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Newly-assessed fungicides for the control of cypress canker caused by *Seiridium cardinale*

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Summary. Bark canker caused by *Seiridium cardinale* is the most destructive disease of *Cupressus* and several Cupressaceae in many temperate regions and particularly in the Mediterranean area. Chemical prevention represents the most effective and essential means of control to protect plant production in nurseries and young plantations. The European Directive 2009/128/CE and CE 1107/2009 application have drastically reduced the number of chemicals that can be used in agriculture, including the benzimidazolic compounds that had shown the best results in preventing *S. cardinale* canker. It is therefore urgent to find alternative fungicides to replace the banned compounds. The purpose of the present work was to assess some fungicides through *in vitro* tests and pre- and post-inoculation sprayings on *Cupressus sempervirens*, for the control of *S. cardinale*. The active ingredients boscalid, fosetyl-aluminium, triadimenol and azoxystrobin were compared with thiophanate-methyl as reference. The effectiveness of thiophanate-methyl in reducing canker development was confirmed especially when it was applied to trees before they were inoculated with *S. cardinale*. Azoxystrobin was as effective as thiophanate-methyl in the pre-inoculation trials. boscalid prevented conidial germination and mycelial growth of *S. cardinale in vitro* and appeared a promising contact fungicide for the prevention of cypress canker. Azoxystrobin and boscalid are listed in a less hazardous class than thiophanate-methyl, and has less risk for the environment and for users.

Key words: *Cupressus sempervirens*, thiophanate-methyl, boscalid, azoxystrobin, chemical prevention.

Introduction

Seiridium cardinale (Wagener) Sutton and Gibson, is a fungal pathogen that causes a bark canker in Cupressaceae, a serious disease that has become pandemic in many temperate regions over the last five decades (Panconesi, 1990; Graniti, 1998). The incidence and severity of *S. cardinale* attacks have been particularly high in the Mediterranean area, where the disease has caused heavy damage especially to *Cupressus sempervirens* L., widely cultivated in woods, windbreaks, ornamental plantations and nurseries, threatening to alter the appearance of the landscape and with heavy economic losses being reported (Xenopoulos and Diamandis 1985; CABEPPO 2003; Tsopelas *et al*., 2007). *S. cardinale* is a typical wound pathogen, which enters and colonises the cortical tissues through physical injury caused to the bark by frost, hail, birds and insects, especially bark beetles of the genus *Phloeosinus*. Often very small lesions occur at the twig axils where the bark is particularly rich in parenchymal tissues. Control of the disease has been based on integrated pest management strategy which includes sanitation aimed at reducing the inoculum sources in woods and plantations by eliminating infected trees or by cutting off infected organs

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Table 1. Fungicides assessed for control of *S. cardinale* cypress canker and used in *in vitro* trials and in tree sprayings. Concentration of the active ingredient in the commercial product is shown in parentheses. The product trade names are: 1. Enovit Metil® Sipcam; 2. Cantus® Basf; 3. Aliette® Bayer; 4. Bayfidan® Bayer; 5. Quadris® Syngenta.

Active ingredient	Mode of action	Fungicide concentration (L^{-1})[a]
1. Thiophanate-methyl (70%)	Contact and systemic	1.50 g
2. Boscalid (50%)	Contact and translaminar	1.20 g
3. Fosetyl-aluminium (80%)	Systemic	0.96 g
4. Triadimenol (22.9%)	Systemic	0.20 mL
5. Azoxystrobin (22.9%)	Contact and systemic	1.00 mL

[a] Maximum dosage recommended on the product label.

(Parrini, 2003), genetic improvement of cypresses by selecting for resistant genotypes (Raddi *et al*., 1990; Pichot *et al*., 1999; Danti and Panconesi, 2003; Danti *et al*., 2006), and chemical prevention for the protection of young trees in nurseries and plantings. Chemicals are still irreplaceable in the nursery where production of Cupressaceae is economically significant in the Mediterranean area (Ciuti and Biagioni, 1991).

