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Fusarium rot of *Orobanche ramosa* parasitizing tobacco in southern Italy

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Summary. In tobacco crops grown in the province of Caserta (southern Italy), we noted, for the first time in Italy, very many broomrape (*Orobanche ramosa*) plants exhibiting mycosis caused by a strain of *Fusarium oxysporum* that is not pathogenic to tobacco. After a brief description of the symptoms of the disease and its incidence in the field, we discuss, on the basis of the observations made and the data supplied by the literature, the feasibility of using this fungus in programmes to control *Orobanche*.

Key words: *Fusarium oxysporum*, branched broomrape, biological control, pest control.

Introduction

During the spring-summer of 2002, in an extensive area around Recale in the province of Caserta (southern Italy) where tobacco has been grown for over 30 years, a series of field observations were carried out to establish the occurrence of viral infections in tobacco. At the end of June very extensive populations of broomrape (*Orobanche ramosa* L.) were noted that were showing diffuse necrosis with such rapid acropetal progression that often the flowers differentiated but seed capsules were not produced (Fig. 1).

The necrosis caused high mortality in *O. ramosa* but the roots of tobacco plants affected with diseased broomrape were undamaged, because of the extremely quick necrosis of the broomrape tissues.

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The undeniable potential utility of this phenomenon, the lack of information to be found on the subject in the relevant literature, and the interest currently shown by the scientific community in alternative ways to control crop pathogens all justify this contribution to the subject.

Materials and methods

The pathogen was isolated in pure culture on potato dextrose agar (PDA Difco) in Petri dishes. To study the incidence of the disease only in 2002, we chose a field that was intensely and widely affected, and just over 1 ha of which was given over to tobacco cultivation. Observations were made in three areas of 3 m^2 each, spaced 3 m apart, positioned along an imaginary diagonal line passing through two of the corners of the field (Fig. 2). Each area consisted of 65 plants spaced 25 cm apart along the rows and with 75 cm between rows. At the two ends of the diagonal we left a buffer zone of 1 m. Counts of tobacco plants parasitized by

Orobanche, and of *Orobanche* with necrosis were carried out at the end of July 2002.

To ensure that the isolate found was not pathogenic to tobacco and was therefore of interest as an alternative way of controlling *O. ramosa*, 20 young tobacco plants approximately 15 cm tall were inoculated by distributing, on the slightly wounded roots and the neck of each plant, 10 ml of a conidial suspension of the fungus in question, containing 1.5×10^6 conidia ml^{-1}. Five plantlets used as controls were given the same treatment without the pathogen.

In the same way, 26 plants of *O. ramosa* growing from seed for about 5 weeks and about 7–8 cm tall were inoculated, in 10 pots each with a tobacco plant, the neck of which had been treated with about 15 ml of a suspension of seeds of *Orobanche*, at a concentration of about 300 seeds ml^{-1}. Six plants of *O. ramosa* were used as controls. The plants were stored in a greenhouse at a temperature between 26 and 28°C.

Since the study arose from a survey on the spread of the virosis, we also sought to ascertain whether in tobacco there was any relation between the presence of a viral infection and parasitisation by *Orobanche* and, if so, which virus was most involved.

Fig. 1. *Orobanche ramosa* infected with *Fusarium oxysporum* in the field.

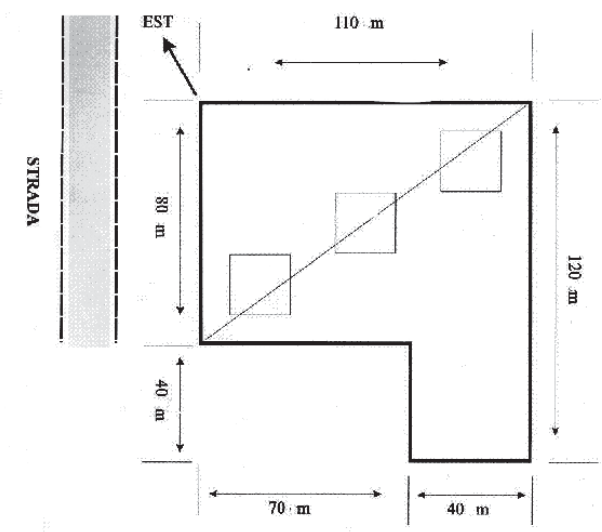

Fig. 2. Plan of the field under study.

Results

Fungal colonies isolated from field-picked *O. ramosa* plants were very frequently found; these colonies were white, tending towards more or less deep purple in the central part, reaching a diame-

Fig. 3. Outcome of artificial inoculation of *Orobanche ramosa* with *Fusarium oxysporum*.

ter of about 8 cm in 7 days on PDA at 23°C, and they produced many oval microconidia with no septa, poor comma-shaped macroconidia with 3–5 septa, and mainly short and flask-shaped conidiophores: these colonies were identified as *Fusarium oxysporum* Schlechtend.: Fr.

Incidence of infection, values in the three plots were as follows: in the first plot we observed 48 parasitized tobacco plants and 39 *Orobanche* plants with necrosis varying from slight hypogeal symptoms to necrosis involving the flowers; in the second plot the respective values were 39 and 38, and in the third 51 and 47.

Tobacco plants inoculated with the isolate in question were completely asymptomatic 50 days after inoculation but inoculated *O. ramosa* plants all showed symptoms similar to those in the field after varying durations starting from 15–20 days after inoculation (Fig. 3). These plants were used to re-isolate the pathogen in question. There was no relation between viral infection of *O. ramosa* and infestation of tobacco by *Orobanche*.

