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Biological activity and physical modes of action of the Q_o inhibitor fungicides trifloxystrobin and pyraclostrobin against *Cercospora beticola*

D.A. Karadimos[a], G.S. Karaoglanidis[b,*], K. Tzavella–Klonari[c]

[a]*Hellenic Sugar Industry S.A., Sugar Factory of Larissa, Plant Protection Department, 41110 Larissa, Greece*
[b]*Hellenic Sugar Industry S.A., Sugar Factory of Platy, Plant Protection Department, 59032 Platy Imathias, Greece*
[c]*School of Agriculture, Plant Pathology Laboratory, Aristotelian University of Thessaloniki P.O. Box 269, 54006, Thessaloniki, Greece*

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Abstract

The effect of trifloxystrobin and pyraclostrobin on spore germination and mycelial growth of *Cercospora beticola*, were determined in vitro. In addition, the protective, curative, translaminar and post-symptom activity against the pathogen was determined on sugar beet plants in growth chambers with difenoconazole and chlorothalonil as standard fungicide treatments. Both pyraclostrobin and trifloxystrobin were highly active causing complete inhibition of spore germination at concentrations of 0.01 and 0.1 μg ml^{-1}, respectively, although higher concentrations were required for complete inhibition of fungal growth. Both fungicides were protective against *C. beticola* when applied 96 h and 24 h before inoculation of plants. Both fungicides, applied at 20 μg ml^{-1}, were superior to difenoconazole applied at 10 μg ml^{-1} and to chlorothalonil applied at 100 μg ml^{-1}. Effective control was obtained when strobilurin fungicides were applied 24 h after inoculation, but were less effective at 96 h after inoculation. Pyraclostrobin at 20 μg ml^{-1} applied 96 h after inoculation, was more effective than difenoconazole while control with trifloxystrobin at 20 μg ml^{-1} applied 96 h after inoculation was similar to that obtained with difenoconazole. Chlorothalonil showed little activity against *C. beticola* in curative treatments. Tests to evaluate translaminar activity showed that disease severity on leaves treated with strobilurin fungicides on the upper leaf surface and inoculated on the opposite surface was similar to that obtained on leaves treated and inoculated on the same surface. Anti-sporulant activity was good with both strobilurin fungicides applied at concentrations of 10 or 20 μg ml^{-1} when applied after the appearance of the symptoms. The anti-sporulant activity of difenoconazole was similar to that of pyraclostrobin and trifloxystrobin applied at 5 μg ml^{-1}, while chlorothalonil did not provide significant antisporulant activity. Such results encourage the evaluation of trifloxystrobin and pyraclostrobin under field conditions to select optimal partner fungicides for use in mixtures.

Keywords: Cercospora leaf-spot; Chemical control; Methoxyacrylates; Sugar beet; Strobilurin fungicides

1. Introduction

Cercospora leaf-spot is the most destructive foliar disease of sugar beet (*Beta vulgaris* L.) in areas with humid and warm summers, e.g. in the Mediterranean basin (Rossi et al., 1995). In the absence of control measures the yield losses range from 25 to 50% (Byford, 1996). In Greece, the disease is mainly controlled by fungicide treatments, whereas resistant varieties and crop rotation merely contribute to successful disease control. The protective fungicides chlorothalonil and maneb, as well as the systemic sterol demethylation inhibitors (DMIs) flutriafol, difenoconazole, tetraconazole and cyproconazole are used to control the disease. However, the prolonged and intensive use of DMIs has lead to a significant decrease in sensitivity of the population of the causal organism *C. beticola* in some

*Corresponding author. Tel.: +30-23330-68249.
E-mail address: gkarao@agro.auth.gr (G.S. Karaoglanidis).

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areas of Northern Greece (Karaoglanidis et al., 2000, 2002). To ensure effective control of the disease, management of resistance to DMIs is a prerequisite and this can be accomplished only by a reduction in the number of DMI spray applications (Karaoglanidis et al., 2001a). Consequently, new compounds with novel mode of action have to be introduced into the spray programs to retain effective control of Cercospora leaf-spot of sugar beet.

