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Reaction of hybrids, inhibition *in vitro* and target spot control in cucumber

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ABSTRACT

The fungus *Corynespora cassiicola* is the causal agent of target spot in cucumber. Under favorable climatic conditions it can cause serious damage in this horticultural crop. In Brazil, there exists not enough knowledge to determine efficient control measures to the disease. This investigation was carried out to evaluate: a) the reactions of nine cucumber hybrids to *C. cassiicola*; b) the sensibility of six isolates of *C. cassiicola* to fungicides *in vitro* (captan, chlorothalonil, mancozeb, azoxystrobin, difenoconazole, carbendazin, tebuconazole and thiophanate-methyl), used in concentrations of 0, 1, 10, 100 and 1,000 µg mL^{-1} of active ingredient and c) protective and curative chemical treatments with the same fungicides used *in vitro* in cucumber plants inoculated with *C. cassiicola*. The cucumber hybrids were evaluated using the scale of notes and diagrammatic of target spot severity. The more resistant hybrids to the pathogen were Taisho, Nikkey, Yoshinari and Safira. The difenoconazole fungicide caused the most mycelial growth inhibition (MGI) and showed the lowest ED_{50}. Thiophanate-methyl was the worst fungicide, it did not inhibit the mycelial growth of the fungus. Azoxystrobin was the most efficient in controlling the disease, although it has to be registered in Ministry of Agriculture, Livestock and Food Supply in Brazil before its recommendation.

Keywords: *Cucumis sativus*, *Corynespora cassiicola*, sensitivity to fungicides, genetic resistance.

RESUMO

Reação de híbridos, inibição *in vitro* e controle da mancha alvo em pepino

O fungo *Corynespora cassiicola*, agente causal da mancha alvo em pepino, pode, sob condições de alta temperatura e alta umidade, causar sérios danos à cultura. No Brasil, não se tem conhecimento suficiente sobre um manejo adequado dessa doença. Este trabalho foi realizado visando avaliar: a) a reação de nove híbridos de pepino desafiados por *C. cassiicola*; b) a sensibilidade *in vitro* de seis isolados de *C. cassiicola* a fungicidas (captan, clorotalonil, mancozeb, azoxystrobin, difenoconazole, carbendazin, tebuconazole e tiofanato-metílico), utilizados nas concentrações de 0, 1, 10, 100 e 1.000 µg mL^{-1} de ingrediente ativo e c) o tratamento químico preventivo e curativo com os mesmos fungicidas testados *in vitro*, em plantas de pepino, inoculadas com *C. cassiicola*. A severidade foi avaliada utilizando escalas de notas e diagramática de severidade da mancha alvo. Os híbridos mais resistentes ao patógeno foram Taisho, Nikkey, Yoshinari e Safira. O fungicida difenoconazole proporcionou as maiores inibições de crescimento micelial (ICM) do patógeno *in vitro* e a menor dose efetiva capaz de inibir o crescimento micelial em 50% (DE_{50}); já tiofanato-metílico foi o pior, sendo incapaz de inibir o crescimento micelial do fungo. Quanto à aplicação dos fungicidas de forma preventiva e curativa, em plantas de pepino, azoxystrobin foi o mais eficiente no controle da doença, porém para sua utilização há necessidade de seu registro junto ao Ministério da Agricultura, Pecuária e Abastecimento.

Palavras-chave: *Cucumis sativus*, *Corynespora cassiicola*, sensibilidade a fungicidas, resistência genética.

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In Brazil, the fungus *Corynespora cassiicola*, pathogen of target spot in the cucumber, has been detected in the States of São Paulo, Paraná and Goiás, in "Japanese-type" cucumbers (Martins *et al*., 2003; Verzignassi *et al*., 2003; Teramoto *et al*., 2006). Disease symptoms appear on the oldest leaves as angular, yellowish spots that grow and become circular with a light brown center and dark edges (Kurosawa *et al*., 2005). Spot coalescing can dry extensive areas of the leaf limbo, with consequent leaf fall from the plant (Verzignassi *et al*., 2003; Kurosawa *et al*., 2005), and can lead to yield losses of up to 60% (Verzignassi *et al*., 2003).

Crop management practices, such as eliminating crop remains, greater between-plant spacing and management of greenhouse side curtains have not significantly reduced the disease severity. Furthermore, the fungicides used to control other leaf spots in cucumber have not presented practical results for the control of this disease (Verzignassi *et al*., 2003).