Many studies have evaluated fungicides for the prevention and cure of *S. cardinale* canker in several Cupressaceae (Moriondo and Bonifacio, 1969; Govi *et al*., 1975; Bartoloni *et al*., 1976; Parrini *et al*., 1976; Parrini and Panconesi, 1977; Govi and Tunioli, 1977; Panconesi and Parrini, 1979; Govi and Deserti, 1980; Marchetti and Zechini d'Aulerio, 1982; Panconesi *et al*., 1984; McCain, 1984; Panconesi and Raddi, 1986; Parrini and Panconesi, 1991; Mallams and Petrick, 2004). Most of these studies reported that the best results in preventing infection of the fungus were achieved with systemic benzimidazolic and thiophanate formulas, mixed or alternated with contact fungicides (Parrini and Panconesi, 1977; Panconesi and Parrini, 1979; Govi and Deserti, 1980; Marchetti *et al*., 1982; McCain, 1984; Panconesi and Raddi, 1986; Parrini and Panconesi, 1991; Mallams and Petrick, 2004). None of these fungicides were ever effective against actively growing cankers.

The repeated revisions of EU Directive 91/414, the recent approval of Directive 2009/128/CE on the sustainable use of pesticides, and Regulation CE 1107/2009 which govern the marketing of plant protection products, have drastically reduced the number of chemical compounds that can be used in agriculture. Also benzimidazolics compounds that are effective against *S. cardinale* bark canker have been banned (Benomyl, Carbendazim, etc.).

Seiridium cardinale continues however to be a very important pathogen of the Cupressaceae in the Mediterranean area (Raft, 2008; Tsopelas *et al*., 2008; Danti *et al*., 2009). Chemical prevention is an important tool for the control of cypress canker, especially in nurseries, where high plant density, watering and fertilization favour the spread of the pathogen and disease (Parrini and Panconesi, 1991). Fungicides are also useful to protect young ornamental plantations or monumental trees, that have particular aesthetic or historic value (Panconesi *et al*., 1984).

The objective of the study was to evaluate the efficacy of some fungicides that have not previously been tested against *S. cardinale* cypress canker. Both *in vitro* trials and stem inoculation on cypress trees were conducted. Our goal was to add new and relatively safe compounds to thiophanate-methyl, the only effective compound that can still be used.

Materials and methods

Active ingredients of the fungicides tested were characterised by different action mechanisms (Table 1). Thiophanate-methyl was included as a reference (Pscheidt and Ocamb, 2001), due to its known effectiveness in preventing cypress canker (Panconesi and Raddi, 1986; Mallams and Petrick, 2004). Boscalid is a new chemical in the anylid class, acting as a contact fungicide, and

characterised by a broad spectrum (active against Oomycetes, Ascomycetes and mitosporic fungi) and a translaminar mobility. Fosetyl-aluminium, which promotes the natural plant defences through the production of phytoalexins, is characterised by rapid absorption and a high mobility in both an acropetal and a basipetal direction. Triadimenol is a broad-spectrum triazolic compound with an acropetal and basipetal systemic diffusion within the plant. Azoxystrobin, a strobilurins analogue, is a synthetic molecule (natural substances produced by saprophytic fungi that live on decomposing wood), which also has a broad-spectrum and which is partially absorbed by the trees. Boscalid, fosetyl-aluminium, azoxystrobin and triadimenol are relatively safe and present low risks for operators and for the ecosystem. Fungicides were used at the maximum concentration recommended by the manufacturers (Table 1) in both the *in vitro* and *in vivo* trials.

***In vitro* trials**

Effect of fungicides on S. cardinale *mycelial growth*

To assess their effect on the growth of *S. cardinale* mycelium *in vitro*, fungicides were blended into 2% molten malt extract agar (MEA) maintained at 40°C in a thermostatic bath, and then poured into 9-cm-diameter Petri dishes. A 5-mm-diameter mycelial disc, taken from the margin of colonies of *S. cardinale* (isolate ATCC 38654) grown on 2% MEA for 2 weeks at 25° C in the dark, was placed in the centre of the fungicide-amended 2% MEA. Ten replications for each fungicide were prepared. Colonies grown on non-amended 2% MEA were used as control. The Petri dishes were incubated in the dark at 25°C, and two perpendicular diameters of the colonies were measured after 3 weeks.

Effect of fungicides on S. cardinale *conidial germination*

Acervuli and conidia of *S. cardinale* were previously obtained in 1% MEA Petri dishes containing autoclaved cypress seeds after 3 weeks of incubation at 18° C with a 12 h day under NUV light. Masses of conidia extruding from 2–3 acervuli were dispersed to a final concentration of 1000 conidia mL^{-1}, to Erlenmeyer flasks each containing 10 mL of 2% ME plus one of the fungicides, and then placed in a stirrer (80 rpm) at room temperature. For each fungicide, after 24 h, three replications of 100 μL of the conidial suspension were separately pipetted on a slide and inspected under a light microscope at 400× to evaluate the number of germinated conidia. Approximately 100 conidia were examined for each replication. Germination of conidia in non-amended 2% ME was evaluated as control in the same way.

