ELSEVIER

Crop Protection 21 (2002) 57–61

Crop Protection

www.elsevier.com/locate/cropro

Efficacy of azoxystrobin and other strobilurins against Fusarium wilts of carnation, cyclamen and Paris daisy

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Received 2 March 2001; received in revised form 27 April 2001; accepted 8 May 2001

Abstract

The strobilurins, azoxystrobin, kresoxym-methyl and trifloxystrobin, were tested in experimental trials carried out in the growth chamber or glasshouse against Fusarium wilts of carnation (*Fusarium oxysporum* f. sp. *dianthi*), cyclamen (*F. oxysporum* f. sp. *cyclaminis*) and Paris daisy (*Fusarium oxysporum* f. sp. *chrysanthemi*), in comparison with benomyl and in some experiments prochloraz. The three strobilurins controlled Fusarium wilt on carnation when applied at transplant at 1–2 g/m^2 as soil drenching. Azoxystrobin, at 250 mg/l of medium controlled also Fusarium wilt on cyclamen and on Paris daisy. Kresoxim methyl at 250 mg/l controlled Fusarium wilt on cyclamen but it was phytotoxic; at the same dosage it was not effective on Paris daisy. Trifloxystrobin was only partially or not effective in controlling Fusarium wilt on cyclamen and Paris daisy. Particularly kresoxim-methyl caused chlorosis and plant stunting. This study shows the high efficacy of azoxystrobin against Fusarium wilts of three important ornamental crops. Azoxystrobin provided control similar or better to those shown by benomyl, applied at higher dosages in all trials.

Keywords: Carnation; Cyclamen; Paris daisy; *Fusarium oxysporum*; Azoxystrobin

1. Introduction

Fusarium wilts, incited by different *formae speciales* of *Fusarium oxysporum* can cause serious losses on several ornamentals, such as carnation, cyclamen, chrysanthemum, bulb crops… (Campbell, 1985). Although their severity and even relative importance may vary as a consequence of the introduction of new varieties and/or cropping systems, for most ornamentals the adoption of control measures against Fusarium wilt is necessary in order to reach the high qualitative standards required by consumers.

Fusarium wilt control is difficult to achieve and generally relies on the integration of several control methods, since no single measure is fully effective by itself. Pathogen-free propagation programmes are routinely adopted, especially for carnation and chrysanthemum. Despite the adoption of such a measure, problems may occur from reinfestation during production. Pathogenic strains of *Fusarium oxysporum* are difficult to eradicate by soil steaming or fumigation. Chemical control is complicated by the very limited number of effective chemicals available; partial control is achieved through benzimidazoles and prochloraz (Garibaldi et al., 1988). The best strategy for controlling Fusarium wilts of ornamentals relies on integrated use of resistant varieties (when available), steam disinfestation of planting medium, pathogen-free propagation material, and application of a benzimidazole fungicide, generally at transplant (Garibaldi and Gullino, 1990).

Strobilurins represent a group of fungicides originating from natural products, which include azoxystrobin, kresoxim-methyl, metominostrobin and trifloxystrobin. Taken together, they have a wide spectrum of activity (Gullino et al., 2000a, b). Even more exciting for crop protection, is that this class of compounds possesses a unique spectrum of activity (Ammermann et al., 1992; Godwin et al., 1992; Sauter et al., 1996; Ypema and Gold, 1999).

Such interesting breadth of activity might well represent a breakthrough for crops such as ornamentals,

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0261-2194/02/$ - see front matter
PII: S0261-2194(01)00066-7

which traditionally lack registered fungicides (Gullino and Wardlow, 1999).

Azoxystrobin showed promise against *Fusarium oxysporum* f. sp. *dianthi*, the causal agent of Fusarium wilt of carnation, in preliminary trials (Gullino et al., 2000a, b). The current work was undertaken to evaluate the efficacy of the three strobilurins, azoxystrobin, kresoxim-methyl, and trifloxystrobin, in controlling Fusarium wilt on carnation, cyclamen and Paris daisy (*Argiranthemum frutescens*), three ornamental crops chosen because of their economical importance in Italy and of the relevance of losses caused by Fusarium wilts.

2. Material and methods

2.1. Growth chamber and field experiments

Azoxystrobin, kresoxym-methyl, and trifloxystrobin were tested in experimental trials in growth chambers of DI.VA.P.R.A., University of Torino, and in the greenhouses of the Centro Regionale di Sperimentazione e Assistenza Agricola of the Chamber of Commerce of Savona, located at Albenga. Strobilurins were tested against Fusarium wilts of carnation, cyclamen, and Paris daisy, as summarized in Table 1, in comparison with benomyl and, in the case of Paris daisy, also with prochloraz.