Research to assess the chemical control of target spot in cucumber has become necessary because most studies have been carried out in other countries, such as, for example, the United States (Jones, 1974; Jones, 1978; Sumner *et al*., 1981), Mexico (Castro, 1979), Japan (Hasama, 1991; Date *et al*., 2004) and Cuba (González, 2005). In Brazil, studies on control of the disease have been limited to assessing product efficiency *in vitro* (Teramoto *et al*., 2004; Ueda *et al*., 2008).

There is also no precise information on the genetic resistance of *C. cassiicola*. There are no descriptions of this characteristic for the cucumber hybrids cultivated in Brazil in the seed company

catalogs, except for the Taisho cucumber hybrid that has been reported with field resistance (www.sakata.com.br) and the Natsuhikari and Tsuyoi hybrids, reported as resistant but whether the resistance is vertical or horizontal has not been specified (www.takii.com.br). Oliveira *et al*. (2006) tested four hybrids against various isolates of the pathogen and reported variation in the severity they presented. The authors stated that the Tsuyataro hybrid was considered the most susceptible to target spot among the hybrids tested and the Natsubayashi hybrid the most resistant.

Thus the objectives of the present study were to assess: a) cucumber hybrid reaction to target spot; b) *in vitro* sensitivity of six *C. cassiicola* isolates to eight fungicides and c) preventive and curative chemical treatment with fungicides in cucumber plants inoculated with *C. cassiicola* in a greenhouse.

MATERIAL AND METHODS

These studies were carried out in the laboratory and experimental area of the Research Nucleus in Plant Pathology, belonging to the Agronomy and Food Engineering College of the Federal University of Goiás, in Goiânia.

Six *C. cassiicola* isolates were used in the experiments, PESP01, PESP02, PESP04, PESP05, PESP06 and PEGO07. The PESP01 isolate came from Indaiatuba, São Paulo State; PESP02 and PESP04 from Promissão, São Paulo State; PESP05 from Piedade, São Paulo State; PESP06 from Mogi das Cruzes, São Paulo State and PEGO07 from Goiânia, Goiás State. All the isolates were obtained from cucumber leaves with typical target spot symptoms, isolated first in agar-agar (AA), followed by replication of the edges of the mycelia growth to potato-dextrose-agar (PDA) and later preserved in PDA covered with mineral oil.

Cucumber hybrid reaction to target spot - The cucumber hybrids were Hokuho, Natsusuzumi, Nikkey, Rensei, Safira, Supremo, Taisho, Tsuyataro and Yoshinari. Except for Safira, which is land race type, and Supremo, an industrial type, the others are Japanese-type cucumbers. A complete randomized experimental design was used with nine treatments (hybrids) and four replications, and each one consisted of one pot containing two plants. The 1.0 L pots were filled with sterilized soil. When the cucumber plants had two true leaves, they were inoculated by spraying a suspension of 10^4 conidia/mL of *C. cassiicola* of the PESP04 isolate. This isolate was chosen because it was sporulating abundantly and the conidia were sprayed until there was surface runoff. After inoculation, the pots were kept in a wet chamber for 24 hours covered with plastic bags and then placed in a simple arch-type greenhouse with the roof covered with transparent polyethylene and the sides with insect-proof screening.

The disease severity (% diseased area) was assessed on the two oldest leaves of the plant after the appearance of the first symptoms, seven, 11 and 15 days after plant inoculation, using the modified Horsfall-Barratt scale (Campbell & Madden, 1990) with the following scores: 0: no symptoms, 1: <1% of the leaf area with symptoms (las), 2: 1-3% las, 3: 3.1-6% las, 4: 6.1-12% las, 5: 12.1-25% las, 6: 25.1-50% las and 7: >50.1% las. The obtained data were submitted to analysis of variance using the SISVAR 5.1 program (Ferreira, 2008) and when significant, the means of the treatments were discriminated by the Scott-Knott test at 5% probability.

