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Resistance to benzimidazole fungicides in pathogens of *Ascochyta* diseases of peas

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Summary

The antifungal action of three fungicides of the benzimidazole family (benomyl, carbendazim, thiabendazole) were studied on the mycelial growth of *Ascochyta pisi, Ascochyta pinodes* and *Phoma medicagenis* var *pinodella* collected principally in 1990 and 1991 in France. It seemed that resistance to these fungicides existed for the two major pathogens of *Ascochyta* diseases of peas. For these two fungal species, the frequency of resistant isolates was high enough to cause resistance in practice. It reached 65% in *A. pinodes* and 100% in *A. pisi*. The existence of a negative cross resistance between fungicides of the benzimidazole family and diethofencarb in *Botrytis cinerea* has not been checked for certain *A. pinodes* isolates, which excludes the use of Sumico, a fungicide which associates carbendazim and diethofencarb.

Introduction

In peas, *Ascochyta* diseases can be caused by *Ascochyta pisi, Ascochyta pinodes* (sexual form: *Mycosphaerella pinodes*) and *Phoma medicagenis* var. *pinodella* (*Ascochyta pinodella*). These fungi present on seeds and in the soil cause primary contaminations, which sometimes lead to damping-off. Secondary contaminations, through pycnospores (for the three parasites) or through ascospores (only for *Mycosphaerella pinodes*) cause symptoms over all the parts of the plants (leaf, stem and (or) pod). In the event of major infestations after flowering, seed filling and yield are affected). *Ascochyta* diseases of the pea are widespread in Europe and in temperate regions in general. A recent study (Tivoli and Lemarchand, 1992) points out that these diseases are present throughout all regions of high protein pea cultivation.

Chemical control of *Ascochyta* diseases is achieved by seed treatments or foliar spraying. In seed treatments, research carried out by Champion *et al.* (1984) showed that benzimidazole fungicides (benomyl, carbendazim, thiabendazole) are highly effective against *A. pisi* and *A. pinodes* (the two main pathogens found on seeds) when applied on seeds. For more than ten years, benzimidazoles have been used on peas but over the last two years there have been reports of decreased effectiveness. As resistance to these fungicides has been observed with other plant pathogens (Leroux, 1983) we decided to determine whether this phenomenon also concerned pathogens of *Ascochyta* diseases of peas.

Materials and methods

Fungi
The fungi were isolated infected pea seeds, stems, leaves or tendrils and maintained on a malt agar medium (10 g malt, 20 g agar per litre). Among the 21 isolates of *A. pisi*, which were all from France, 19 were collected in 1991 and one in 1990. The 21st isolate was given to us by C. Allard (INRA, Phytopathologie, Versailles) and was isolated before the introduction of benzimidazole fungicides. 92 isolates of *A. pinodes* were studied, 89 were collected in France (81 in 1991, 6 in 1990 and 2 by C. Allard before 1990) and 3 in England in 1990. Only 10 isolates of *A. pinodella* were tested; 5 were collected in France (1 in 1990 and 4 in 1991) and the others were from England in 1990.

Fungicides
The products used were benomyl (Dupont de Nemours, USA), carbendazim (BASF, Germany), diethofencarb (Sumotono Chemical, Japan) and thiabendazole (Merk, USA).

Antifungal assays
For all the isolates of *Ascochyta*, the antifungal activity of carbendazim and thiabendazole was evaluated by measuring the inhibitory action of three concentrations (0.5, 5 and 50 mg/litre) in malt agar media upon the growth of the various fungal isolates. The fungicides (technical grade) were added to the malt agar medium as suspended solutions in ethanol. The final alcohol concentration, identical in the controls and treatments, was 0.5%. After homogenization, the medium was poured into 9 cm Petri dishes (20 ml dish), inoculated with a mycelial plug and incubated at 20 °C in the [illegible]. The diameter of the fungal colony was measured after six and nine days. For each fungicide concentration (three replications), growth was expressed as a percentage of control.

For a certain number of isolates sensitive and resistant to carbendazim and thiabendazole, further tests were carried out:
- to better estimate the concentration causing a 50% reduction in mycelial growth (EC 50).
- to better understand the behaviour of these isolates by testing a third fungicide of the benzimidazole family, benomyl, and a fungicide known for its negative cross resistance with carbendazim in *Botrytis cinerea*, diethofencarb.

Results

Generally, whatever the *Ascochyta* species, the various isolates had one of the following reactions:
- isolates which were totally inhibited by 5 mg/litre of carbendazim or thiabendazole

Table 1. Characteristics of mycelial growth of *Ascochyta pisi* isolates after six and nine days expressed as percentage of control (extreme values).

Phenotype	Number of strains	Carbendazim (mg/l)			Thiabendazole (mg/l)		
		0,5	5	50	0,5	5	50
S	1*	86	0	0	90	0	0
R	20	70–100	70–100	70–100	60–100	60–100	30–55

* Sensitive strain collected before the benzimidazoles use.

were considered as being sensitive (S) to benzimidazoles.

