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Chemical and biological treatments for control of gummy stem blight of greenhouse cucumbers

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Abstract

Experiments were conducted to determine the effects of chemical and biological treatments on gummy stem blight of cucumber caused by *Didymella bryoniae* in vitro and under greenhouse conditions. Eleven strains of *Bacillus subtilis*, strain AGB10 of *B. cereus*, and strain B8Fr of *Enterobacter agglomerans* produced antagonistic zone against *D. bryoniae* in vitro. Of four experiments conducted, the chemical treatments 'Nova',[1] kresoxim-methyl, and azoxystrobin controlled the disease in three experiments and the biological treatments *E. agglomerans* (B8Fr), *B. subtilis* (AGS-4), and lysozyme in one experiment when applied as sprays on lesions caused by *D. bryoniae* on cucumber plants under greenhouse conditions. Fruit rot of cucumber was significantly reduced when the fruit was treated with 'Nova' or kresoxim-methyl. These results suggest the potential of azoxystrobin, kresoxim-methyl, *E. agglomerans* (B8Fr), and *B. subtilis* (AGS-4) applied post-inoculation to control gummy stem blight on greenhouse cucumbers.

Introduction

Gummy stem blight of cucumber (*Cucumis sativa*) is caused by *Didymella bryoniae* and its anamorph *Phoma cucurbitacearum* (Boerema and van Kesteren, 1972; Farr et al., 1989). The first symptom on greenhouse cucumber is lesions on stubs left after the removal of fruit, tendrils, or lateral shoots (Menzies et al., 1994). These lesions elongate and girdle the stem, causing wilt and eventual death of the plant. It is a serious disease of greenhouse-grown cucumbers in The Netherlands, where it causes fruit rot (van Steekelenburg, 1982). Gummy stem blight is also a problem in other European countries, including the UK and Denmark. A small to moderate degree of resistance does exist in cucumber germplasm (St. Amand and Wehner, 1995a; Wehner and St. Amand, 1993), but no gene for resistance has yet been identified (Punithalingam and Holliday, 1972). Variations among isolates of *D. bryoniae* were observed for pathogenicity on cucumber. St. Amand and Wehner (1995b) observed that the two isolates of *D. bryoniae* from The Netherlands and Sweden were more virulent than the USA isolates. Adequate control is hard to achieve with present fungicides because plants grow fast, have dense foliage, and are continuously being wounded as a result of picking and trimming.

Since 1997, this disease has become a serious problem in commercial greenhouses in British Columbia, Canada. Pesticides 'Rovral',[2] 'Benlate',[3] 'Dyrene'[4] are registered for control of this disease in Canada. However, these pesticides are not very effective in controlling this disease. This may be due to the development of resistant pathotypes. Azoxystrobin, which is a highly active fungicide providing a broad spectrum of disease control against ascomycete, basidiomycete, deuteromycete, and oomycete plant pathogens

[1] 'Nova' is a trademark of Rohm & Haas Inc.

[2] Rovral is a trademark of Rhone Polenc Inc.

[3] Benlate is a trademark of E.I. DuPont and Company.

[4] Dyrene is a trademark of Bayer AG of Germany.

(Godwin et al., 1992), is not registered for use by greenhouse growers in Canada. Experiments were designed to determine the antagonistic ability of 14 bacterial agents from three genera against *D. bryoniae* in vitro and to determine effects of various chemical and biological agents on disease development of *D. bryoniae* gummy stem blight on cucumber plants and fruits under near-commercial greenhouse conditions.

Materials and methods

In vitro *experiment*

The purpose of this experiment was to determine the antagonistic ability of three bacterial genera against *D. bryoniae*. One strain of *Pseudomonas putida*, 11 of *Bacillus subtilis*, one of *B. cereus* and one of *Enterobacter agglomerans* were evaluated separately for antagonism to a virulent strain of *D. bryoniae* in a dual culture test. The dual culture test was used to determine if antibiotic production was responsible for control of the disease. *Pseudomonas putida*, strain R-20, which was isolated from the rhizosphere of a lima bean (*Phaseolus lunatus*) was obtained from M. Schroth, Department of Plant Pathology, University of California, Berkeley, California 94720, USA. The *B. subtilis* strains were isolated from the soils in the Fraser Valley of British Columbia. The *E. agglomerans* strain B8Fr was isolated from soils in the Okanagan valley of British Columbia. These bacteria were stored at −4 °C. Inoculum was prepared for each of the bacterial strains by transferring one loop of bacteria from a PDA slant into 25 ml of potato dextrose broth (PDB) which was incubated at room temperature (22 °C) on a shaker at 200 rpm for 24 h. Petri dishes (100 mm × 15 mm) with PDA were inoculated by placing a 4-mm diameter disk from an actively growing *D. bryoniae* pathogen and a loop of bacteria spread in line 75 mm apart on the agar surface. The control dishes consisted of a plug of *D. bryoniae* grown alone on PDA. All dishes were incubated at 22 °C. The width of the widest part of inhibition zones was measured when the fungal growth on control dishes had reached the other side of the dish. Each test was replicated five times and repeated once.