All carnation experiments were carried out on the cv Indios. In trial 1 and 2 carnation plants, grown in pots (3.5 l volume, 4 plants/pot), were maintained in growth chamber; in trials 3 and 4 carnations were grown on benches ($1.35\,m^2$/plot, 55 plants/plot) under glasshouse conditions.

Cyclamen (cv Concerto F1) was grown in clay pots (2 l volume, one plant/pot) and maintained throughout the experiment in the open field under shade. Each treatment consisted of 15 plants, with 4 replicates.

Paris daisy plants, belonging to the cultivars Europa, Camilla, Veronica, and Carla were grown in plastic pots (0.4 l volume, 1 plant/pot) under glasshouse conditions. Ten plants/treatment, with 4 replicates, were used.

All plants were grown according to traditional cultural practices and maintained under conditions very favorable to wilt development. Particularly, in order to favor disease development, they were fertilized every 15 days with a high nitrogen ratio ($N:P_2O_5:K_2O = 2.5:1:1$).

2.2. Artificial infestation with the different pathogens

All media used were steam disinfested and artificially infested with selected isolates of the three pathogens (*F. oxysporum* f. sp. *dianthi*, *F. oxysporum* f. sp. *cyclaminis* or *F. oxysporum* f. sp. *chrysanthemi*), applied as chlamydospores suspended in talcum powder (Locke and Colhoun, 1974) or as propagules obtained from infected plants.

Soil artificially infested with *F. dianthi* (race 2, strain 75), by adding 5×10^3/ml of soil chlamydospores prepared in talcum powder; soil artificially infested with 1×10^3 colony forming units (CFU)/ml of soil with a strain of *F. oxysporum* f. sp. *cyclaminis,* isolated from infected cyclamen; soil artificially infested with 1×10^3 CFU/ml of soil of a strain of *F. oxysporum* f. sp. *chrysanthemi* isolated from Paris daisy wilted plants, were used for carnation, cyclamen and Paris daisy, respectively.

2.3. Chemical treatments

The following fungicides and rates (Tables 2–5) were used: azoxystrobin (YF 10537 or Ortiva, 25% a.i.Syngenta Limited), benomyl (Benlate, 50% a.i., Dupont

Table 1
Outline of the different trials

Trial	Trial site	T (°C)	Crop (target pathogen)	Number of		Artificial soil infestation and fungicide treatment date	Transplant date	Termination date
				Replicates	Plants/treatment			
1	Growth chamber	27	Carnation (*F. oxysporum* f. sp. *dianthi)*	4	16	11/20/99	11/20/99	01/03/00
2	Growth chamber	28	Carnation *(F. oxysporum* f. sp. *dianthi*))	4	16	05/24/00	05/25/99	08/04/00
3	Glasshouse	15–38	Carnation *(F. oxysporum* f. sp. *dianthi*))	3	55	05/19/99	05/20/99	08/03/99
4	Glasshouse	15–23	Carnation (*F. oxysporum* f. sp. *dianthi))*	3	55	09/29/99	09/30/99	03/27/00
5	Under shade	15–34	Cyclamen (*F. oxysporum* f. sp. *cyclaminis)*	4	15	06/05/99	06/15/99	09/23/00
6	Glasshouse	15–38	Paris daisy (*F oxysporum* f. sp. *chrysanthemi)*	4	10	05/15/99	05/26/99	08/20/99

Table 2
Effectiveness of different treatments against *F. oxysporum* f. sp. *dianthi* on carnation, cv Indios, in two trials carried out in a growth chamber

Treatment	Dosage g a.i./m^2	Dead plants (%) at days after transplant			
		Trial 1		Trial 2	
		34	45	64	71
Healthy control	—	0.0 a[a]	0.0 a	0.0 a	0.0 a
Inoculated control	—	43.8 c	50.0 d	43.8 c	68.8 b
Azoxystrobin	1+1	18.8 a–c	25.0 a–d	n.t.[b]	n.t.
Azoxystrobin	2.0	25.0 a–c	25.0 a–d	25.0 bc	25.0 a
Kresoxim-methyl	1+1	12.5 ab	18.8 a–c	n.t.	n.t.
Kresoxim-methyl	2.0	18.8 a–c	18.8 a–c	n.t.	n.t.
Trifloxystrobin	1+1	12.5 ab	18.8 a–c	n.t.	n.t.
Trifloxystrobin	2.0	12.5 ab	12.5 ab	12.5 ab	18.8 a
Benomyl	2.5+2.5	18.8 a–c	37.5 b–d	n.t.	n.t.
Benomyl	5.0	31.3 bc	37.5 b–d	25.0 bc	31.3 a

[a] Numbers followed by the same letters are not significantly different within each column according to Duncan's Multiple Range Test ($P = 0.05$).
[b] Not tested.