In vitro Corynespora inhibition - The following protective fungicides were tested for *in vitro* inhibition in the laboratory: captan, chlorothanonil and mancozeb and the systemic fungicides azoxystrobin, difenoconazole, carbendazin, tebuconazole and thiophanate-methyl at the concentrations 0, 1, 10, 100 and 1.000 µg mL^{-1} active ingredient (a.i.). Most of these fungicides are registered in the Ministry of Agriculture, Livestock and Supply, for the cucumber crop, but for other fungus diseases. Only captan and carbendazin are registered for other crops and other pathogens. The fungicides were prepared previously in 10 mL stock solution in sterilized water of each concentration of each product in test tubes, before being incorporated into the PDA culture medium. The 1.000 µg mL^{-1} concentration was the first fungicide to be prepared and the calculated quantity of fungicide was added to the sterilized water, shaken, and then 1.0 mL^{-1} of this was transferred to another tube containing 9 mL water (dilution in series). This procedure was repeated until the lowest concentration was obtained (1 µg mL^{-1}). The control plates contained only PDA. After preparing the culture medium with the respective fungicide concentrations, 6 mm mycelia discs of the PESP01, PESP02, PESP04, PESP05, PESP06 and PEGO07 *C. cassiicola* isolates were removed from the edges of the colonies when approximately 10 days old and transferred to the different culture media (with and without fungicide). The plates were incubated at 25°C in continuous darkness. Each treatment consisted of three replications and each plate was one replication. A complete randomized design was used. The mycelia growth was calculated by the mean of the radius of two transverse diameters every two days for 10 days. The mycelia growth inhibition was determined with the data obtained from the last reading: MGI= 100-(concentration radius i x 100)/concentration radius 0, where i corresponds to the concentration radius tested. Next the effective dose capable of inhibiting mycelia growth by 50% (ED_{50}) was estimated for each treatment using the parameters calculated by the regression of the MGI versus $\log_{10}$ of the fungicide concentration. The ED_{50} was calculated for each isolate corresponding to the fungicide. The fungicides were ranked for toxicity according to the parameters adopted by Edgington & Klew (1971), who considered highly toxic the fungicide that obtained ED_{50} <1 µg mL^{-1}; from 1-50 µg mL^{-1}, moderately toxic, and, >50 µg mL^{-1}, non toxic. This experiment was repeated twice.

In vivo target spot control - The *in vivo* chemical control was carried out in a protected environment and the same fungicides were tested as in the *in vitro* test at the concentrations recommended by the manufacturers for the cucumber crop, but for other fungus diseases. A 250 mL solution

was prepared of each fungicide at the following concentrations: azoxystrobin (7.5 g a.i. 100 L^{-1} H_2O); captan (113.5 g a.i. 100 L^{-1} H_2O); carbendazin (250 g a.i. 400 L^{-1} H_2O); chlorothanonil (150 g a.i. 100 L^{-1} H_2O); difenoconazole (5 g a.i. 100 L^{-1} H_2O); mancozeb (2.0 kg a.i. 100 L^{-1} H_2O); tebuconazole (200 g a.i. 500 L^{-1} H_2O) and thiophanate-methyl (50 g a.i. 100 L^{-1} H_2O).

Two plants of the Tsuyataro and Nikkey cucumber hybrids were used per 1.0 L pot filled with sterilized soil and each replication consisted of three pots. These hybrids were chosen because they belong to the group that presented greatest and least susceptibility, respectively, when challenged by *C. cassiicola*. A complete randomized design was used.

a) Preventive treatment - When the plants reached two true leaves they were sprayed with the contact and systemic fungicides quoted previously. They were inoculated 24 hours after product application by spraying with a spore suspension of the PESP04 isolate of *C. cassiicola*, at the concentration of 10^4 conidia/mL, until there was surface runoff. This isolate was chosen because it was sporulating abundantly. After inoculation, the pots were kept in a moisture chamber for 24 hours and then in a greenhouse until the end of the experiment.

The disease severity (% diseased area) was assessed on the two oldest leaves of each plant, seven and 14 days after inoculation, using the diagrammatic scale set out by Teramoto *et al*. (2011) that has seven severity levels: 0.3; 0.8; 2; 5; 11.5; 25 and 46%.

The data obtained were transformed in $(x + \alpha)^{½}$ and submitted to analysis of variance. When significant the means were discriminated by the Tukey test at 5% probability. This experiment was repeated twice.

Curative treatment - To assess the curative effect of the systemic fungicides, as soon as the plants reached two true leaves they were inoculated with a suspension of spores of the PESP04 *C. cassiicola* isolate, as reported previously. After inoculation, the pots remained in a moisture chamber for 24 hours and were kept in a greenhouse until the end of the experiment.

