiązana z jego antagonistycznym działaniem w stosunku do grzyba *Diplodia mutila* wywołującego choroby u wielu gatunków dębów. W warunkach *in vitro* wykonano antagonistyczne badania hodując oba grzyby na pożywce PDA.

A. mucronatum testowano w różnych stężeniach zarówno w formie grzybni jak i wodnej zawiesiny zarodników konidialnych. Zawiesiny zarodników były wprowadzane w formie kropel bezpośrednio na powierzchnię grzybni *D. mutila* lub do wyciętych w pożywce wklęśnięć o średnicy 0,5 cm.

Hamujące efekty w rozwoju patogena były obserwowane w obu rodzajach doświadczeń. Wyraźny antagonistyczny efekt uwidaczniał się w przypadku bezpośredniego kontaktu dwóch grzybni. Następowało wtedy wyraźne zahamowanie patogenicznego grzyba *D. mutila*, podczas gdy grzyb *A. mucronatum* kontynuował normalny rozwój przerastając *D. mutila*, aż do całkowitego zarośnięcia powierzchni pożywki. Wydaje się, że *A. mucronatum* może być skutecznym grzybem w biologicznym zwalczaniu patogenicznego grzyba *D. mutila*.

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