All the *in vitro* trials were conducted twice.

***In vivo* trials**

Cypress trees were tested in a greenhouse (Temp. 20±5°C; RH, ~80%) to evaluate *in vivo* the effect of each fungicide on *S. cardinale* canker in stem-inoculated ramets of the *C. sempervirens* susceptible clone PM 296 (IPP-CNR collection). A group of 192 3-year-old, 80-100-cm-tall grafted saplings were grown in pots containing peat, compost, and perlite (3:1:1, v:v:v). The saplings were subdivided into two groups of 96 each, one for pre-inoculation treatment and one for post-inoculation treatment.

Pre-inoculation treatment

In October 2009, each of the five tested fungicides was separately sprayed to run off on a subgroup of 16 saplings. Another subgroup of 16 saplings was sprayed with distilled water as a control. For each subgroup, eight saplings were stem-inoculated with *S. cardinale* mycelium 10 days after treatment; the other eight were stem-inoculated 20 days after treatment. Inoculations were made where the stem had a diameter of about 1 cm, using *S. cardinale* isolate ATCC 38654. A 3-mm-diameter circular plug of bark was removed with a cork borer and replaced with a plug of the same size from the margin of a colony of the fungus grown on potato dextrose agar (PDA) for 2 weeks at 25° C in the dark. The inoculation site was covered with cotton and wrapped with tape, both of which were removed after 1 week (Danti *et al*., 2006).

Canker length and width (along the stem circumference), were measured 4 months after inoculation by removing the outer bark with a scalpel to expose the necrotic cortical tissue underneath. The canker area was measured using the formula: $\pi \cdot a \cdot b$ where a and b are the semi-major and the semi-minor axis of an ellipse respectively.

Post-inoculation treatment

In October 2009, 96 cypress saplings were stem-inoculated with *S. cardinale* as above. Five

Table 2. Diameters of *Seiridium cardinale* colonies on 2% MEA amended with fungicides after 3 weeks at 25°C. The control was non-amended 2% MEA. Percentage growth inhibition was determined for each fungicide with reference to growth on non-amended MEA (subtracting the diameter of the mycelium plugs). The mycelium plugs used to initiate colonies were 5 mm in diameter.

Active ingredient	Colony diameter (mm)	SE	Mycelial growth inhibition (%)
Thiophanate-methyl	5.0 a[a]	0	100
Boscalid	5.3 a	0.13	99.6
Fosetyl-aluminium	18.6 b	0.91	81
Triadimenol	24.1 c	0.62	68.5
Azoxystrobin	25.4 c	0.54	71.4
Control	76.2 d	0.81	_

[a]Mean values with different letters differ significantly ($P<0.01$) according to the one-way ANOVA HSD test.

subgroups of 16 saplings where then sprayed to run off, each, with one of the fungicide. Eight of the saplings were sprayed 10 days after inoculation, and the other eight 20 days after inoculation. A control group of 16 saplings was sprayed with sterilized water, half 10 days and half 20 days after inoculation.

Phytotoxicity

The phytotoxic effect of the fungicides was assessed as follows: six saplings were sprayed to run off with one of the fungicides three times in a month (ten-day intervals), placed outdoors, and their crown regularly inspected for foliage discoloration, foliage and bark lesions or dead tips. As a control, distilled water was sprayed at the same intervals on another group of six saplings.

Data were subjected to one-way analysis of variance (ANOVA), Tukey HSD (Honestly Significant Difference) was performed for a post-ANOVA pair-wise comparison using STATISTICA® 6.0 software. Percentages were converted with the Bliss formula prior to analysis.

Results

***In vitro* trials**

Effect of fungicides on S. cardinale *colony growth*

All the fungicides significantly reduced ($P<0.01$) the colony diameter of *S. cardinale* on 2% MEA, as compared to the control (Table 2). Thiophanate-methyl and boscalid completely prevented colony growth.

Effect of fungicides on S. cardinale *conidial germination*

All the fungicides substantially reduced germination of conidia compared to the control ($P<0.01$) (Table 3). Boscalid was the most effective completely preventing conidial germination. The least effective was Axoxystrobin which reduced germination by 23.8%.