The disease severity was assessed on the two oldest leaves of each plant, seven and 14 days after inoculation, using the scale elaborated by Teramoto *et al*. (2011).

The data obtained were transformed in $(x + \alpha)^{½}$ and submitted to analysis of variance. When significant, the means were discriminated by the Tukey test at 5% probability. This experiment was repeated twice to confirm the results.

RESULTS AND DISCUSSION

Cucumber hybrid reaction to target spot - Considering the modified Horsfall-Barratt (Campbell & Madden, 1990) scale, three different susceptibility groups were observed seven days after inoculation (DAI): a) susceptible, Tsuyataro and Supremo; b) moderately susceptible, Hokuho and Natsusuzumi and, c) moderadately resistant, Rensei, Yoshinari, Safira, Nikkey and Taisho. At 11 DAI, only Rensei went to the group of moderately susceptible, the other cultivars maintained the previous classification. At 15 DAI, only Taisho continued as moderately resistant and Yoshinari, Nikkey and Safira went to the moderately susceptible group. Hokuho, Rensei and Natsusuzumi became part of the susceptible group together with Tsuyataro and Supremo (Table 1).

These results corroborated the data obtained by Oliveira *et al*. (2006), who also observed greater susceptibility of the Tsuyataro cultivar to the isolates and hybrids tested. In the present experiment, in addition to the Tsuyataro cultivar, the Supremo cultivar was shown to be one of the most susceptible up to 11 DAI. After 15 days, other cultivars were included in the greater susceptibility group, such as Hokuho, Rensei and Natsusuzumi.

Regarding the experiments carried out by Oliveira *et al*. (2006), the most resistant cultivar was Natsubayashi with severity scores ranging from 2.0-2.33. In contrast in the present experiment, the most resistant cultivars at seven DAI were Taisho, Nikkey, Safira, Yoshinari and Rensei, and all except for Rensei presented scores well below 2.0, thus demonstrating that the cultivars tested had a good resistance level.

The Taisho cultivar, reported with field resistance to *C. cassiicola*, really

Table 1. Severity of target spot caused by *Corynespora cassiicola* in cucumber hybrids at seven, eleven and fifteen days after inoculation (severidade da mancha alvo causada por *Corynespora cassiicola* em híbridos de pepino aos sete, 11 e 15 dias após a inoculação). Goiânia, UFG, 2007.

Cucumber hybrids	Severity[1]		
	7 days	11 days	15 days
Yoshinari	1.5[2] c	3.0 c	4.0 b
Rensei	2.2 c	4.8 b	6.5 a
Hokuho	4.2 b	5.2 b	5.8 a
Taisho	0.5 c	0.8 c	1.5 c
Safira	1.5 c	3.0 c	4.7 b
Nikkey	1.3 c	2.5 c	4.5 b
Supremo	5.2 a	6.8 a	7.0 a
Natsusuzumi	3.3 b	4.8 b	6.5 a
Tsuyataro	5.8 a	6.5 a	6.8 a
CV (%)	45.7	37.8	33.5

[1]Scale of notes: 0= no symptoms; 1= <1% of foliar area with symptoms (fas); 2= 1 to 3% of fas; 3= 3.1 to 6% of fas; 4= 6.1 to 12% of fas; 5= 12.1 to 25%; 6= 25.1 to 50% of fas; 7= >50.1% of faz; [2]Means followed by the same letters in the column are not statistically different by Scott-Knott ($p<0.05$); Means of three repetitions ([1]escala de notas: 0= ausência de sintomas; 1= <1% de área foliar com sintomas (afs); 2= 1 a 3% de afs; 3= 3,1 a 6% de afs; 4= 6,1 a 12% de afs; 5= 12,1 a 25% de afs; 6 = 25,1 a 50% de afs; 7= >50,1% de afs; [2]Médias seguidas de mesma letra na coluna não diferem entre si pelo teste de Scott-Knott a 5% de significância; Média de três repetições).

Table 2. Effective doses able to inhibit 50% of mycelial growth (ED_{50}) of six isolates of *Corynespora cassiicola* from cucumber (dose efetiva capaz de inibir o crescimento micelial em 50% (DE_{50}) de seis isolados de *Corynespora cassiicola* provenientes de pepineiro). Goiânia, UFG, 2007.

