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Short Communication

Resistance to benomyl in *Pyrenopeziza brassicae*

(Kerintangan *Pyrenopeziza brassicae* terhadap benomil)

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Key words: benomyl, oil-seed rape, resistance, *Pyrenopeziza brassicae*

Abstrak

Lapan asingan *Pyrenopeziza brassicae* Sutton dan Rawlinson yang diperoleh dari tanaman 'oil-seed rape' di United Kingdom telah diuji kepekaan terhadap benomil dengan mengkaji pertumbuhan konidia dalam medium agar yang dirawat dengan racun kulat. Walaupun terdapat perbezaan pada pertumbuhan konidia, kesemua asingan adalah peka kepada benomil pada kadar 20 mg/L. Keputusan menunjukkan bahawa benomil boleh digunakan untuk mengawal penyakit tompok daun pada tanaman 'oil-seed rape' secara berkesan.

Abstract

Eight isolates of *Pyrenopeziza brassicae* Sutton and Rawlinson, collected from oil-seed rape crops in the United Kingdom, were tested for sensitivity to benomyl by studying germination of conidia in fungicide-amended agar. Although variations in germination of conidia were found, all isolates were sensitive to benomyl at 20 mg/L. Results indicated that benomyl could be used for effective control of light leaf-spot disease of oil-seed rape.

Introduction

One of the main problems in chemical control of plant diseases is the development and emergence of insensitive strains of certain pathogens that will result in reduced efficacy of chemicals used to control them. Continuous and repeated use of a particular type of fungicide, especially the systemic fungicide, was found to have caused most of the resistance problems (Brent 1987). Brent (1987) also showed that *Phytophthora infestans* acquired resistance to acylalanine in about 2 years after its commercial use. Rapid development in resistance to other systemic fungicides like benzimidazoles was found in *Spaerotheca fuliginea* (Schroeder and Provvindenti 1969), *Botrytis cinerea* (Bollen and Scholten 1971) and *Fusarium oxysporum* f. sp. lycopersici (Thanassoupoulos et al. 1971).

In view of such build up in resistance in fungal pathogen populations, it is essential to test the sensitivity of pathogens to fungicide. Results of such tests would enable us to assess the level of resistance developed, hence a better choice of fungicide can be used to provide satisfactory control of the disease. In this paper, results of a sensitivity test on several isolates of *Pyrenopeziza brassicae* by using a laboratory method developed by Gullino and Garibaldi (1986) are reported.

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Materials and methods

Fungal isolates

Eight isolates of *P.brassicae* from oil-seed rape, *Brassica napus* sp. oleifera, from various parts of the UK (*Table 1*) were tested for resistance to benomyl.

Sensitivity tests

The tests were based on methods developed by Gullino and Garibaldi (1986). Benomyl stock suspension was prepared from commercial *Benlate* (50% benomyl) in sterile distilled water to give 2 g/L benomyl. Amended potato dextrose agar (PDA) was prepared by adding 10 mL of stock suspension to 1 L of agar (autoclaved and cooled to about 50 °C) to give a final concentration of 20 mg/L of benomyl. Streptomycin (10 mL/L) was also added to the agar at the same time. The molten agar was poured into three-sector petri dishes. Each sector contained about 8 mL of agar.

Conidial suspension

Spore suspensions were prepared directly from tissues bearing sporulating lesions. Lesions were cut out from infected leaf or pod samples and transferred into beakers, each containing 5 mL sterile distilled water, 0.05 mL streptomycin and 0.05 mL *Tween 20*. After shaking, 0.1 mL of suspension (about 3 x 10^5 conidia/mL) was spread over the agar in each sector of the petri dishes. Two petri dishes were used for each isolate. All petri dishes were incubated at 25 °C. Germination of conidia and germ tube elongation was observed using a light microscope (24 h after incubation). In each assay, 100 conidia were examined. Germination was considered to have occurred when germ tubes exceeded the length of conidia.

Benomyl range test

The stock suspension containing 2 g/L benomyl was used. Appropriate quantities of the suspension were added to PDA (autoclaved and cooled at 50 °C) to give a final concentrations of 0.0625, 0.125, 0.25, 0.5, 0.1 and 2.0 mg/L benomyl. A volume of 25 mL of each benomyl supplemented agar was poured into each petri dish. All petri dishes containing different levels of benomyl were carefully marked and 0.1 mL of conidial suspension was spread over the agar in each petri dish. Two petri dishes of each level of benomyl were used for each isolate. All petri dishes were incubated at 25 °C. After 24 h, germination of conidia was observed and recorded in the usual way.