***In vivo* trials**

Pre-inoculation treatment

When fungicides were sprayed 10 days before pathogen inoculation, the resulting cankers were significantly smaller ($P<0.01$) in trees sprayed with thiophanate-methyl, azoxystrobin and boscalid than in the control trees (Table 4). Triadimenol and fosetyl-aluminium did not significantly affect the canker size (Table 4).

When inoculations were made 20 days after treatment, the smallest cankers again occurred in trees treated with thiophanate-methyl; however, these cankers did not differ significantly in size from cankers in trees sprayed with triadimenol or azoxystrobin (Table 4). Canker size in trees sprayed with boscalid did not differ significantly from that in the control trees.

In trees sprayed with thiophanate-methyl, the mean canker area was significantly smaller ($P<0.01$) when trees were inoculated 20 days after treatment than in plants inoculated 10 days after spraying than when they were inoculated 20 days after spraying. (Table 4). In contrast, with boscalid the cankers were significantly smaller ($P<0.05$)

Table 3. Mean percentages of germinated conidia of *Seiridium cardinale* after 24 h at room temperature in 2% ME amended with one of each fungicide. The control was non-amended 2% ME. Mean values with different letters were significantly different ($P<0.01$) according to the ANOVA one-way HSD test.

Active ingredient	Germinated conidia (%)	SE
Thiophanate-methyl	7.9 a[a]	0.14
Boscalid	0.0 a	0.00
Fosetyl-aluminium	7.2 a	0.05
Triadimenol	11.8 ab	0.10
Azoxystrobin	23.6 b	0.05
Control	77.1 c	0.08

[a] See Table 2.

Table 4. Pre-inoculation spraying. Mean area of cankers on the stems of *Cupressus sempervirens* saplings 4 months after inoculation with *Seiridium cardinale*. Plants were sprayed with fungicides 10 or 20 days before being pathogen-inoculated. Mean values with different letters within each column were significantly different ($P<0.01$) according to the ANOVA one-way HSD test.

Fungicide	Canker size (mm^2)			
	Inoculation made 10 d after fungicide spraying	SE	Inoculation made 20 d after fungicide spraying	SE
Thiophanate-methyl	69.3 a [a]	8.07	23.7 a [a]	3.36
Boscalid	302.8 ab	19.12	709.5 cd	8.06
Fosetyl-aluminium	787.1 d	16.36	495.2 bc	6.23
Triadimenol	408.7 bc	17.44	245.2 ab	10.18
Azoxystrobin	60.7 a	7.48	264.9 ab	9.01
Control	700.0 cd	9.51	962.3 d	3.52

[a] See Table 2.

when trees where inoculated 10 days after spraying than in trees inoculated 20 days after spraying.

Post-inoculation treatment

Cankers in trees sprayed with Thiophanate-methyl and Azoxystrobin 10 days after inoculations were significantly smaller ($P<0.01$) than cankers in trees sprayed with the other fungicides and than those in control trees. In trees sprayed with triadimenol, boscalid or fosethyl-aluminium, the mean canker size did not differ significantly from that of the control trees (Table 5). When trees were sprayed 20 days after inoculations, canker areas did not differ significantly from that of the control trees (Table 5).

Phytotoxicity

None of the fungicides produced visible symptoms of toxicity in the trees such as discoloured foliage, dead tips, lesions on the foliage or cracks in the bark, when sprayed three times a month (10-day intervals), at the concentrations indicated in Table 1.

Discussion

Copper compounds were not considered here, since previous studies have yielded conflicting results on their effectiveness against bark canker caused by *S. cardinale* (Govi *et al*., 1975; McCain, 1984; Panconesi and Raddi, 1986; Mallams and

Table 5. Post-inoculation spraying. Mean area of cankers measured on the stems of *Cupressus sempervirens* saplings 4 months after inoculation with *Seiridium cardinale*. Plants were sprayed with fungicides 10 or 20 days after being inoculated. Mean values with different letters within each column were significantly different ($P<0.01$) according to the ANOVA one-way HSD test.