Greenhouse Experiment 1

The purpose of the following four experiments was to determine the repeatability of the effect of various chemical and biological agents on disease development caused by *D. bryoniae* on cucumber plants. Cultivar 'Enigma' was sown on June 15, 1998 into rockwool cubes and transplanted to sawdust bags on July 9, 1998. Each plant received the complete nutrient solution ($Ca(NO_3)_2$, 1005 g; $MgSO_4$, 405 g; KH_2PO_4, 270 g; KNO_3, 825 g; KSO_4, 54 g; minor elements, 150 ml (Plant Products Brand); iron EDTA (7%), 75 ml; and, 160 ml of 10 N H_2SO_4 per 1000 l). The pH of the nutrient feed was adjusted to 6.0 and the electric conductivity was maintained at 1.7. One drip tube was placed at each plant to dispense nutrient solution. All plants were grown in the greenhouse at 17–21 °C with 290–2200 J cm^{-2} light, and 60–80% relative humidity. Thrips and other insect pests were controlled as recommended in the Greenhouse Vegetable Production Guide for Commercial Growers 1997 (BCMAFF, 1997). Pre-infection treatments were applied at 2.5 ml per plant as a spray by a hand sprayer on August 12, 1998 and the *D. bryoniae* inoculum was applied about 1 h later. Post-infection treatments were applied as a spray on August 13 and repeated several times. Stem wounds were made by pruning leaves off close (10 mm) to the stem, 5 per plant, evenly spaced up the plant from the base of stem to about 2 m above. Pesticides and biological agents were applied to control white flies, thrips, and other insect pests as recommended in the Greenhouse Production Guide for Commercial Growers (BCMAFF, 1997). The plants were supported using nylon cord that was attached to a horizontal wire 2 m from the ground. The plants were pruned and trained using the 'Umbrella' system (Klieber et al., 1993).

Ten days old cultures of *D. bryoniae* grown on potato dextrose agar were used to prepare the inoculum. The concentration of the *D. bryoniae* inoculum was 7.5×10^5 spores per ml determined by dilution plating. It was prepared as a suspension of spores in sterile distilled water and applied as a spray. Each lesion received about 15,000 spores. The bacterial treatments were prepared as follows: the bacteria, from five nutrient agar (NA) plates of 7–10 day old cultures, were scraped from plates and suspended in sterile distilled water. Concentrations were determined by dilution plating. The bacteria were sprayed on to each lesion and surrounding tissue until run-off. Each lesion area received about 4 ml of suspension.

The treatments were: control (*D. bryoniae* inoculum only), 'Nova' (Myclobutanil, Rohm & Haas Inc. Philadelphia, PA, USA, 0.3 g product per l, post-inoculation treatment on August 13, September 8,

and October 2), 'Rovral' (Iprodione 50 wp, Rhone-Poulenc, Mississauga, Ont., Canada, 1.0 g product per l, post-inoculation treatment on August 13, September 8, and October 2), Agral (Amway Canada Inc., surfactant, at 1 ml per l, post-inoculation treatment on August 13 , September 8, and October 2), azoxystrobin (Amistar, Syngenta Crop Protection Inc., Guelph, Ont., 1 g per l, post-inoculation treatment on August 13, September 8, and October 2), $CaNO_3$ (BDH Inc. Toronto, Ont., 50 mM $CaNO_3 \cdot 4H_2O$, pre- and post-inoculation treatment on August 12 and 13), Stylet oil (JMS FlowerFarms Inc. Vero Beach, Florida, 1 g per l pre- and post-inoculation treatment on September 8 and October 2), *E. agglomerans* (B8Fr at 2×10^8 colony forming units (CFU) per ml, pre- and post-inoculation treatment on August 12, 14, and 24), *B. subtilis* (AGS1, 10^8 CFU per ml, pre- and post-inoculation treatment on September 8, 25, and October 9), *B. subtilis* (AGS4 at 10^8 CFU per ml, pre- and post-inoculation treatment on September 8, 25, and October 9). The treatments were arranged in a completely randomised design with 10 replicate plants per treatment. Fruit weights were recorded twice a week from August 15 to October 15, 1998. Lesion lengths were measured on October 16, 1998.

