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Sensitivity of *Sclerotinia homoeocarpa* isolates from turfgrass in Italy to demethylation-inhibiting (DMI) fungicides and iprodione

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Summary. *Sclerotinia homoeocarpa* F.T. Bennett is one of the most important pathogens affecting high maintenance turfgrass in Italy. Control of this pathogen still relies on the use of fungicides. Twenty nine isolates of *S. homoeocarpa* collected from 13 different golf courses in northern and central Italy were tested *in vitro* for their sensitivity to propiconazole, tebuconazole and iprodione, fungicides registered for use on turfgrass in Italy. Three isolates from untreated turfgrasses were use as reference isolates. The tests were carried out on an agar medium amended with 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 μg mL^{-1} of the active ingredient of each fungicide. Most of the isolates showed reduced sensitivity to the tested fungicides, while maintaining their virulence when inoculated on potted *Agrostis stolonifera* L. Fungicide resistance in this pathogen has increased in Italy compared with the situation 10 years previously.

Key words: turfgrass, fungicide resistance, dollar spot.

Introduction

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is one of the most common diseases on turfgrass in Italy, and worldwide. The attacks are severe on high maintenance turfgrasses, such as greens, tees and fairways of the golf courses, where the pathogen can affect the playability of the surfaces (Beard, 2002). Dollar spot occurs primarily on cool-season grasses, in particular *Agrostis stolonifera* (bentgrass) and *Poa annua,* which are used in all the golf course greens in northern and central Italy. *Lolium perenne* and *Festuca arundinacea* are less sensitive to the pathogen than bentgrass (Watschke *et al*., 1995). The disease symptoms, which appear in early spring and can persist until October, are straw-coloured small patches (2.5–5 cm in diameter) in closely mowed turfs and larger patches up to 25 cm in diameter in less closely mowed lawns. On individual leaves, lesions appear as small spots, with typical reddish-brown borders (Smiley *et al*., 2005).

The control strategies in the case of golf greens and professional football fields, where it is important to maintain turfgrass completely free from spots, rely on the use of fungicides combined with adequate cultural practices, in particular fertilisation programmes and reduction of the duration of leaf wetness. In Italy, dollar spot management is complicated by the scarcity of chemicals registered for use on turf: at present propiconazole, tebuconazole and iprodione are registered for the control of the disease (Mocioni and Gullino, 2009).

Resistance of *S. homoeocarpa* populations to different fungicides has been reported. The resistance of *S. homoeocarpa* to benomyl was observed in North America during the 1970s (Warren *et al*., 1974), followed by resistance to iprodione (Detweiler and Vargas, 1982) and to demethylation inhibitor fungicides (DMI) (Vargas *et al*., 1992;

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Golembiewski *et al*., 1995; Hsiang *et al*., 1997; 2006). More recently, resistance to both thiophanate-methyl and propiconazole has been observed in Ohio (Jo *et al*., 2006), Wisconsin, Massachusetts (Koch *et al*., 2009) and in the north-eastern United States (Putman *et al*., 2010). In Italy, reduced sensitivity to benomyl and in few cases to propiconazole, tebuconazole and iprodione has also been reported (Mocioni *et al*., 2001).

After the last investigation carried out in Italy almost 10 years ago (Mocioni *et al*., 2001), the aim of the present study was to determine the sensitivity of *S. homoeocarpa* isolates from golf courses in northern and central Italy to propiconazole, tebuconazole and iprodione, the only fungicides registered against dollar spot in Italy. In addition the presence of multiple resistance in the same isolates was considered.

Materials and methods

Collection of *Sclerotinia homoeocarpa* isolates

In spring and summer 2009, 29 samples of *Agrostis stolonifera* and *Poa annua,* showing distinct dollar spot lesions, were collected from 13 golf courses located in northern and central Italy. In 12 out of 13 golf courses DMIs and iprodione had been applied in the last 5 years more than five times for the control of dollar spot (Table 1).

Sclerotinia homoeocarpa was isolated from diseased leaf tissues, disinfested in a 0.5% sodium hypochlorite solution, rinsed in sterile water and then placed on potato dextrose agar (PDA) amended with 0.1 g L^{-1} streptomycin sulfate. The plates were kept for 3 days at room temperature, then the colonies resembling to *S. homoeocarpa* were transferred onto clean PDA. The isolates were transferred on PDA slants for storage at 12°C.

