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Research Note

Epicoccum nigrum LINK: A biological control agent of *Plasmopara viticola* (BERK. et CURT.) BERL. et DE TONI ?

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K e y w o r d s : downy mildew, Plasmopara viticola, Epicoccum nigrum, hyperparasite.

Introduction: The downy mildew fungus of grape, *Plasmopara viticola* (BERK. et CURT.) BERL. et DE TONI, is still one of the most important grapevine pathogens causing great yield losses without protective treatments. However, some fungi are able to prevent the development of other microorganisms due to their rapid growth or the production of lytic enzymes or other toxic compounds. The development of *P. viticola* could be reduced, e.g., by weekly postinfection with *Fusarium proliferatum* microconidia (FALK *et al.* 1996). Germination and germ-tube elongation of conidia of *Botrytis cinerea*, another grape pathogen, was diminished by the biocontrol agent *Trichoderma harzianum* (ZIEMAND *et al.* 1996). HEINTZ and BLAICH (1990) reported inhibition of growth and spread of grape powdery mildew (*Uncinula necator*) on leaf discs coinfected with *Verticillium lecanii* which led to a penetration of oidiospores by hyphae of this fungus (e.g. via appressoria) and thus to the death of oidiospores.

Sometimes a pink or red colored mycelium can be observed on hyphae and sporangiophores of *P. viticola* growing out of the leaf stomata of field-grown leaves (unpublished observations, GEHMANN 1983). In these areas the growth of hyphae and sporangiophores seems to be inhibited. The aim of this work was to identify this fungus and to study interactions with *P. viticola.*

Material and methods: Sporangia of *P. viticola* were collected from infected leaves of two susceptible *Vitis vinifera* varieties (Kerner and Riesling, vineyards of the Institute of Grapevine Breeding Geilweilerhof), dried at room temperature and stored at -25 °C. Sporangia were cultured and propagated on aseptically-grown *in vitro* plants (cv. Vidal). The unknown fungus, growing on the surface of the *P. viticola*-infected leaves of field-grown plants, was isolated and transferred onto Petri dishes containing potato dextrose agar (PDA, pH 5.6; Oxoid, Hampshire, UK). After purification through some subcultures, the mycelium was incubated at 20-22 °C and daylight. After 2-3 months a production of spores on the surface of the mycelium became visible. These spores were used for fungus identification. Aseptically outgrown sporangia were used to reinfect NaOCl-sterilized leaves of greenhouse-grown Riesling plants. After 4-5 d these leaves were inoculated with mycel segments of one-month-old cultures of the second fungus. For scanning electron microscopy (SEM) leaf samples were frozen in liquid propane, transferred into cold ethanol and stored at -25 °C until they turned white. Fixed leaf segments were transferred into dioxan of rising concentrations, vacuum dried and sputter coated on aluminium stubs under argon atmosphere using an Edwards sputter coater (Edwards, West Sussex, UK).

Results: The unknown fungus was identified as *Epicoccum nigrum* LINK on the basis of spores produced on PDA. *E. nigrum* spores (Figure, b) are multicellular, spherical, with a dark-brown jagged outer wall they are produced in sporodochia (Figure, c) on the surface of the mycelium (Figure, a). The mycelium is characterized by its red, pink or orange colour. The produced pigment also diffuses into the agar leading to a dark-red colour. On the leaf surfaces infected with *P. viticola* and *E. nigrum* a hyphal growth became visible on and between hyphae and sporangiophores of *P. viticola* as evident from light and scanning electron microscopy (Figure, d, f). Sporangiophores of *P. viticola* were rapidly surrounded by hyphae of *E. nigrum* whereas control leaves (not infected with *P. viticola*) showed no or only little growth of *E. nigrum* (data not shown). Although *Plasmopara* sporangia are often fully encased by hyphae of *E. nigrum* an infection of sporangia was observed only rarely. The spread of sporangia was inhibited because mature sporangia could not break off from sporangiophores due to the tight contact of sporangia and hyphae of *E. nigrum.* Germinated sporangia could not infect leaf cells by formation of germ tubes due to the long distance to the leaf surface. In the case of successful infection deformation of sporangia due to the loss of cytoplasm was observed by SEM (Figure, e). Nevertheless we noted intact and mature sporangia showing normal morphology as well (Figure, f).

Discussion: *E. nigrum* is a widespread and common saprophyte on dying plant organs but also a parasite on different hosts (e.g. apple, millet), especially on seeds of cereals (WEBSTER 1983). MULDER and PUGH (1971) reported a parasitism on spores and mycelium of *Helminthosporium sativum* and an inhibition of the spread of *Alternaria tenuis* or *Cladosporium herbarum* in dual culture by the rapid growth of *E. nigrum.* Although *E. nigrum* is efficient in controlling several other pathogenic fungi due to its production of numerous antifungal compounds such as flavipin (MADRIGAL and MELGAREJO 1994), which prevents the germination of conidia of *Botrytis allii* (BAMFORD *et al.* 1961), it does not seem to be antagonistic to *P. viticola.* Although *E. nigrum* was able to infect and destroy leaves of *in vitro* plants no lesions were observed on infected leaves of greenhouse- or field-grown grapevine plants, whose well developed cuticle seems to represent an efficient protective barrier against the fungus.