Discussion

Orobanche incidence on tobacco reached very high levels, but since more than 90% of the *O. ramosa* plants in the sampled areas were infected with the *Fusarium,* damage to the tobacco crop was substantially reduced. The virulence of the pathogen to *O. ramosa* led to a significant reduction in damage caused by this plant due most likely to the decrease in inoculum potential of *O. ramosa* caused by the *Fusarium*.

This *Fusarium* infection of *O. ramosa* is reported for the first time in Italy.

In light of the results and the findings of Mazaheri *et al*. (1991), the fungus is completely harmless to tobacco crops while it is lethal to *O. ramosa*: this would make this fungus a candidate for possible use in pest control programmes against *Orobanche*. However, although there are reports of fungi that parasitize *Orobanche* spp., as specified below, there is as yet no technique to use these fungi against *O. ramosa* in the field (Amsellem *et al*., 2001). In-depth studies have however been conducted on the biological and pathogenetic characteristics of the fungus in question (Murasheva, 1995a, b) on ways to enhance its conidiogenesis (Bedi and Donchev, 1995) and on ensuring its conservation (Amsellem *et al*., 1999).

In Iran, pest control trials have shown that *F. oxysporum* reduces the amount of tobacco plant parasites by over 70% and achieves yield increases of approximately 80% (Mazaheri *et al*., 1991). In Nepal, when fungi were isolated from visibly infected plants, it was found that over 70% of the isolates were from the genus *Fusarium* (Thomas *et al*., 1999). In Hungary, during trials to control *O. ramosa* with strains of *F. solani* and *F. oxysporum* isolated from the plant test, a single treatment achieved a mortality of over 90% in the first year and about 97% in the second, showing the capacity of these fungi to survive in the soil and to maintain or even enhance their virulence over time, especially in the case of *F. oxysporum* (Hodosy, 1981). Equally encouraging results were obtained in India against *O. cumana* (Bedi and Donchev, 1995) while in Egypt some fungi isolated from the fruit of *O. crenata*, including the fungus in question here, reduced seed viability in *O. crenata* by 21–85% (Al-Menoufi, 1986).

Very encouraging results were recently achieved by Amsellem *et al*. (2001) who obtained two isolates, one of *F. arthrosporioides* and one of *F. oxysporum*, that parasitized plants of *O. ramosa*, *O. aegyptiaca* and *O. cernua* but not *O. cumana* nor the various crop plants tested: this result, reached through a series of artificial inoculations, was confirmed with an in-depth bio-molecular analysis (RFLP and RAPD) which showed the considerable genetic difference between these two isolates, and between these isolates and many other isolates of *Fusarium* spp. and *formae speciales* of *F. oxysporum*, including an isolate of *F. oxysporum* parasitizing *O. cumana*. Amsellem *et al*. (2001) also found that tomato plants, whose root apparatus had been treated with a conidial suspension and hyphal fragments of the two isolates and had then been planted in soil infested with *O. ramosa*, were completely protected for 6 weeks (Amsellem *et al*., 2001).

Fusarium oxysporum, together with *F. semitectum*, was also reported as a possible pest control agent for *Cuscuta* (Stojanovic and Boric, 1981). Other *Fusarium* spp. have also been indicated as possible control agents of *Orobanche*. Bozoukov and Kouzmanova (1994) tested *F. lateritium* (*Gibberella baccata*) against *O. ramosa* and *O. mutelii* on tobacco; he found that the fungus was more effective when it was distributed as conidia or in the

plant's hypogeal phase, especially between germination and tubercle formation, and that it remained effective longer if it was distributed in irrigation water.

Besides the species named, only a few other fungi are reported to be parasites on *Orobanche* spp. and hence are potential pest control agents; the list seems limited to *Botrytis cinerea* on *O. fasciculata* near Washington (USA) (Shaw, 1973; Farr *et al*., 1989), *Thielaviopsis basicola* (=*Chalara elegans*) on *O. ramosa* in the Ukraine (Popova, 1929), *Colletotrichum lagenarium* on *O. aegyptiaca* in eastern Europe (Stankevich, 1971; Prokudina, 1973), *Sclerotium rolfsii* on *O. cernua* in Indian tobacco crops (Raju *et al*., 1995), and *Ulocladium atrum* isolated, together with a *Fusarium* sp. and an *Alternaria* sp., from plants of *O. crenata* and *O. minor* picked in Syria, Morocco and France, and which destroyed the tubercles in the soil or the sprouts which had just emerged (Linke *et al*., 1992).

Considerable attention is currently being focused on the micromycetes that control pests and parasites biologically (Sauberborn, 1993), although recent research undertaken in Hungary has shown the scant efficacy of such methods of control with certain plant-pathogen associations, including *Fusarium* spp. and *Orobanche* spp. (Beres *et al*., 2000).

In examining whether certain fungi will control weeds or plant parasites, two different approaches can be followed, as reported by Müller-Schärar (2000): either a particularly efficacious isolate can be distributed in the field and its effect optimised; or alternatively a better understanding of the relationship between a crop plant, its parasite and any natural antagonists or pathogens of the latter may make it possible to act on the environmental conditions so as to maximise the spread and impact of one of the native antagonists or pathogens of the parasite. Moreover, we need to bear in mind that, despite the promise offered by *F. oxysporum* to control *O. ramosa*, the fungus may yet be found to be harmful to some crop species, making it impossible to use it as a biocontrol agent (Murasheva, 1995a).

Our survey produced encouraging data on the possibility of using micro-organisms in the biological control of *O. ramosa* in the not too distant future. This view is shared by researchers in the University of Rehovot (Israel) who are now working to produce transgenic parasites, especially fungi, that are particularly virulent against *Orobanche* spp. (Benvenuti, 2002).

Further research on the pathogenesis of the disease here described is in progress.

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