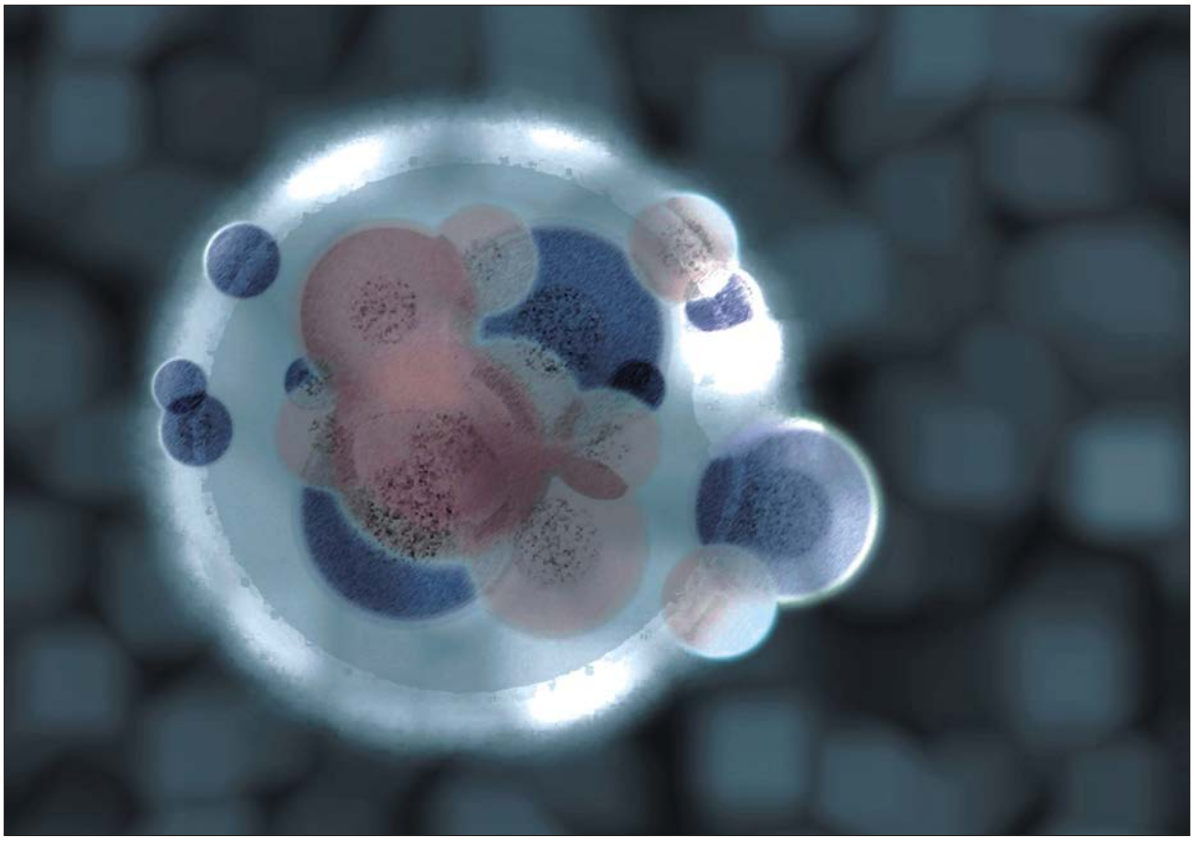

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Biological and Chemical Control of Fruit Rot in Greenhouse Sweet Peppers (*Capsicum annum* L.) Caused by *Fusarium subglutinans*

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Abstract: Experiments were conducted for two years to evaluate biologicals and chemicals for control of internal fruit rot of peppers caused by *Fusarium subglutinans* under greenhouse conditions. *Fusarium subglutinans* inoculum was pipetted on flowers of sweet peppers cv. Sympathy one day after applications of chemical and biological treatments. Pepper fruits were assessed for disease incidence and fruit weight sixty days after inoculation of flowers. Pepper fruits in PreStop®, Rovral® , BASF-516 and Quadra-137 treatments were significantly less infected than those observed in inoculated control treatment for 3 inoculations dates in both years. Treatments with Mycostop® and PlantShield® showed significantly less infected fruits compared with the control on 2 inoculations dates in both years. Flowers treated with Rovral® had significantly higher fruit weight compared with the control on 4 inoculation dates in 2003 and for 3 inoculation dates in 2004. Treatments with PreStop® and Quadra-137 produced heavier fruit than the control treatment for 3 inoculation dates in both years. These results suggest that these chemical and biological treatments have the potential to prevent internal fruit rot caused by *F. subglutinans* on peppers under greenhouse conditions.