Fungicides	ED_{50} (µg mL^{-1})					
	PESP01[1]	PESP02	PESP04	PESP05	PESP06	PEGO07
Azoxystrobin	87.2	$5.8x10^{4}$	$1.2x10^{4}$	$1.0x10^{25}$	$3.7x10^{8}$	$2.5x10^{2}$
Captan	32.5	29.6	23.2	40.2	64.4	31.4
Thiophanate-methyl	$6.8x10^{56}$	$1.0x10^{4}$	$2.1x10^{12}$	$6.7x10^{16}$	$1.4x10^{28}$	$4.4x10^{10}$
Chlorothalonil	$5.6x10^{4}$	$6.0x10^{5}$	$2.2x10^{3}$	$2.9x10^{7}$	$3.7x10^{12}$	$6.2x10^{2}$
Carbendazin	$2.4x10^{4}$	$1.4x10^{8}$	$4.8x10^{3}$	$4.4x10^{3}$	$6.7x10^{4}$	$1.9x10^{2}$
Mancozeb	12.8	20.7	11.4	5.0	1.9	20.8
Tebuconazole	$2.2x10^{-2}$	3.2	0.6	1.4	2.9	$2.4x10^{-5}$
Difenoconazole	$2.1x10^{-14}$	$5.3x10^{-3}$	$2.4x10^{-3}$	$7.7x10^{-6}$	$2.5x10^{-7}$	$9.61x10^{-5}$

[1]PESP01 to PESP06 were from São Paulo State, PEGO07, from Goiás State (PESP01 a PESP06 foram provenientes do estado de São Paulo, PEGO07, do estado de Goiás).

presented itself as one of the least susceptible to the disease in all the assessment periods and at 15 DAI it was the only cultivar remaining in the moderately resistant group.

Thus for a future recommendation for commercial planting of target spot resistant hybrids, these materials should to be tested in the field until the production phase but, based on the preliminary results of the resistance of these hybrids to target spot, in the greenhouse, there are strong indications that the Taisho, Nikkey, Safira and Yoshinari hybrids will perform well in the field.

In vitro *Corynespora. cassiicola* inhibition - Difenoconazole, of the eight fungicides tested *in vitro*, most inhibited mycelia growth (MGI= 74.6%) at the concentration 1 µg mL^{-1} a.i. Chlorothanonil, captan, thiophanate-methyl and carbendazin resulted in the lowest MGIs (data not shown).

At the 10 µg mL^{-1} a.i. concentration, difenoconazole and tebuconazole obtained the highest MGIs and thiophanate-methyl and carbendazin the lowest MGIs (data not shown).

At the 100 µg mL^{-1} a.i. concentration, difenoconazole, tebuconazole, mancozeb and captan gave the best results. At the same concentration, thiophanate-methyl, chlorothanonil and carbendazin obtained the lowest MGIs (data not shown). A similar result to that of carbendazin was reported by Hasama (1991), who concluded that the effectiveness of the benzimidale fungicides had decreased against the disease. For this, the sensitivity of 419 *C. cassiicola* isolates collected in cucumber fields was tested with benomyl and carbendazin, and verified that 330 were highly resistant to values greater than 100 µg mL^{-1}.

Tebuconazole and mancozeb were the only fungicides that completely inhibited mycelia growth of the *C. cassiicola* fungus isolates at the 1.000 µg mL^{-1} a.i. concentration (data not shown). Thiophanate-methyl performed worst among the products, followed by carbendazin, chlorothanonil and azoxystrobin. Reinforcing these results, Date *et al*. (2004) tested the sensitivity of 193 *C. cassiicola* cucumber isolates, using the minimal inhibitor concentration method (MCI) and concluded that 29 isolates were highly resistant to thiophanate-methyl and diethofencarb and one isolate was resistant to azoxystrobin. This result was due to the fact that the thiophanate-methyl, belonging to the benzimidales group, has been used very frequently (Delen & Tosun, 2004).