Greenhouse Experiment 2

Cultivar Enigma was sown on April 1, 1999 into rockwool cubes and transplanted to sawdust bags on April 22, 1999. Pruning, training, and maintenance of the crop was as described in Experiment 1. Stem wounds were made and the concentration of the *D. bryoniae* inoculum (1.0×10^5 spores per ml) was prepared and applied as described in Experiment 1. The bacterial treatments were prepared, concentrations determined, and sprayed as described for Experiment 1. Treatments were applied 2 h after inoculation of wounds with *D. bryoniae*. Subsequent bacterial and chemical treatments were applied on June 2, 3, 10, 25, and July 16, 1999. There were six treatments with six replications arranged randomly in the greenhouse. The treatments were: control (*D. bryoniae* inoculum only), 'Nova' (0.3 g product per l), azoxystrobin (Amistar) (1 g product per l), *E. agglomerans* (B8Fr at 1.5×10^8 CFU per ml), *B. subtilis* (S 30, at 2.5×10^8 CFU per ml), kresoxim-methyl (Stroby, BASF Canada Inc. Toronto, Ont., 0.5 g product per l). Fruit yield was recorded twice a week beginning June 10 until August 13, 1999. Lesions were measured on August 9, 1999.

Greenhouse Experiment 3

Cultivar Enigma was sown on August 10, 1999 into rockwool cubes and transplanted to sawdust bags on September 2, 1999. Pruning, training, and maintenance of the crop was as described in Experiment 1. Lesions were made and the concentration of the *D. bryoniae* inoculum (1.0×10^5 spores per ml) was prepared and applied as described in Experiment 1. Each lesion received about 75,000 spores on September 29, 1999. Post-inoculation treatments were applied on October 1, 14, and 29, 1999. There were four treatments with seven replications arranged randomly in the greenhouse. The treatments were: 'Nova' (0.3 g product per l), azoxystrobin (Amistar) (1.0 g product per l), kresoxim-methyl (Stroby) (0.5 g product per l), and inoculated control (water). Fruit yields were recorded twice a week beginning October 12 until December 3, 1999. Lesions were measured on December 3, 1999.

Greenhouse Experiment 4

Cultivar Enigma was sown on December 31, 1999 into rockwool cubes and transplanted to sawdust bags on January 27, 2000. Pruning, training, and maintenance of the crop was as described in Experiment 1. Stem wounds were made and the concentration of the *D. bryoniae* inoculum (1.0×10^5 spores per ml) was prepared and applied on March 7, 2000 as described in Experiment 1. Post-inoculation treatments were applied on March 9, 24, and April 13, 2000. There were five treatments with seven replications arranged randomly in the greenhouse. The treatments were: 'Nova' (0.3 g product per l), kresoxim-methyl (Stroby, 0.5 g product per l), lysozyme (0.5 g product per l, Inovatech Canada Inc.), lysozyme (1.0 g per l), and inoculated control (water). Fruit yields were recorded twice a week beginning March 20 until June 2, 2000. Lesions were measured after 10 weeks since inoculation.

Greenhouse Experiment 5

The purpose of this experiment was to determine the effect of control treatments on the infection of cucumber fruits inoculated with *D. bryoniae* after harvest. Fruit was harvested leaving a long stem on the fruit. For inoculation, the stems were re-cut, leaving a 1 cm long stub, and immediately dipped in a suspension of 1×10^6 *D. bryoniae* conidia per ml, then allowed to dry for about 30 min. The inoculated ends were dipped in

the treatment solution, prepared at the same concentrations as described in Experiment 4. Non-treated fruits were dipped in sterile water. The fruits were allowed to dry, then placed in a plastic bag in an incubator at 15 °C. Each treatment was tested separately by comparing in each test 10 treatment fruits with 10 control fruits. Each treatment was tested twice. Fruits were rated for internal browning using the following scale: 0 = no browning, 1 = slight browning, 2 = moderate browning, 3 = severe browning.