E. nigrum is not an obligate parasite, as it grows on PDA, and it is uncertain whether this fungus is a hyperparasite in the case of coinfection or rather a saprophyte growing on decomposing cells of *P. viticola.* Thus, a successful biological control of *P. viticola* in the field by *E. nigrum* is questionable.

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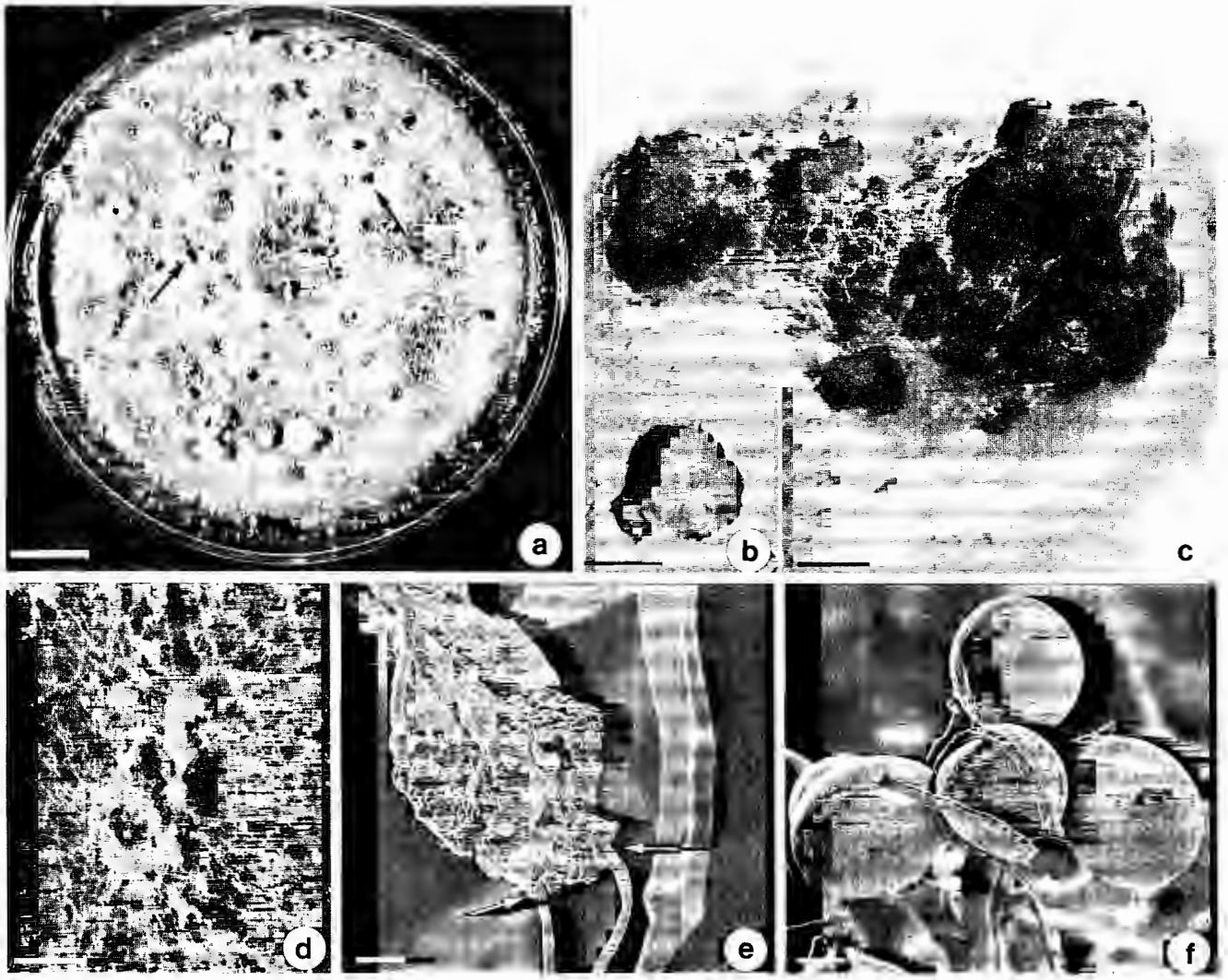

Figure: **a**) Two-month-old culture of *E. nigrum* on PDA. Some drops of liquid as well as sporodochia (arrows) are visible. Bar = 1 cm. **b**) Single isolated, multicellular spore with a rough and jagged crust-like cell wall. Bar = 10 µm. **c**) Detail of a, showing some dark sporodochia. Bar = 0.25 mm. **d**) Surface of a Riesling leaf showing sporangiophores of *P. viticola* which are surrounded by hyphae of *E. nigrum*. Bar = 0.5 mm. **e**) Two hyphae of *E. nigrum* attached on a collapsed sporangium of *P. viticola* (arrows). Bar = 2 µm. **f**) Some turgescent, mature sporangia of *P. viticola* which are surrounded by hyphae of *E. nigrum*. Bar = 5 µm.

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