Pathogenicity test

The capability of reproducing dollar spot symptoms of all isolates was evaluated by inoculating 1-month-old *A. stolonifera* cv. Penncross plants, grown in pots (10×10 cm). Inoculum was prepared by transferring mycelial plugs of isolates of *S. homoeocarpa* from colonies on PDA to flasks containing moist autoclaved (30 min at 121°C) wheat grain; flasks were incubated for 10 days at 25°C. Infested grains were then removed and used to in-

Table 1. Locations, golf course surfaces and fungicides usage at sites where *Sclerotinia homoeocarpa* isolates were obtained.

Site (province)	No. of isolates	Isolated from	Fungicide(s) applied	No. of treatments in the last 5 years
Pecetto (Torino)	3	Green, Collar	Propiconazole, iprodione	10
Sutri (Viterbo)	4	Green	Propiconazole – tebuconazole, iprodione	5
Rome (Rome)	1	Green	Propiconazole, tebuconazole, iprodione	20
Avigliana(Torino)	5	Green, tee	Propiconazole, tebuconazole, iprodione	10
Avigliana (Torino)	1	Nursery	None	-
Albisola (Savona)	2	Collar	None	-
Grugliasco (Torino)	2	Green	Propiconazole	5
Montorfano (Como)	2	Green, fairway	Propiconazole, iprodione	15
Solbiate Olona (Varese)	2	Green	Propiconazole, tebuconazole, iprodione	15
Casale Monferrato (Alessandria)	1	Green	Propiconazole	7
Cassina Rizzardi (Como)	2	Fairway	Propiconazole	5
Fiano (Torino)	1	Collar	Propiconazole, iprodione	20
Garlenda (Savona)	2	Fairway	Propiconazole, tebuconazole, iprodione	5
Peschiera (Brescia)	1	Green	Propiconazole, tebuconazole, iprodione	20

oculate grass plants by placing five wheat kernels on the soil surface in each pot. Three replicates, arranged in a randomized block design, were used for each isolate. Pots were maintained in a greenhouse at the average temperature of 22°C. Seven days after the inoculation, dollar spot severity was assessed, using a scale from 0 (100% healthy plants) to 5 (100% diseased plants). Data obtained were subjected to analysis of variance and Tukey's Test (P=0.05). The trial was carried out twice, and the data presented are the average of the two trials.

Fungicide screening

Fungicide suspensions were prepared using commercial formulations of propiconazole (Tilt 25 EC, Syngenta Crop Protection, 25% a.i.), tebuconazole (Folicur WG, Bayer CropScience, 25% a.i.) and iprodione (Chipco Green, Bayer CropScience, 25% a.i.). The fungicides were suspended in water and added to molten PDA after sterilisation. To determine the sensitivity of the different isolates of *S. homeocarpa* to the fungicides, 6 mm agar plugs, cut from 1 week old colonies of *S. homoeocarpa* isolates, were transferred onto plates (one plug/plate) containing PDA amended with the different fungicides at 0, 0.01, 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300 μg mL^{-1}. After incubation for 5 days at 21°C, the colony diameter was measured. The results are expressed as the relative growth (colony diameter on fungicide amended medium divided by the diameter on non amended medium multiplied by 100) for each isolate. Then the ED_{50} and ED_{90} values (estimated fungicide concentration needed to inhibit growth by 50 and 90% of control growth respectively) were calculated by regressing the relative growth against the log of the fungicide concentration. The experiments were carried out twice and no differences were observed between the two trials.

Results and discussion

Pathogenicity test

All the isolates of *S. homoeocarpa* reproduced symptoms of dollar spot on potted *A. stolonifera* plants; only one isolate, coming from Montorfano on a fairway, showed a disease index lower than 2. The isolate number 12 showed the highest disease index and it was able to completely kill the plants in the three replications (Table 2).