Table 3
Effectiveness of different treatments against *F. oxysporum* f. sp. *dianthi* on carnation, cv Indios, in two trials carried out in a glasshouse

Treatment	Dosage g a.i./m^2	Dead plants (%) at days after transplant			
		Trial 3		Trial 4	
		66	74	73	128
Inoculated control	—	35.8 c[a]	64.2 b	21.5 b	31.6 c
Azoxystrobin	1.0	9.3 ab	23.5 a	8.5 a	9.7 ab
Azoxystrobin	2.0	8.6 ab	23.5 a	3.6 a	5.5 a
Kresoxim-methyl	1.0	16.0 ab	22.8 a	0.6 a	3.0 a
Kresoxim-methyl	2.0	6.8 a	21.6 a	2.4 a	4.2 a
Trifloxystrobin	1.0	6.2 a	21.6 a	1.8 a	3.6 a
Trifloxystrobin	2.0	6.8 a	14.8 a	2.4 a	3.7 a
Benomyl	4.0	16.7 b	29.6 a	13.9 ab	24.2 bc

[a] Numbers followed by the same letters are not significantly different within each column according to Duncan's multiple range test ($P = 0.05$).

Table 4
Efficacy of different treatments against *Fusarium oxysporum* f. sp. *cyclaminis* on cyclamen (cv. Concerto)

Treatment	Dosage g a.i./litre of soil	Dead plants (%) at days after transplant		
		44	64	86
Inoculated control	—	38.6 c[a]	81.8 f	88.6 d
Azoxystrobin	0.125	9.1 a	20.5 ab	31.8 ab
Azoxystrobin	0.250	2.3 a	18.2 bc	36.4 b
Kresoxim-methyl	0.125	6.8 a	11.4 ab	38.6 b
Kresoxim-methyl	0.250	0.0 a	0.0 a	16.8 a
Trifloxystrobin	0.125	22.7 b	47.7 de	71.4 d
Trifloxystrobin	0.250	4.5 a	32.5 cd	67.3 cd
Benomyl	1.000	6.8 a	59.1 e	86.4 d

[a] Numbers followed by the same letters are not significantly different within each column according to Duncan's Multiple Range Test ($P = 0.05$).

Table 5
Efficacy of different treatments against *Fusarium oxysporum* f. sp. *chrysanthemi* on different cultivars of Paris daisy (*Argyranthemum frutescens*)

Treatment	Dosage g a.i./litre of soil	Dead plants at the end of the trial on the cultivar (%)			
		Europa	Camilla	Veronica	Carla
Control	—	86.7 bc[a]	86.7 cd	93.3 de	93.3 bc
Azoxystrobin	0.250	20.0 a	20.0 a	6.7 a	35.0 a
Azoxystrobin	0.125	13.3 a	13.3 a	53.3 bc	73.3 bc
Kresoxim-methyl	0.250	100.0 c	100.0 d	100.0 e	100.0 c
Kresoxim-methyl	0.125	100.0 c	93.3 d	93.3 de	100.0 c
Trifloxystrobin	0.250	100.0 c	100.0 d	100.0 e	100.0 c
Trifloxystrobin	0.125	100.0 c	93.3 d	93.3 de	100.0 c
Benomyl	0.500	66.7 b	53.3 b	66.7 cd	80.0 bc
Benomyl	0.250	86.7 bc	73.3 bcd	86.7 de	70.0 b
Benomyl	1.125	80.0 bc	60.0 bc	73.3 cde	71.7 b
Prochloraz	1.000	20.0 a	20.0 a	26.7 ab	43.3 a

[a] Numbers followed by the same letters are not significantly different within each column according to Duncan's multiple range test ($P = 0.05$).

de Nemours), kresoxim-methyl (BAS 49002F, 50% a.i.), prochloraz (Octave, 46.1% a.i., Aventis), trifloxystrobin (A 9360 B or Flint, 50% a.i., Bayer). Chemical treatments were carried out immediately after transplant as a soil drench, by applying 5 l of fungicide suspension/m^2. In most cases only one application was carried out, at transplant. In trial 1, two applications were made, the second one 21 days after the first one.

Those fungicides supplied as wettable powder or granule formulations were manually mixed with the potting media or applied by dusting the soil surface in benches. Emulsifiable concentrate formulations were diluted in 20 l of water/m^3 of substrate for pot application or applied by drenching the soil (5 l/m^2) in the case of benches.