Fungicide	Canker size (mm^2)			
	Fungicide spraying 10 d after pathogen-inoculation	SE	Fungicide spraying 20 d after pathogen-inoculation	SE
Thiophanate-methyl	107.5 a[a]	11.40	743.8 a[a]	13.32
Boscalid	830.5 d	1.56	853.3 a	11.44
Fosetyl-aluminium	394.1 bc	2.32	307.2 a	21.14
Triadimenol	470.2 bc	13.25	825.4 a	22.46
Azoxystrobin	187.2 ab	6.48	531.2 a	23.34
Control	582.3 cd	18.45	582.3 a	18.45

[a] See Table 2.

Petrick, 2004). Copper compounds were also at times toxic to cypress (Govi and Tunioli, 1977; Mallam and Petrick, 2004). Moreover, in *in vitro* tests, *S. cardinale* showed low sensitivity to cupric ions, which had no effect on either its spore germination or on radial colony growth even when the compounds were applied at concentrations as high as 100 g L^{-1} (McCain, 1984).

All fungicides tested prevented or slowed down significantly the radial growth of *S. cardinale* colonies on 2% MEA. Only thiophanate-methyl was previously reported to inhibit the growth of *S. cardinale* mycelium *in vitro* (Panconesi *et al*., 1984; Mallams and Petrick, 2004). The other products tested in this study, have not been tested on *S. cardinale* before.

The low conidial germination achieved with Thiophanate-methyl contrasts with previous studies on this fungicide and other systemic benzimidazolics which found that these fungicides almost completely inhibited conidial germination of *S. cardinale* (McCain, 1984; Panconesi *et al*., 1984). In those studies these fungicides exerted their effect almost exclusively during the processes of post-germinative synthesis by mean of a biotransformation *in vivo*. This discrepancy between our findings and earlier studies may be due to differences in the experimental conditions. In the present work, the germination test was done on a liquid substrate containing the fungicide at the concentration of 1.5 g L^{-1}, while in Panconesi *et al*. (1984) the suspension containing the fungicide was sprayed over solidified agar surface on which the conidia had been placed, and concentrations from 0.05 to 1.0 g L^{-1} were used. It is probable that the addition of the fungicide to the liquid substrate favoured its absorption by the conidia (during the swelling) and thus made its effect more rapid, hampering germination of the conidia.

The effectiveness of Thiophanate-methyl as a preventive agent has been reported in several studies (Govi *et al*., 1975; Parrini *et al*., 1976; Mallams and Petrick, 2004). In the present work a single application of this fungicide only produced small necrosis. This may have been due to the different experimental procedures adopted here. In our study, trees where sprayed with fungicides and were stem-inoculated with *S. cardinale* 10 and 20 days later. In Mallams and Petrick (2004) trees were stem-wounded and treated with fungicide 24 h before being inoculated, while Parrini *et al*. (1976) and Govi *et al*. (1975) inoculated the trees with *S. cardinale* and then sprayed them with the fungicide at regular intervals.

In this work when Thiophanate-methyl was sprayed 20 days before *S. cardinale* inoculation smaller cankers were produced than when it was sprayed only 10 days before, thus indicating that the effectiveness of the product was time-dependent. Thiophanate-methyl sprays must have been absorbed, transformed and accumulated in the cortical tissues to a greater extent, after 20 days than 10 days after the treatment. This supposition is supported by several studies which reported that the fungicidal effect of the benzimidazolics and thiophanate-methyl on the incidence and de-

velopment of various diseases on apple tree, sugar cane, cowpea, tomato persisted for some weeks or even six months (Cirulli and Montemurro, 1978; Sharma and Sohi, 1980; Clifford *et al*., 1987; Malathi *et al*., 2004).

When sprayed 10 days before *S. cardinale* inoculations azoxystrobin showed results similar to those of thiophanate-methyl but it was less persistent. Compared to the other fungicides, azoxystrobin was more effective when applied to trees, than in the *in vitro* trials probably because it was more easily absorbed by the trees and more active in living tissues. Reuveni and Sheglov (2002) similarly reported that *in vitro* Azoxystrobin was less effective in inhibiting mycelial growth and conidial germination of *Alternaria alternata* than other fungicides, while it was very effective in the field when sprayed on trees to control apple decay caused by the same fungus. Aguín *et al*. (2006) reported that, 1 g L^{-1} Azoxystrobin added to PDA inhibited *Armillaria mellea* mycelium just as well as it inhibited *S. cardinale* in the present study. Azoxystrobin (and all strobilurins analogues) inhibits mitochondrial respiration by blocking electron transfer at the cytochrome bc1 complex (Anke, 1995). Some pathogens induce an alternative oxidase respiratory pathway making their mycelium less sensitive to the strobilurins (Olaya *et al*., 1998; Reuveni and Sheglov, 2002). This alternative oxidase respiratory pathway is utilized by fungi growing on agarised media, and this could explain why *S. cardinale* is less sensitive to azoxystrobin when growing on MEA amended with this compound.