Q_o inhibiting fungicides (strobilurin and strobilurin-related fungicides) constitute a relatively new fungicide class that has been developed from natural fungicidal derivatives such as strobilurin A, oudemansin A and myxothiazol A (Bartlett et al., 2002). These derivatives are produced in nature by the Basidiomycete fungi *Strobilurus tenacellus* and *Oudemansiella mucida* and the bacterium *Myxococcus fulvus*. Compounds belonging to this class of fungicides include kresoxim-methyl (Ammermann et al., 1992), azoxystrobin (Godwin et al., 1992), metaminostrobin (Hayase et al., 1995), trifloxystrobin (Margot et al., 1998), fenamidone (Mercer et al., 1998), picoxystrobin (Godwin et al., 2000), pyraclostrobin (Ammermann et al., 2000), famoxadone (Sternberg et al., 2001) and fluoxastrobin (Haeuser–Hahn et al., 2002). These fungicides inhibit mitochondrial respiration by binding at the Q_o site of cytochrome *b*. Inhibition of mitochondrial respiration is achieved by blocking the electron transport between cytochrome *b* and cytochrome c_1, which in consequence leads to a disruption of the energy cycle (Anke, 1995; Bartlett et al., 2002). An important, for crop protection, feature of this fungicide class is that it possesses an extremely broad spectrum of activity including *Ascomycetes*, *Deuteromycetes, Basidiomycetes* and *Oomycetes* (Ammermann et al., 1992; Heaney and Knight, 1994; Margot et al., 1998; Ypema and Gold, 1999; Wong and Wilcox, 2001).

Trifloxystrobin and pyraclostrobin are recently developed strobilurin fungicides and till now no information on their efficacy against Cercospora leaf-spot of sugar beet has been published. The current study was conducted: (a) to test the biological activity of these two fungicides against *C. beticola* in vitro and (b) to test the physical modes of action in planta under controlled environment conditions. The DMI fungicide difenoconazole and the protectant fungicide chlorothalonil were included in the study as standard fungicide treatments to allow direct comparisons.

2. Materials and methods

2.1. Fungicides

Trifloxystrobin (Flint 50 WG, Bayer Hellas) and pyraclostrobin (F-500 25 EC, BASF Hellas) were compared with chlorothalonil (Daconil 75 WP, Syngenta Hellas) and difenoconazole (Score 25 EC, Syngenta Hellas). Chlorothalonil was used in the study as a fungicide of known protective activity against *C. beticola* while difenoconazole combines both protective and curative activity.

2.2. Plant material

All the in planta experiments were conducted on *Beta vulgaris* L. cv. Rizor plants, a cultivar which is very susceptible to Cercospora leaf-spot. Plants were grown in the greenhouse (18–26 °C), in plastic pots (18 cm diameter) containing a 2:1 (v : v) mixture of peat and perlite. Plants were fertilized once per week with 1% N:P:K (20:20:20) solution. Each pot contained two plants. Plants used for inoculations were 7–8 week-old, at the stage of 8–10 fully expanded leaves.

2.3. Pathogen

A *C. beticola* isolate, collected during the summer of 2003 from a sugar beet field in Imathia area of Northern Greece, was used to determine the effects of trifloxystrobin and pyraclostrobin on spore germination and mycelial growth. The fungal strain was isolated and maintained on asperigillus completed medium (ACM) composed of 20 g agar (Oxoid, Unipath Ltd, Basingstoke, England), 10 g dextrose (Merck, Darmstadt, Germany) and 1 g yeast extract (Oxoid) per litre.

For the in planta experiments inoculum was obtained from a heavily infected field in the area of Imathia, N. Greece. Diseased leaves were transferred into the laboratory and conidia were washed off from sporulating lesions and used to inoculate the test plants.

2.4. Effects on spore germination and mycelial growth

Autoclaved ACM was amended with 0.0005, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5, 1, 2, 5 and 10 μg a.i. ml^{-1} trifloxystrobin or pyraclostrobin, by adding appropriate volumes of the fungicide stock solutions into the medium, while it was still liquid. Control medium was not amended with fungicides.

To measure spore germination, spores were produced according to a method described previously by Karaoglanidis et al. (2001b). Droplets of conidial suspensions (10×0.1 ml) were transferred on to the medium surface by using a precision pippette. Conidia with germinating tubes longer than the half of the conidium length, were counted after incubation for 24 h at 25 °C, in the dark. One hundred conidia were counted per plate and two replicate plates were prepared for each fungicide concentration tested.

Mycelial growth, was measured using a sensitivity assay technique described previously (Karaoglanidis et al.,