Fungicide sensitivity

Out of the 29 isolates tested, numbers 1, 2 and 8 are considered reference isolates, since in these cases, the populations of *S. homeocarpa* had not been exposed to the tested fungicides: in the case of Albisola (isolates 1 and 2), where a local law has forbidden the use of fungicides due to the presence of water wells for public use, and Avigliana, where one sample (isolate 8) was collected from a nursery, on a 3-month-old turfgrass. The results obtained for these isolates showed ED_{50} values of 0.01 μg mL^{-1} for propiconazole, between 0.01 and 0.03 μg mL^{-1} for tebuconazole, and between 0.85 and 0.95 μg mL^{-1} for iprodione. The ED_{90} values for these isolate were between 0.53 and 0.74 μg mL^{-1}, 2.18 and 3.79 μg mL^{-1}, 15.91 and 17.26 μg mL^{-1} respectively (Table 3).

Most of the isolates tested gave ED_{50} and ED_{90} values greater than those measured for the three reference isolates (Table 3). For propiconazole, the greatest value of ED_{50} was observed in isolate 3 from Avigliana, but all the isolates from the same golf course (isolates 4, 5, 6 and 7) gave values between 0.09 and 0.28 μg mL^{-1}, and also gave high ED_{90} values. The isolates from Solbiate Olona (isolates 25 and 26), Cassina Rizzardi (isolates 19 and 20) and Montorfano (isolate 29) had reduced sensitivities to propiconazole, with ED_{50} values between 0.05 and 0.18 μg mL^{-1} (Table 3). In the same isolates reduced sensitivity to tebuconazole was observed, as well as in the isolates 22 and 24, which were partially sensitive to the other fungicides, with ED_{50} values between 0.03 and 0.04 μg mL^{-1} for propiconazole and 0.94 and 1.14 μg mL^{-1} for iprodione. ED_{50} and ED_{90} values were in general greater for tebuconazole, as previously observed by Hsiang *et al.* (1997).

In the case of iprodione, the situation was more uniform, with the ED_{90} values between 14.06 μg mL^{-1} (isolate 11) and 36.14 μg mL^{-1} (isolate 21). Half of the isolates gave ED_{50} values greater than 1 μg mL^{-1} and six isolates (isolates 3, 7,19, 20, 21 and 26) gave values greater than 2 μg mL^{-1}, more than double the values for the reference isolates. Isolate 21, which had the greatest ED_{50} value (4.08 μg mL^{-1}), was sensitive to DMI fungicides (Table 3).

The reduced sensitivity to the fungicides can be explained with the increased number of treatments that golf course superintendents are carry-

ing out in the practice with unsatisfactory results. In the last 5 years on the golf course in Rome where isolate 21 was obtained, 20 treatments with the three registered products have been carried out; the same situation occurred for the Peschiera golf course green where isolate 24 was obtained (Table 1).

The isolates 28 and 29, coming from the Peschiera golf course but in different areas (fairway and green), gave different sensitivities to the three fungicides: the isolate from a green (isolate 29) gave greater ED_{50} and ED_{90} values (Table 3).

Table 2. Pathogenicity of *Sclerotinia homoeocarpa* isolates on *Agrostis stolonifera*, expressed as mean disease index, from 0 to 5 (see text).

Isolate number	Disease index
1	3.3 b[a]
2	4.0 b
3	2.7 b
4	3.3 b
5	3.3 b
6	3.0 b
7	4.0 b
8	2.7 b
9	2.3 ab
10	3.7 b
11	4.0 b
12	5.0 bc
13	3.3 b
14	2.3 ab
15	2.3 ab
16	2.7 b
17	4.3 bc
18	3.0 b
19	4.0 b
20	3.7 b
21	3.3 b
22	2.7 b
23	2.3 ab
24	4.3 bc
25	2.7 b
26	2.7 b
27	2.3 ab
28	1.7 ab
29	2.7 b

[a] Means followed by the same letter(s), are not significantly different, Tukey's Test (P=0.05)

Isolates 3, 4, 5, 6, 7 (isolated from Avigliana) 19, 20 (from Cassina Rizzardi), 25 and 26 (from Solbiate Olona) showed doubled resistance to DMIs and iprodione.

Comparing present results with those obtained 10 years previously (Mocioni *et al*., 2001), the problem of the presence of resistance is now more widespread: some golf courses that did not show fungicide resistance, including those at Solbiate Olona, Montorfano and Avigliana, currently have problems of reduced efficacy of some fungicides, because *S. homoeocarpa* populations are now resistant to the registered chemicals. The number of fungicide treatments carried out in recent last years, creating greater selection pressure, increases the risk of reduce sensitivity: in Sutri, where the use of fungicides is minor in comparison with golf courses located in northern Italy, the isolates did not show resistance in the present study and also 10 years previously. Where fungicides have been applied more intensively, such as on greens compared with fairways at Montorfano, the isolates that showed tolerance to DMIs fungicides were also resistant to iprodione.

