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# Isolation and identification of hyperparasitic fungi associated with Erysiphaceae

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**Abstract**

In the vicinity of Bonn a limited survey was conducted on the occurrence of hyperparasites on powdery mildew species on some Papilionaceae. Eight parasitic fungi were isolated among which *Dissoconium aciculare,* a hitherto unknown deuteromycete.

*Additional keywords:* powdery mildew, hyperparasitism.

## Introduction

Hyperparasitism, the phenomenon that one fungus parasitizes another, is rather widespread in nature. The subject has been reviewed by Barnett (1963, 1964), Boosalis (1964), Barnett and Binder (1973), Cooke (1977) and most recently and comprehensively (though only dealing with imperfect hyperparasites) by Hawksworth (1981).

Though hyperparasitism is a rather common phenomenon, the hyperparasites reported from Erysiphaceae are few in number. The most common species is *Ampelomyces quisqualis* Ces., which is almost exclusively restricted to Erysiphales. The few other hosts are species of Mucorales and *Plasmopara.* Other parasites listed by Hawksworth are *Acremonium byssoides* W. Gams & Lim and *Aphanocladium album* (Preuss) W. Gams which is mainly myxomyceticolous. Furthermore, *Cladosporium oxysporum* Berk. & Curt. and *Verticillium lecanii* (Zimm.) Viégas are reported to have adverse effects upon powdery mildews (Hawksworth, 1981).

During a study of powdery mildews on Papilionaceae in autumn 1981 a hitherto undescribed hyperparasite *Dissoconium aciculare* (De Hoog et al., 1983) was discovered. This stimulated the search for other fungi parasitizing Erysiphaceae. In addition to this fungus, seven other fungal species, all necrotrophic, were found to colonize powdery mildews.

## Materials and methods

Leaves of some Papilionaceae, predominantly *Medicago lupulina* and *Lupinus polyphyllus,* infected with powdery mildews, mainly *Erysiphe martii,* were collected in the field in the vicinity of Bonn at regular intervals and inspected for hyperparasites

either at once or after some days incubation in petri dishes in a climate chamber with artificial light at 20°C. Hyperparasites were isolated from infested mildew colonies by plating infected material onto water agar containing 200 μg streptomycin per ml and incubation at 23°C. After a colony had been formed, mycelium was transferred to malt agar and maintained on this medium.

To check pathogenicity of the isolated fungi mycelial disks of the suspected hyperparasites were placed on powdery mildew-infected leaves (mainly cucumber leaves of ± 10 cm diameter) near the petiole and incubated in petri dishes in a climate chamber with artificial light at 20°C. Five replicates per tested fungus were used.

A fungus was considered to be pathogenic when conidia and mycelium were completely destroyed under the conditions of the experiment. In due time fungi were reisolated from destroyed conidia at a distance of about 5 cm from the inoculation place and compared with the original isolate.

## Results

*Internal parasites.* During the survey on the occurrence of parasites of powdery mildews several fungi were isolated (Table 1). *Ampelomyces quisqualis* Ces., a fungus with intramycelial growth, was most frequently observed in the present investigation. It seems to be the most common and exclusive parasite of Erysiphales though it has been reported to infest species of Mucorales and *Plasmopara* also (Hawksworth, 1981). Because of the attention already paid to this fungus (Blumer, 1967; Beuther et al., 1981) it was not investigated further.

*Tilletiopsis minor* Nyland was discovered as a parasite with intraconidial growth. This basidiomycete yeast was isolated from *E. martii* on *L. polyphyllus.* It was pathogenic to sporulating mycelium of *Microsphaera alphitoides* on oak and was reisolated from it. In greenhouse experiments the fungus showed promising results in the biological control of *Sphaerotheca fuliginea* on cucumber (Hijwegen, to be published). The fungus produced a tremendous amount of banana-shaped ballistospores, forming numerous sub-colonies in culture. Growth on malt agar was rather

Table 1. Hyperparasites isolated from Erysiphaceae.