Statistical analysis

All data were subjected to an analyses of variance by using the general linear model (GLM) procedure of SAS (SAS GLM procedure, SAS Institute, Cary, NC). The Waller Duncan K-ratio t-test was used to compare significant differences ($P = 0.05$) among treatment means. Data in Experiment 5 was statistically compared using a t-test at $P = 0.05$.

Results

All of the bacteria tested, except *B. subtilis* strain AG-7, developed an antagonistic zone against *D. bryoniae* (Table 1). Azoxystrobin, 'Nova', and *E. agglomerans* (B8Fr), and *B. subtilis* (AGS-4) applied as a post-inoculation spray significantly reduced the lesion length caused by *D. bryoniae* compared with the water control in Experiment 1 (Table 2). No significant differences in lesion length were observed between 'Rovral', Agral, stylet oil, calcium nitrate, and *B. subtilis* (AGS-1) treatments and inoculated control in Experiment 1. Fruit yield was significantly higher in treatments strain AGS-1 of *B. subtilis* (5.30 kg per plant), Agral (4.97 kg per plant), and calcium nitrate (4.76 kg per plant) compared with the inoculated control (3.59 kg per plant) in Experiment 1. Azoxystrobin, 'Nova', and kresoxim-methyl applied as a post-inoculation spray significantly reduced lesion lengths compared with the control and other treatments in Experiments 2–4 (Table 2). Lysozyme applied as post-inoculation spray also significantly reduced the lesion length compared with the inoculated control treatment in Experiment 4 (Table 2). Fruit rot of cucumber was significantly ($P = 0.01$) less when the fruit was treated with 'Nova' or kresoxim-methyl compared with inoculated controls (Table 3). Lysozyme did not reduce cucumber fruit rot compared to inoculated controls.

Table 1. Antagonism between bacterial strains and *Didymella bryoniae*, causal agent of gummy stem blight of cucumber under *in vitro* conditions

Plant growth-promoting-rhizobacteria	Strain	Concentration of bacteria (CFU per ml $\times 10^8$)	Antagonistic zone (mm)
Pseudomonas putida	R-20	100	0.0 a*
Bacillus subtilis	AGS-2	1	15.0 b
B. subtilis	AGS-6	0.5	16.0 b
B. subtilis	AG-7	20	0.0 a
B. subtilis	AGSK	1	10.6 b
B. subtilis	AGS-4	1.7	15.0 b
B. subtilis	BACT-0	0.2	12.6 b
B. subtilis	AG-3	100	15.6 b
B. subtilis	AGS-3	65	12.3 b
B. subtilis	AGS-1	1	15.5 b
B. cereus	AGB-10	0.1	14.8 b
B. subtilis	BACT-10	250	15.2 b
B. subtilis	AG-1	140	13.8 b
Enterobacter agglomerans	B8Fr	20	14.4 b
Control		0	0.0 a
S.E.			1.89

*Values with same letters are not significantly ($P = 0.05$) different according to the Waller Duncan K-ratio t-test. There were five replications per test.

Discussion

'Rovral', 'Nova', 'Benlate', and 'Dyrene' are registered for use by growers for gummy stem blight of cucumber in Canada. Azoxystrobin and kresoxim-methyl controlled the disease in three experiments. The effect of azoxystrobin and kresoxim-methyl on *D. bryoniae* lesion expansion indicate that these fungicides were active both in the prevention of infection and in restricting the mycelial growth of *D. bryoniae* when infections become established, indicating that azoxystrobin and kresoxim-methyl can affect several stages of the fungal life cycle. The risk of this pathogen developing resistance to the strobilurins is unclear. However, the introduction of selectively active, site-specific fungicides into crop production systems has been followed by the development of resistance to them by some target organisms (Eckert, 1988; Sisler, 1988). Continuous use of 'Rovral', 'Nova', 'Benlate', and 'Dyrene' would result in development of resistance by *D. bryoniae* to these fungicides. Azoxystrobin is currently used on a number of agronomic and horticultural crops worldwide. The use of strobilurins fungicides to

Table 2. Effect of chemical and biological treatments applied as a spray on lesion length (mm) on cucumber plants inoculated with *Didymella bryoniae*