In the last monitoring carried out at Avigliana, the reduced activity of iprodione was related to accelerated degradation of the fungicide, possibly by soil micro-organisms (Negre *et al*., 1997). Now resistance to the three tested fungicides has been described in all the isolates isolated from that golf course.

The presence of multiple resistance to DMIs and iprodione, the only chemicals registered in Italy for control of dollar spot, suggests that dollar spot management strategies should be modified, reducing or avoiding the use of fungicides but increasing cultural practices. These could include balanced nitrogen fertilisation, modified mowing frequency and clipping collection, and removing dew and guttation water from turf creating a nonconductive environment for the pathogen and development of dollar spot (Mocioni *et al*., 2004). The possible future registration of new compounds, such as boscalid, for control of *S. homoeocarpa*, will be important in order to reduce the risk of fungicide resistance, with the potential for rotation of fungicides with different modes of action.

Table 3. Estimated ED_{50} and ED_{90}, values, expressed as μg mL^{-1}, to propiconazole, tebuconazole and iprodione, for 29 isolates of *Sclerotinia homoeocarpa* isolated from 13 Italian golf courses.

Isolate number	Location	Isolated from	Propiconazole		Tebuconazole		Iprodione	
			ED_{50}	ED_{90}	ED_{50}	ED_{90}	ED_{50}	ED_{90}
1	Albisola	Collar	0.01	0.53	0.01	2.18	0.95	16.83
2	Albisola	Collar	0.01	0.67	0.03	3.79	0.91	17.26
3	Avigliana	Green	0.44	23.01	2.35	42.41	2.41	31.06
4	Avigliana	Green	0.09	20.80	7.88	46.27	1.48	26.56
5	Avigliana	Green	0.19	21.30	0.93	33.82	1.58	28.76
6	Avigliana	Tee	0.28	21.71	2.15	50.63	1.38	26.07
7	Avigliana	Tee	0.27	18.39	0.96	40.16	2.11	28.67
8	Avigliana	Nursery	0.01	0.74	0.02	3.12	0.87	15.91
9	Casale	Green	0.02	5.73	0.04	9.43	0.36	14.15
10	Pecetto	Collar	0.02	0.95	0.01	2.30	0.67	15.86
11	Sutri	Green	0.01	1.80	0.04	3.72	0.76	14.06
12	Sutri	Green	0.01	1.14	0.03	2.84	0.88	15.73
13	Sutri	Green	0.01	1.48	0.02	1.97	0.72	14.13
14	Sutri	Green	0.01	0.74	0.01	0.91	0.64	16.55
15	Garlenda	Fairway	0.03	0.57	0.02	3.21	0.74	14.54
16	Garlenda	Fairway	0.02	0.87	0.01	2.95	1.07	17.53
17	Grugliasco	Green	0.01	1.36	0.02	3.43	0.88	16.58
18	Grugliasco	Green	0.01	1.03	0.05	4.85	1.02	18.30
19	Cassina Rizzardi	Fairway	0.07	8.92	0.90	36.85	3.35	27.95
20	Cassina Rizzardi	Fairway	0.05	9.49	0.17	14.55	3.64	28.62
21	Rome	Green	0.02	2.69	0.03	6.39	4.08	36.14
22	Pecetto	Green	0.03	4.61	0.22	9.54	1.14	16.67
23	Pecetto	Green	0.04	6.31	0.03	2.91	1.14	17.81
24	Peschiera	Green	0.04	5.18	0.15	10.18	0.94	17.84
25	Solbiate Olona	Green	0.18	15.58	0.77	38.04	1.20	27.65
26	Solbiate Olona	Green	0.16	13.89	1.06	40.33	2.14	28.39
27	Fiano	Collar	0.01	1.29	0.02	1.35	1.60	18.89
28	Montorfano	Fairway	0.02	0.58	0.02	2.84	0.81	18.44
29	Montorfano	Green	0.07	12.58	0.39	30.17	0.77	16.23

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