There was significant isolates x fungicides interaction, therefore the ED_{50} had to be calculated separately for each isolate. Difenoconazole fungicide was considered highly fungitoxic, following classification by Edgington & Klew (1971), and the ED_{50} value ranged from $2.4x10^{-3}$ to $2.1x10^{-14}$ µg mL^{-1}, followed by tebuconazole, where three values were classified as highly fungitoxic ($2.2x10^{-2}$, 0.6 and $2.4x10^{-6}$) and another three as moderately toxic (3.2, 1.4 and 2.9). Other fungicides considered as moderately toxic were mancozeb (1.9-20.7) and captan (23.2-40.2), and one isolate of the fungus presented ED_{50} greater than 50, a value classified as non-toxic, and the non-toxic: carbendazin, azoxystrobin, chlorothanonil and thiophanate-methyl (Table 2).

Thus difenoconazole and tebuconazole were the most efficient presenting high MGI *in vitro C. cassiicola* values. Both the fungicides belong to the triazoils chemical group that act on demethyilation of the lanosterol to intermediate compounds, ergosterol precursors.

Mancozeb and captan were considered moderately toxic. Mancozeb, from the dithiocarbamate chemical group, interferes in energy production and can be considered as a non-specific or multiple action inhibitor. Captan, belonging to the heterocyclic nitrogens chemical group, inactivates essential enzymes that interfere in the fungus vital processes (Azevedo, 2003).

Azoxystrobin, carbendazin, chlorothanonil and thiophanate-methyl were not considered fungitoxic for the *C. cassiicola* isolates tested that means that these isolates are insensitive to these fungicides in artificial and *in vitro* incubation conditions.

Azoxystrobin belongs to the

Table 3. Protective effect of contact and systemic fungicides on the management of target spot in Nikkey and Tsuyataro cucumber hybrids, at seven and fourteen days after inoculation with *Corynespora cassiicola* (efeito protetor de fungicidas de contato e sistêmico no manejo da mancha alvo em híbridos de pepino Nikkey e Tsuyataro, aos sete e 14 dias após a inoculação com *Corynespora cassiicola*). Goiânia, UFG, 2008.

Treatments	Research 1 and 2 (Nikkey)		Research 1 (Tsuyataro)		Research 2 (Tsuyataro)	
	14 days	14 days	7 days	14 days	7 days	14 days
Control	13.25b[1,2]	3.07a	12.33a	96.75a	3.45ab	77.5a
Azoxystrobin	0.16c	0.00b	0.00c	12.67de	0.01d	0.98b
Captan	56.67a	2.65a	13.25a	84.50ab	2.15abcd	50.33a
Thiophanate-methyl	3.41bc	1.42ab	6.46ab	93.58a	4.94a	86.58a
Chlorothalonil	1.67bc	0.29ab	0.15c	7.83e	0.28cd	36.24ab
Carbendazin	6.19bc	2.70a	5.95ab	90.33a	2.03abcd	71.87a
Mancozeb	2.47bc	0.48ab	2.68bc	45.83c	1.15bcd	63.91a
Tebuconazole	4.76bc	0.03b	2.27bc	48.25cd	0.97bcd	60.0a
Difenoconazole	10.88bc	0.41ab	6.54ab	57.67bc	1.91abc	78.46a
CV(%)	44.4	40.3	40.1	19.6	35.4	27.2

[1]Means of six repetitions; [2]Means followed by the same letter in the column are not statistically different by Tukey ($p<0.05$); Results of two trials ([1]média de seis repetições; Médias seguidas pela mesma letra na coluna não diferem entre si pelo teste Tukey ($p<0.05$); Resultados de dois ensaios).

Table 4. Curative effect of systemic fungicides on management of target spot in cucumber hybrids Nikkey and Tsuyataro expressed in percent of damaged foliar area, at seven and fourteen days of inoculation with *Corynespora cassiicola* (efeito curativo de fungicidas sistêmicos no manejo da mancha alvo em híbridos de pepino Nikkey e Tsuyataro, expressa em porcentagem de área foliar afetada, aos sete e 14 dias após a inoculação com *Corynespora cassiicola*). Goiânia, UFG, 2008.