Treatment	Rate	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Azoxystrobin	1.0 g l^{-1}	1.0 c*	8.0 b	11.0 b	—
Kresoxim-methyl	0.5 g l^{-1}	—	11.8 b	8.4 b	1.9 b
'Nova'	0.3 g l^{-1}	2.0 bc	9.2 b	6.9 b	1.9 b
E. agglomerans (B8Fr)	2×10^8 CFU ml^{-1}	3.0 bc	79.3 a	—	—
B. subtilis (AGS-1)	1×10^8 CFU ml^{-1}	6.0 abc	86.2 a	—	—
B. subtilis (AGS-4)	1×10^8 CFU ml^{-1}	3.0 bc	—	—	—
'Rovral'	1.0 g l^{-1}	6.0 abc	—	—	—
'Agral'	1 ml l^{-1}	8.0 abc	—	—	—
Stylet oil	10 ml l^{-1}	9.0 a	—	—	—
Calcium nitrate	11 g l^{-1}	11.0 a	—	—	—
Lysozyme	0.5 g l^{-1}	—	—	—	4.7 b
Control	Water	11.0 a	78.7 a	30.0 a	10.9 a
S.E.		1.98	2.59	1.73	1.23

— not tested. *Values in the column followed by the same letter are not significantly ($P = 0.05$) different according to the Waller Duncan K-ratio t-test.

Table 3. Effect of chemical and biological treatments applied as a dip treatment on severity of cucumber fruit rot caused by *Didymella bryoniae*

Treatment	Rate	Treated fruits	Non-treated fruits	t-test
'Nova' (run 1)	0.3 g l^{-1}	0.3	1.7	**
'Nova' (run 2)	0.3 g l^{-1}	0.7	2.7	***
Kresoxim-methyl (run 1)	0.5 g l^{-1}	0.7	2.4	**
Kresoxim-methyl (run 2)	0.5 g l^{-1}	1.6	2.5	**
Lysozyme (run 1)	0.5 g l^{-1}	2.6	2.3	ns
Lysozyme (run 2)	0.5 g l^{-1}	2.1	2.0	ns
Lysozyme (run 1)	1.0 g l^{-1}	1.8	1.8	ns
Lysozyme (run 2)	1.0 g l^{-1}	2.5	2.3	ns

** and *** significant at ($P = 0.01$ and 0.001) according to the t-test. ns = not significant.

control gummy stem blight of cucumber would be very useful to greenhouse growers in Canada to manage the fungicide resistance.

The biological agents *E. agglomerans* (B8Fr) and *B. subtilis* (AGS-4) controlled the disease and increase the fruit yield in one experiment only. These bacteria are naturally found in the Okanagan Valley and Fraser Valley soils, respectively. These bacteria were observed to be antagonistic to *D. bryoniae* in a dual culture *in vitro* test. It appears that antibiotic production by these bacteria may be involved in the control of gummy stem blight of cucumber (Utkhede and Li, 1989).

Agral applied as sprays on wound sites increased fruit yield but did not affect the lesion length in the first year of the study. This may be because of very high inoculum (1×10^5 CFU ml^{-1}) was applied before the treatment. Stanghellini et al. (1996) demonstrated that amending the nutrient solution with Agral 90 (ICI) at 20 ppm resulted in control of the spread of the fungus *P. aphanidermatum* in a cucumber crop. Agral was shown to kill fungal structures such as zoospores of a pathogen. However, we did not find any effect of Agral on disease development of gummy stem blight.

Post-infection sprays of calcium nitrate significantly increased fruit yield but did not reduce the lesion lengths of *D. bryoniae* on cucumbers in the first year only. Volpin and Elad (1991) showed that roses grown with fertilizer containing 408 mg l^{-1} of calcium sulphate had 30% less Botrytis gray mold and when used in the dip water exhibited a 45% reduction of Botrytis gray mold compared to untreated controls. They suggested that calcium treatments work by suppressing production of the plant hormone ethylene which makes plants more susceptible to attack by fungal pathogens. Grey mold on eggplant caused by *B. cinerea* was significantly reduced by sprays of calcium nitrate (Yunis et al., 1991).

In summary, 'Nova', azoxystrobin, and kresoxim-methyl have the potential to control the gummy stem blight of cucumber. The chemical 'Nova' is a triazole compound used as a systemic, protectant, and curative fungicide and is registered for use by greenhouse growers. Azoxystrobin and kresoxim-methyl are not registered for control of gummy stem blight caused by *D. bryoniae* on greenhouse cucumber in Canada. It is important to maintain the efficacy of new alternatives such as Azoxystrobin and kresoxim-methyl as a means of avoiding fungicide resistance.

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