When sprayed after inoculation of *S. cardinale* fungicides generally had no or only minimal effect on active growing bark cankers. Only thiophanate-methyl and azoxystrobin when sprayed 10 days after *S. cardinale* inoculation significantly reduced the canker size as compared with the control. Thiophanate-methyl and azoxystrobin, in addition to a preventive action, seem to slow down canker growth at the early stages of bark colonization by *S. cardinale*. When sprayed 20 days after *S. cardinale* inoculation, none of the fungicides had a significant effect on canker size as compared to the control. Panconesi and Parrini (1979) reported that inoculations that didn't result in symptom development were 92, 42, 10 and 12% when thiophanate-methyl was sprayed at intervals of 3, 7, 14 and 21 days after inoculation respectively; thus this fungicide have some therapeutic effect when applied very frequently.

Fosetyl-aluminium was more effective when tested *in vitro* than when it was sprayed on the trees. This fungicide is well-known for stimulating the production of phytoalexins by the tree, thus promoting its defence mechanisms, but it might require repeated sprayings over time to be effective. The fact that cankers in trees sprayed 20 days before *S. cardinale* inoculation were smaller than cankers on trees sprayed only 10 days before inoculation seems to support this hypothesis.

Boscalid is a contact and translaminar fungicide, and in the *in vitro* tests inhibited both colony growth and conidial germination of *S. cardinale*. Trees sprayed with boscalid reduced canker size somewhat when it was sprayed 10 days before inoculation. Boscalid may, at least in partly be absorbed into the living tissues of the bark, and act against the fungus there. Boscalid did not affect canker size when sprayed 20 days before the inoculation, probably because, as a contact and translaminar fungicide, it was not absorbed into the inner bark and its fungicidal effect decreased quickly with time.

In previous studies thiophanate-methyl, Chlorotalonil, copper hydroxide and calcium polysulphide were toxic on young cypress trees (Govi *et al*., 1975; Mallams and Petrick, 2004). In Mallams and Petrick (2004) thiophanate-methyl caused toxic symptoms (bark cracks) in *Chamaecyparis lawsoniana* plants, but the concentration of the active ingredient sprayed was higher (1.8 g L^{-1}) than that in our study (1.5 g L^{-1}). In this study none of the fungicides were toxic to *C. sempervirens* at the maximum dosage recommended by the manufacturers (Table 1).

Although the tested fungicides generally did not prevent cankers on inoculated stems, cankers that arose were smaller when azoxystrobin, boscalid or thiophanate-methyl were sprayed on the trees 10 days before inoculations. The fungicides thus acted against *S. cardinale* within the tree. The fact that azoxystrobin acts inside the cortical tissues is important, since infections are often caused by the bark beetle vector *Phloeosinus* sp. (Covassi *et al*., 1975), which tunnels into the bark and thus inoculates the tree from within. Boscalid strongly inhibited both mycelial growth and conidial ger-

mination of *S. cardinale in vitro* and may inhibit *S. cardinale* inoculum on plant surfaces. Boscalid may also be effective in disinfecting wounds during sanitation.

Probably, therefore regular spraying every 15–20 days during the seasons most favourable to *S. cardinale* spread (spring and autumn), will reduce the number of new infections. Azoxystrobin and boscalid are in a class of less hazardous fungicides, and are relatively safe for the environment and for operators. We suggest that sprays with azoxystrobin, a systemic fungicide, should be alternated with boscalid, to reduce the likelihood of mutants in the pathogen population that overcome the protection provided by a single product. In fact a lowered sensitivity to azoxystrobin correlated with some site-specific pathogen mutations has already been detected on *Alternaria solani* (Rosenzweig *et al*., 2008), *Magnaporte grisea* (Avila-Adame and Köller, 2003) and *Penicillium digitatum* (Zhang *et al*., 2009) after they were treated repeatedly with azoxystrobin and on *Alternaria alternata* (Avenot and Michailides, 2007) after it was treated repeatedly with boscalid.

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