2.4. Disease assessment

Disease incidence was evaluated at regular intervals by counting and eliminating dead plants. Data are expressed as percentage dead plants. All experiments were planned as randomized block design, with three or four replicates (Table 1). The values from the different replicates per treatment in each experiment were arcsin-transformed and analyzed with Duncan's multiple comparison test ($P = 0.05$).

3. Results

3.1. Carnation

The three strobilurins performed well against *F. oxysporum* f. sp. *dianthi*, in the presence of a relatively high disease incidence in most trials, both in growth chamber and under glasshouse. Only in trial 4, was disease pressure lower. They provided good disease control at 1 g/m^2, with results similar or superior to those offered by benomyl at 4 or 5 g/m^2 (Tables 2 and 3). Two applications of 1g /m^2, at 3 weeks interval, with the three tested strobilurins gave results similar to one single application of 2 g/m^2 at transplant (Table 2, trial 1). Similar results were obtained by applying the three tested strobilurins at the rates of 1 or 2 g/m^2 (Table 3, trial 4).

Under our experimental conditions, on the cv Indios, no phytotoxicity was observed.

3.2. Cyclamen

In the presence of a high disease pressure, as shown by the percent of dead plants in the control treatment at the end of the trial, azoxystrobin, particularly at 0.250 g/l of media was effective against Fusarium wilt (Table 4). Kresoxim-methyl did reduce disease incidence; however, this product caused, under our experimental conditions, severe chlorosis and plant stunting. Benomyl and trifloxystrobin at 1 g/l of soil offered unsatisfactory disease control (Table 4).

3.3. Paris daisy

Azoxystrobin provided the best Paris daisy wilt control on all cultivars, in the presence of a high disease pressure at 0.250 g/l of soil (Table 5). Kresoxim-methyl and trifloxystrobin were not effective and caused a severe chlorosis and plant stunting.

In the presence of a high disease pressure (Table 5), benomyl, at 0.5 g/l of soil, only partially controlled the disease. Prochloraz, at 1 g/l of soil was as effective as azoxystrobin, but did cause some slight phytotoxicity symptoms at the tested dosage (Table 5).

4. Discussion

Strobilurins represent a unique new class of fungicides, which inhibit mitochondrial respiration (Mansfield and Wiggins, 1990; Ypema and Gold, 1999). Although primarily developed for control of foliar diseases of major crops, such as grapevine and apple, and more recently investigated for their activity against foliar diseases of vegetables (Gullino, 2000), their unique broad spectrum of activity leaves room for possible interesting applications on a number of other pathogens on several other crops. Although soilborne pathogens are not considered as major targets for strobilurins (Gullino et al., 2000a, b), the good activity shown by such group of fungicides against Fusarium wilts, which are important diseases on a broad range of crops, is of interest for specialty crops.

This study shows the high efficacy and selectivity of azoxystrobin against Fusarium wilts of three major ornamental crops. Of special interest appears to be its activity against wilt of *A. frutescens*, which developed only recently (Garibaldi et al., 1998), causing major concern among Italian growers, due to its rapid spread.

Among the three tested strobilurins, azoxystrobin provided the best and most consistent results, without exhibiting phytotoxicity.

Azoxystrobin and trifloxystrobin were very effective on carnation, with a single soil treatment, for the control of *F. oxysporum* f. sp. *dianthi*, *Rhizoctonia solani* and *Phytophthora nicotianae* (Gullino et al., 2000a, b). The possibility of controlling three important soilborne diseases of carnation with only one treatment carried out at transplant with this group of fungicides looks very promising and interesting, in a cropping system where the level of disease control achieved must be very high. A single treatment at transplant is a practice easily carried out in floriculture.

The effectiveness of azoxystrobin on a number of pathogens affecting ornamental crops has been reported: among others, *Phytophthora cryptogea* on gerbera and *Peronospora statices* on *Goniolimon tataricum* (Skrzypczak and Orlikowski, 1998), *Puccinia horiana* on chrysanthemum (O'Neill and Pye, 1997), *Puccinia lagenophorae* on common daisy (Gullino et al., 1999), *Rhizoctonia solani*, *Pythium* spp. and *Microdochium nivale* on turf (Frank and Sanders, 1994).

Since the Italian floricultural industry is in urgent need of fungicides with novel modes of action, the possibility of applying azoxystrobin is very interesting. The broad activity demonstrated for azoxystrobin would appear to make it an ideal fungicide for many sectors of the industry. In the case of soilborne pathogens, good control (and multiple control in the case of carnation) obtained with a single soil treatment is very interesting and should not lead to overuse of such compound.

Acknowledgements

Work supported by a grant from the University of Torino.

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