Treatments	Research 1 and 2 (Nikkey)		Research 1 (Tsuyataro)		Research 2 (Tsuyataro)	
	14 days	14 days	7 days	14 days	7 days	14 days
Control	13.25a	3.07a	12.33 c[1,2]	96.75 ab	3.45 a	77.50 a
Azoxystrobin	0.48b	0.00b	0.98 c	81.10 ab	0.01 c	0.94 b
Tiophanate-methyl	9.88ab	0.42ab	47.25 ab	99.92 a	1.13 abc	60.23 a
Carbendazin	6.08ab	1.39ab	54.75 a	84.17 ab	1.64 abc	66.37 a
Tebuconazole	2.09ab	0.29ab	16.47 bc	74.58 b	0.43 bc	48.92 a
Difenoconazole	9.69ab	0.87ab	9.37 c	84.75 ab	2.07 ab	62.29 a
CV (%)	55.5	44.9	39.0	10.5	34.3	27.1

[1]Means of six repetitions; [2]Means followed by the same letter in the column are not statistically different by Tukey ($p<0.05$); Results from two trials ([1]média de seis repetições; [2]Médias seguidas pela mesma letra na coluna não diferem entre si pelo teste Tukey ($p<0.05$); Resultados de dois ensaios).

strobilurin chemical group, quinone inhibitors that are toxic because they inhibit the respiratory chain at the Complex III level (Ghini & Kimati, 2000). In Japan, the occurrence has been detected of *C. cassiicola* isolates derived from cucumber, resistant to strobilurin (Date *et al*., 2004; Ishii, 2006) and thiophanate-methyl (Date *et al*., 2004). Carbendazin, from the benzimidales chemical group, acts on fungi by inhibiting specific proteins, α and β tubulins (Coutinho *et al*., 2006). Chlorothanonil belongs to the nitrile chemical group and acts on fungus cell respiration (Azevedo, 2003). Thiophanate-methyl, also from the benzimidales chemical group, acts by interfering in the DNA synthesis or with the cell or nuclear division process (Picinini, 1994).

Several recent studies carried out in Brazil with other pathosystems such as *Didymella bryoniae*-watermelon where isolates were detected with crossed resistance to thiophanate-methyl and carbendazin (Santos *et al*., 2006); *Guignardia citricarpa*-citrus where selection pressure was tested using benzimidales fungicides in the citrus producing regions (Rodrigues *et al*, 2007) and *Lasiodiplodia theobromae*-papaya, where high estimated ED_{50} values were observed for all the fungicides tested, especially the benzimidales, that indicated selection pressure in the field resulting from the intensive use of this fungicide (Pereira, 2009).

The results of the *in vitro* experiments serve to indicate the sensitivity of the cucumber-derived isolates to the chemical molecules but the physiological performance of these strains might be expressed differently and contradictorily in interactions in the field.

a) In vivo target spot control - In

the first experiment, the two hybrids tested performed differently at seven days after inoculation (DAI): low disease severity was observed in the Nikkey cultivar in all the treatments, that it did not differ statistically from the control (data not shown). In the Tsuyataro cultivar, the greater severity was observed but in the treatments with azoxystrobin, chlorothanonil, mancozeb and tebuconazole there was low severity. At 14 DAI, the severities increased in the two hybrids except for the treatment where azoxystrobin-based fungicide was applied to the Nikkey cultivar and azoxystrobin, chlorothanonil, tebuconazole, mancozeb and difenoconazole were applied to the Tsuyataro cultivar (Table 3).

In the second experiment, at seven days, the severity observed on the Nikkey cultivar leaves was less than in the previous experiment but no treatment differed from the mean of the control (data not shown). In the Tsuyataro cultivar, the severity was also less, but lower severities were observed in treatments with azoxystrobin and chlorothanonil, that differed from the control but did not differ from the other products (captan, carbendazin, mancozeb and tebuconazole). At 14 days, severity increased in Nikkey that allowed assessment of lesser severity in the treatments with azoxystrobin and tebuconazole. For Tsuyataro, only azoxystrobin maintained low disease severity in the plants (Table 3).

Generally, azoxystrobin was more efficient in controlling the disease. Although it was considered not fungitoxic by the Edgington & Klew (1971) criterion, it was the product that gave the best results in the present experiment. The dose used in the preventive treatment was 75 µg mL^{-1} and ED_{50} $1.2x10^4$, that is, a dose 167 times greater had effective action on the pathogen in the plant. Perhaps because of the fact that the fungicide is mesostemic, that is, had an affinity with the leaf surface and was absorbed by the wax layer, forming a deposit on the surface of the susceptible organ that could later be redistributed on the plant surface in the vapor phase. The mesostemic substance penetrates tissues presenting translaminar activity (Azevedo, 2003; Reis & Bresolin, 2007), thus performance in culture medium may not reflect the action of the product in the plant and vice versa.