- isolates which developed on 50 mg/litre of carbendazim and thiabendazole were considered as being resistant (R) to benzimidazoles. At this dose of fungicide, the percentage of fungi growth in the presence of carbendazim reached 70 to 100% of the control, and in the presence of thiabendazole the percentage of growth was 60% of the control.
- For *A. pinodella:* the isolates collected in 1990 and 1991 (in France and in England) were all sensitive to benzimidazoles.
- For *A. pisi:* the isolates found in France in 1990 and 1991 were resistant to these two fungicides, mycelial growth was slower than that of *A. pinodes* and *P. medicagenis* var. *pinodella,* and in the presence of 50 mg/litre of thiabendazole the growth of *A. pisi* isolates is between 20 and 55% of the control. Only the isolate collected 25 years ago by C. Allard (Table 1) was sensitive to benzimidazole.
- For *A. pinodes:* among the isolates collected in 1990 and 1991 (in France and England) 35% were sensitive to benzimidazole. The growth of cultivated resistant isolates in a medium containing 50 mg/litre of thiabendazole was between 40 and 60% that of the control (Table 2).

The EC50 values for isolates in the presence of carbendazim, thiabendazole and benomyl were very close, and in sensitive isolates, whatever the fungal species, the action of the fungicide was similar. As far as isolates resistant to benomyl and carbendazim were concerned, their EC50 was higher than 50 mg/litre. For these same isolates in the presence of thiabendazole, a difference according to the fungal species was

Table 2. Effects of carbendazim and thiabendazole on the mycelial growth of *Mycosphaerella pinodes* isolates after an incubation time of 9 days. Results expressed as percentage of control (extreme values).

Phenotype	Number of strains	Carbendazim (mg/l)			Thiabendazole (mg/l)		
		0,5	5	50	0,5	5	50
S	32*	73–100	0–10	0	83–100	0–5	0
R	60	85–100	85–100	80–100	80–100	80–100	40–60

* 3 isolates from England.

Table 3. Effect of fungicides on mycenial growth of pathogen isolates of *Ascochyta* diseases of peas.

Fungi		Fungicides			
Species	Phenotype	benomyl	carbendazim	thiabendazole	diethofencarb
P. medicaginis var. *pinodella*	S	2.5 [2–3]	1.15 [0.8–1.5]	2 [1.5–2.4]	>25
A. pisi	S	1.2	1.7	1.5	>25
	R1	>50	>50	26 [20–40]	2.25 [2–2.5]
M. pinodes	S	3.25 [2–4.5]	1,7 [1–2.4]	1.95 [1.5–2.4]	>25
	R1	>50	>50	>50	2.05 [1.7–2.5]
	R2	>50	>50	>50	>25

EC50 in mg/litre (fungicides concentration inhibiting the mycelial growth by 50%). The extreme values observed are brackets.

observed: the EC50 value of the *A. pinodes* isolates was higher than 50 mg/litre and that of *A. pisi* isolates was between 20 and 40 mg/litre.

All the isolates of *A. pisi, M. pinodes* and *P. medicagenis* sensitive to benzimidazoles were tolerant to diethofencarb (type S). The resistant isolates had a behaviour which varied according of the fungal species: all the *A pisi* isolates and most of the *M. pinodes* isolates which were resistant to benzimidazoles were sensitive to diethofencarb (type R1). Only a few *A. pinodes* isolates were resistant to both benzimidazoles and diethofencarb (type R2), (Table 3).

Discussion

From the samples collected in 1990 and 1991, it appeared that resistance to benzimidazole fungicides (benomyl, carbendazime, thiabendazole) existed in the two most important pathogens of *Ascochyta* diseases: *A. pisi* and *M. pinodes*. For these two fungal species, the frequency of resistant isolates was high enough to cause resistance in practice. It appeared that at present in France, resistance of *M. pinodes* to benzimidazoles reached 65% and that of *A. pisi* 100%.

The differences observed between the various *Ascochyta* species, in connection with their resistance to benzimidazoles, were probably additional proof of their biological and epidemiological differences.

Considering these results, it seems that the use of benzimidazole fungicides to control the two major pathogens of *Ascochyta* diseases of peas, *A. pisi* and *M. pinodes*, is challenged. Sumico, a fungicide which combines two active ingredients having a negative cross reaction in *B. cinerea*, carbendazim and diethofencarb, cannot be used since *M. pinodes* isolates are resistant to both products.

In seed treatment, these fungicides can be replaced by iprodione (Champion *et al*, 1984). Several experimental phenylpyrroles tested by Ciba Geigy (Leadbeater A. J. *et al*, 1990 and Nevill, D. *et al.*, 1988) – fenpiclonil, CGA 1730506 – whose mode of action is close to that of iprodione seem to be good candidates (Leroux, 1991). As foliar sprays, several multisites, fungicides including chlorothalonil and sterol biosynthesis inhibitors such as flutroafol and prochloraze are very effective (Daguenet *et al.*, 1990).

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