| Hyperparasite | Host fungus |
|---|---|
| *Acrodontium crateriforme* | *Erysiphe pisi*[1] and *Pseudopeziza trifolii* on *Medicago lupulina* |
| *Ampelomyces quisqualis* | *Erysiphe* spp. on *Lupinus, Medicago, Trifolium* |
| *Aphanocladium album* | *Sphaerotheca fuliginea* on *Cucumis sativus* |
| *Dissoconium aciculare* | *E. pisi*[1] on *Medicago lupulina* |
| *Paecilomyces farinosus* | *E. martii* on *Lupinus polyphyllus* |
| *Ramichloridium apiculatum* | *E. pisi*[1] on *Medicago lupulina* |
| *Tilletiopsis minor* | *E. martii* on *Lupinus polyphyllus* |
| *Trichothecium roseum* | *E. martii* on *Lupinus polyphyllus* |

[1] According to Blumer (1967) *Medicago lupulina* is parasitized by *E. pisi.* Since no perithecia were found, the identity of our isolate could not be verified unequivocally.

slow. It was conspicuous that even under favourable conditions the hyphae became very soon emptied, protoplasm usually only being present at the tips of the hyphae.

*Contact parasites.* *E. pisi* on *M. lupulina* was parasitized by *Dissoconium aciculare* a fungus not recorded before. A full discription of this species of a new genus is given by De Hoog et al. (1983). The fungus was pathogenic to sporulating mycelium of *E. martii* on *L. polyphyllus* and was reisolated from it. It was also parasitic to *E. betae* on *Beta vulgaris.* In culture it was lysed and subsequently overgrown by *Fusarium oxysporum* and *Penicillium* cf. *variabile,* both possibly being parasitic. Growth on malt agar and oat meal agar (as well as on the host fungus) was rather slow. The aerial mycelium was woolly. Germination hyphae often did not grow horizontally on the agar plate, but negatively geotropically, ascending.

The mycelium on agar had a very strong tendency to anastomosis. Later on the mycelium was fragmenting to yeast-like particles, sometimes cemented together with all the transition states to true sclerotia. On the natural substrate 'spore-bridges' were formed, arising from germinating conidia still attached to the conidiophores, contacting with other hyphae or powdery mildew mycelium thus providing an elaborate network. Growth was only very rarely intraconidial, and no specialized structures could be observed, though hyphae were growing in close contact with powdery mildew conidia. When the fungus was grown on a slide under moist conditions and powdery mildew conidia dusted on it, the conidia in contact with the mycelium became emptied, whereas the others remained unaffected. Parasitism is probably effected by means of enzymes or toxins.

*Acrodontium crateriforme* (Van Beyma) De Hoog was isolated firstly from a colony growing on top of a well developed apothecium of *Pseudopeziza trifolii* f. sp. *medicaginis-lupulinae* on *M. lupulina.* On the same leaf it also parasitized *E. pisi.* It was pathogenic to sporulating mycelium of *S. fuliginea* on cucumber and was reisolated from it. Growth on malt agar was rather slow, the fungus combining bundles of hyphae to branch-like structures bearing numerous conidiophores, rich in conidia. Infection of mildew colonies proceeded rather slowly, the fungus leaving little more behind than some dark brown, very flattened debris. Attack of the powdery mildew conidia was extramycelial by means of hyphae forming appressorium-like structures. Only very rarely haustoria were formed. The fungus has been reported from various substrates, among others, insects and spiders (De Hoog, 1972). Recently *A. myxomyceticola* Crane & Schoknecht was found on *Stemonitis fusea* Roth (Crane & Schoknecht, 1982).

*Ramichloridium apiculatum* (Miller et al.) De Hoog was isolated from parasitized *E. pisi* on *M. lupulina.* Because the isolate was lost after determination it could not be studied any further. However, isolate CBS 156.59 obtained through the courtesy of G.S. de Hoog (Centraal Bureau voor Schimmelcultures, Baarn) proved to be slightly pathogenic to *S. fuliginea* on cucumber. After inoculation it did not destroy the mildew colonies completely, but it could be reisolated from destroyed conidia. Until now, the fungus has been reported to be saprophytic only, but two related species, *R. epichloes* (Ellis & Dearn.) De Hoog and *R. meliolae* (Hansf.) De Hoog are hyperparasites (De Hoog, 1977).