Chlorothanonil was also classified as non fungitoxic and a 1500 µg mL^{-1} dose was used and ED_{50} 2224. The dose applied was only 1.5 times greater and performed well against the pathogen. This good performance may be explained by the chlorothanonil action mechanism and because it is retained and redistributed in the plant. It has excellent leaf adherence and retention, so that the product provides safe protection capacity to the plant, even in conditions favorable to the disease development (Azevedo, 2003).

Captan, a fungicide classified as moderately toxic, did not differ from the control and performed poorly, while the protective effect of mancozeb was clear in experiment 1 with the Tsuyataro hybrid. This fact can be explained because in the protective form the toxic action is partially exercised on the sporulation, germination tube and appressorium formation, resulting from inhibition of the haustorium and/or mycelia development inside the host tissues (Forcelini, 1994).

The fungicides that were classified as highly toxic, tebuconazole and difenoconazole, when sprayed preventively did not control the pathogen as well as expected. The dose used for difenoconazole was 50 µg mL^{-1} and 400 µg mL^{-1} for tebuconazole, much greater values than those calculated for the ED_{50} 0.62 and $2.4x10^{-4}$.

Only thiophanate-methyl, considered as not fungitoxic obtained a corresponding performance, because it did not differ from the control in any of the severity means. The fact that this fungicide is widely used may have caused a higher selection pressure (Parreira *et al*., 2009). All the results indicated that the isolates used were resistant to this fungicide although it has been used to control other pathogens in the cucumber crop.

b) Curative treatment - In the first experiment, no fungicide was efficient for the curative treatment for Tsuyataro at seven DAI because although there were low severity percentages for the treatment with azoxystrobin, it was not significantly different from the control. The severity means of the treatments with thiophanate-methyl and carbendazin were higher than the control. At 14 DAI, no treatment differed from the control and high severities were observed in all the treatments. Only azoxystrobin was efficient in reducing the infection caused by the pathogen for the Nikkey cultivar at 14 DAI (Table 4).

In the second experiment, the efficiency of azoxystrobin and tebuconazole was observed for the Tsuyataro cultivar at seven days, although they differed from the control but not from the other treatments. At 14 days, only azoxystrobin was able to maintain the disease severity at low levels. The same result was obtained for the Nikkey cultivar (Table 4).

Generally, the fungicides applied as protection were more effective than those applied to cure. The preventive application ensured low severities at least in the first seven days after application, even under conditions extremely favorable to the pathogen and to the most susceptible hybrid (Tsuyataro).

The fungicides applied to cure, that is, applications after plant infection by the pathogen, were not efficient, except for azoxystrobin, regardless of whether the hybrids used were more or less susceptible. Thus it was concluded that the best measure to take is to avoid planting very susceptible hybrids in seasons of the year when conditions favor the plant pathogen development, because even applying preventive and efficient fungicides for disease control, 14 days after application the disease severity reached very high numbers (96.75% of the control).

Tiophanate-methyl and carbendazin were not efficient in controlling target spot that may have been the result of the appearance of resistance on the part of the pathogen, because these two products belong to the benzimidazole chemical group and high risk resistance group (Ghini & Kimati, 2000; Delen & Tosun, 2004), although only thiophanate-methyl is recommended for the cucumber crop, but against another

pathogen.

Thus the results obtained *in vitro* did not correlate with the *in vivo* results because the fungicides most efficient *in vitro* were tebuconazole, difenoconazole, mancozeb and captan, and the most efficient *in vivo* was azoxystrobin. The results were similar only for the benzimidales and were very inefficient in *in vitro* and *in vivo* target spot control.

Thus it was concluded that the results of sensitivity for the *C. cassiicola* isolates to different fungicides cannot be used as recommendation for chemical control of the disease in the field, because in the field there is pathogen-environment-host interaction. The results of the chemical control experiments supplied more consistent data for a future recommendation but there should also be more field experiments with the plants in the production phase. Thus it can be assumed that azoxystrobin fungicide has great potential for use in control of the disease, but it needs to be registered in the Ministry of Agriculture, Livestock and Food Supply to be able to be recommended in the control of *C. cassiicola* in the cucumber crop.

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