Results and discussion

Results from sensitivity tests showed that there was no conidial germination for all isolates at 20 mg/L benomyl (*Table 2*). In tests using a range of concentrations of benomyl, conidial germination was inhibited in most isolates at 1.0 mg/L benomyl (*Table 3*). There was no germination of conidia in isolate 292 in even the lowest concentration of benomyl tested, indicating that the isolate was very sensitive to the fungicide. However, isolate 304 was found

Table 1. Origin of *Pyrenopeziza brassicae* isolates

Isolate no.	Origin
292	Northumberland
296	Tenterden
299	Chichester
301	Chichester
302	Chichester
303	Bristol
304	Bridgwater
305	Bristol

Table 2. Sensitivity of *P. brassicae* isolates to benomyl

Isolate no.	Conidial germination (%)	
	Control	Benomyl (20 mg/L)
292	100	0
296	100	0
299	100	0
301	100	0
302	100	0
303	100	0
304	100	0
305	100	0

to germinate in media containing 2 mg/L benomyl. This isolate could be considered to exhibit some insensitivity to benomyl. However, it must be emphasized that all isolates were inhibited by benomyl at 20 mg/L as used in most sensitivity tests.

This method of determining the level of resistance in *P. brassicae* may not provide a complete assessment of resistance of the pathogen to benomyl, even though it was successfully used to test for such resistance in *Botrytis cinerea* on tomatoes by Gullino and Garibaldi (1986). This is because sensitivity of a fungus to a fungicide such as benomyl could be more accurately determined by assays designed to test mycelial growth rather than by conidial germination (Clemons and Sisler 1971). Failure of conidia to germinate could be due to reduced viability rather than fungitoxicity.

King and Griffin (1985) considered sensitivity tests using mycelial growth of *Pseudocercosporella herpotrichoides* to be a more reliable estimate of carbendazim resistance which was likely to influence disease control and to be of practical significance to growers. Despite this, the Gullino and Garibaldi technique can be regarded as a rapid method of testing for fungicide resistance in slow growing pathogens such as *P. brassicae*, which are difficult to isolate in pure culture.

From this survey, it was found that there has been no development of resistance towards benomyl to levels which were likely to prevent satisfactory control of *P. brassicae*. The absence of highly resistant strains could be due to the use of benomyl on most of the oil-seed rape crops from which the isolates were collected. As such, it is concluded that carbendazim-based fungicides can be used to give satisfactory control of light leaf-spot disease. However, as a precautionary measure of a possible breakdown in control due to build-up in resistance, the carbendazim-based fungicides should not be used repeatedly. Other fungicides that have been tested and found to be effective for controlling the disease should be used alternately with benomyl. Some of these fungicides are propiconazole and vinclozolin (Anon. 1986). These fungicides applied alternately with benomyl at different stages of growth should provide effective control of the disease.

References

Anon.(1986). *Plant disease survey* U.K.: Agricultural Development and Advisory Services (ADAS)

Bollen, G. J. and Scholten, G. (1971). Acquired resistance to benomyl and some other systemic fungicides in a strain of *Botrytis cinerea* in cyclamen. *Netherlands Journal of Plant Pathology* 77: 83

Brent, K. J. (1987). Fungicide resistance in crops and its practical significance and management. In *Rational pesticide use* (Brent, K. J. and Atkin, R., ed.) p.137- 49. London: Press Syndicate, University of Cambridge

Table 3. Conidial germination of *P. brassicae* isolates at various concentrations of benomyl

Isolate no.	Conidial germination (%) at 7 benomyl conc. (mg/L)						
	0	0.0625	0.125	0.25	0.5	1.0	2.0
292	100	0	0	0	0	0	0
296	100	22	17	15	0	0	0
299	100	94	88	60	0	0	0
301	100	90	75	0	0	0	0
302	100	80	40	34	0	0	0
303	100	14	12	12	9	0	0
304	100	15	15	12	9	8	3
305	100	10	9	8	8	7	0

Clemons, G. P. and Sisler, H. D. (1971). Localisation of the site of a fungitoxic derivative. *Pesticidae biochemistry and physiology 1*: 32

Gullino, M. L. and Garibaldi, A. (1986). Fungicide resistance monitoring as an aid to tomato grey mould management. *Proc. British Crop Protection Conf. on Pests and Diseases* 1986, p. 499–504. Brighton: British Crop Protection Council

King, J. E. and Griffin, M. J. (1985). Survey of benomyl resistance in *Pseudocercosporella herpotrichoides* in winter wheat and barley in England and Wales in 1983. *Pl. Path. 34:* 272–83

Schroeder, W. T. and Provvindenti, R. (1969). Resistance to benomyl in powdery mildew of cucurbits. *Plant Disease Reporter 53*: 271

Thanassoupoulos, C. C., Giannopolitis, C. N. and Kitsos, G. T. (1971). Evaluation of sensitiveness and development of resistance of *Fusarium oxysporum* f. spp. *lycopersici* to benomyl. *Phyt. Zeitschr 70*: 114

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