*Trichothecium roseum* (Pers.) Link ex Gray was also found to parasitize *E. martii* on *L. polyphyllus.* Its action against powdery mildews has been investigated by Dar-

poux and Faivre-Amiot (1952). In our own experiments spores and mycelium of *S. fuliginea* on cucumber and *Puccinia horiana* on chrysanthemum were destroyed to the greater extent, but not completely. The fungus was phytotoxic, causing broad necrotic zones around infected colonies.

*Paecilomyces farinosus* (Holm ex Gray) A.H.S. Brown & G. Sm. (teleomorph: *Cordyceps memorabilis* Ces.) was isolated as a parasite from *E. martii* on *L. polyphyllus.* It was pathogenic to sporulating mycelium of *S. juliginea* on cucumber and was reisolated from it. Hitherto the fungus has been reported as a parasite of insects (Samson, 1974) and it was also isolated from aecidiospores of *Cronartium comandrae* (Powell, 1971). It was the fastest grower of all the parasites observed in the present survey, in culture as well as on natural substrate.

*Aphanocladium album* (Preuss) W. Gams parasitized powdery mildews only under very moist conditions. It was also isolated from *S. fuliginea* on cucumber. It was pathogenic to sporulating mycelium of *S. fuliginea* on cucumber and was reisolated from it. This fungus is most common on Myxomycetes but has been reported from *E. cichoracearum* DC. ex Mérat before (Hawksworth, 1981).

The fungus was also found to infect uredo- and aecidiospores of several species of Uredinales, teliospores of Ustilaginales and an *Ascochyta* species (Koç and Défago, 1983). However, their isolate did not infect *E. graminis* and *Podosphaera leucotricha.*

This raises the interesting question, whether there are physiological races among this fungus.

## Discussion

Hyperparasitism of powdery mildews seems to be more widespread than has been realized before, especially in autumn when mildewed material is abundant and the epidemic is bound to cease due to decreasing temperatures and increasing humidity.

The question arises whether in nature there is a succession in the appearance of hyperparasitic fungi. In mid-October *D. aciculare* and *A. quisqualis* were isolated, subsequently *A. crateriforme* and *R. apiculatum,* followed by *T. minor* and *T. roseum* whereas *P. farinosus* was isolated in December at the very end of the season. *A. album* was found throughout the season under very wet conditions.

It would be very interesting to make a detailed inventory at various moments during the autumn to see whether the assumption is correct. This might give us an indication, whether these fungi could be used in biological control and, if so, under which conditions of temperature and humidity. It also might broaden our insight into the breakdown of epidemics under natural circumstances.

In this respect it is worth mentioning that most hyperparasites were isolated from powdery mildew colonies on *M. lupulina* and *L. polyphyllus,* growing in a more or less natural environment at Bonn-Ramersdorf, whereas from an experimental plot at Bonn-Poppelsdorf only *A quisqualis* was isolated.

Cultures of *T. roseum, A. crateriforme, D. aciculare* and *T. minor* became easily contaminated by some fungi causing lysis to the hyperparasites of powdery mildews, most probably brought into the laboratory adherent to powdery-mildew-infested plant material. These fungi were identified as *Fusarium oxysporum, Penicillium* cf. *variabile* and three strains with different cultural characteristics of *P. funiculosum.* However, after 2 years of cultivation only *F. oxysporum* and *P.* cf. *variabile* had re-

tained their activity. All three strains of *P. funiculosum* had lost their pathogenicity for the greater part and were still only slightly parasitic. *P. farinosus* also overgrew *D. aciculare* and *T. minor,* possibly causing lysis.

Research on this subject is continued.

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**Samenvatting**

*Isolering en identificering van hyperparasieten op Erysiphaceae*

In de omgeving van Bonn (Bondsrepubliek Duitsland) werd een onderzoek verricht naar het voorkomen van hyperparasieten van soorten van echte meeldauw. Acht parasitaire schimmelsoorten werden geïsoleerd, waaronder *Dissoconium aciculare,* een tot nu toe nog niet beschreven deuteromyceet.

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