Key words: Biological control, *Capsicum annum*, *Trichoderma harzianum*, *Gliocladium virens*, *Gliocladium catenulatum*, *Bacillus subtilis*, *Streptomyces griseoviridis*, *Rhodotorula mucilaginosa*

INTRODUCTION

Sweet pepper (*Capsicum annum* L.), grown hydroponically in greenhouses, is an important crop in British Columbia and other provinces of Canada. Pepper fruits are susceptible to different fungi that includes stem and fruit rot caused by *Fusarium solani* (Mart.) Sacc; telimorph: *Nectria haemotococca*, Berk and Br.[1] fruit rot caused by *Alternaria alternata* (Fries) Keissler, *A. tennuis*[2-4] and *Verticillium* sp.[1]. Internal fruit rot of greenhouse peppers is caused by *Fusarium subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas[5] . In a commercial greenhouse in British Columbia (BC), number of ripening orange pepper fruits (*Capsicum annum* L.) of cultivar Sympathy were observed to be diseased in 2001. About 40 and 10% orange pepper fruits were infected with the disease in 2001 and 2002, respectively[5] . In the year 2003 the disease was observed on other cultivars namely, 444 and Spirit in commercial greenhouses of BC and Alberta provinces. Infected fruits generally do not show any external symptoms. Internal rot, mycelial growth and sporulation is observed on the seeds, placenta and pericarp of the fruit only when the fruit is cut open. In some fruits, discolored soft patches, browning and necrosis is observed on the fruit surface mostly at the calyx end and sometimes anywhere on the mature fruits. Fruits become unmarketable because of external symptoms and internal rot. The disease appeared on mature fruits at harvest time and affected fruits become unmarketable.

Since internal fruit rot is a new disease on peppers, there are no chemical or biological agents recommended to prevent this disease in greenhouses. The objectives of this study was to evaluate the biological and chemical agents to prevent internal fruit rot of greenhouse peppers caused by *F. subglutinans* under near-commercial greenhouse conditions.

MATERIALS AND METHODS

Inoculum of the pathogen: Cultures from single spore colony of *F. subglutinans* isolated from diseased greenhouse peppers, was used for the experiment. A spore suspension was prepared by flooding 10-day-old cultures of *F. subglutinans* on Potato Dextrose Agar (PDA) dishes with sterilized distilled water, dislodging

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the spores by a glass rod and filtering through five layers of cheesecloth. The suspension was diluted with sterile water to adjust spore concentration to $3x10^6$ Colony Forming Units (CFU) mL^{-1} (determined by hemacytometer and dilution plating) and 0.1 mL spore suspension was applied using a pipette directly on flowers.

Experiment 1-2003: The experiment was conducted under near-commercial greenhouse conditions at the Pacific Agri-Food Research Centre, Agassiz, BC, Canada. Seeds of peppers (cv. Sympathy) were planted on December 2, 2002 in 70 mm rockwool cubes in vermiculite. Plants were fertilized with complete nutrient solution as needed. Nutrient solution consists of $Ca(NO_3)_2$-632 g, $MgSO_4$-270 g, KH_2PO_4-180 g, KNO_3-568 g, K_2S0_4-36 g, micrnutrients 150 mL (Plant Products Brand) and 180 mL of 5 ML^{-1} H_2SO_4 per 1000 L. The pH was adjusted to 6.0. The Electrical Conductivity (EC) of nutrient feed was maintained between 2.5-3.0. Seedlings were raised in the greenhouse at 21°C with 160 watts/cm^2 supplemental light using high pressure sodium lights and 70% relative humidity.

Seedlings were transplanted in yellow cedar sawdust bags (25 L sawdust in each bag, 1 seedling/bag) on January 8, 2003. The sawdust was pre-soaked with nutrient solution 48 h prior to setting the plants out in the bags. One drip tube was placed at each plant to feed nutrient solution. The plants were tied up by the twine to the overhead wire running the length of the row at a height of 3 m. Control of insect pests, training and pruning of plants were followed as recommended in the greenhouse vegetable production guide for commercial growers[6].

Yeast strain of S-33 of *Rhodosporidium diobovatum* Newell and Hunter was selected based on earlier studies[7]. Based on morphological characters and microscopic examination, strain S-33 was initially identified as *R. diobovatum* through conventional identification by Dr. John Bissett of Eastern Cereal and Oilseed Research Centre, National Fungal Identification Service, Ottawa, Canada. However, recently this organism is identified as *Rhodotorula mucilaginosa* (Jörgensen) Harrison and will be referred as *R. mucilaginosa* . The yeast inoculum was prepared by growing yeast for 24 h in nutrient broth with shaking (200 rpm) at 25°C. The other biological treatments were selected based on their availability as a commercial product and/or are registered in some countries for commercial use. The concentrations for application of each treatment were: PlantShield® at 1 g L^{-1} (*Trichoderma harzianum* Rafai strain T-22, Bioworks Inc., Geneva, New York, USA), Rovral® at 1 g L^{-1} (Iprodione, Rhone-Poulenc, Mississauga, Ont, Canada), BASF-516 at 1 g L^{-1} (BASF Canada Inc. Toronto, ON), Strain S-33 of *R. mucilaginosa* (Agriculture and Agri-Food Canada, Agassiz, Canada) at $1x10^7$ CFU mL^{-1} , SoilGard® at 5 g L^{-1} (*Gliocladium virens* Miller, Giddens and Foster strain GL-21, Thermo Trilogy Corporation, Columbia, MD), Prestop® at 10 g L^{-1} (*Gliocladium catenulatum* J.C. Gilman and E.V. Abbott strain J1446, Verdera Oy, Espoo, Finland), Quadra 136 (*Bacillus subtilis* (Ehrenberg) Cohn, AgraQuest Inc., Parma, Idaho, USA) at 20 g L^{-1}, Quadra 137 (*Bacillus subtilis*, AgraQuest Inc., Parma, Idaho, USA) at 10 g L^{-1}, Mycostop® (*Streptomyces griseoviridis* strain K-61, Verdera Oy, Espoo, Finland) at 1 g L^{-1} and inoculated control. Each treatment had 16 replicate plants. The treatments and controls were arranged in a Completely Randomized Design in the greenhouse. To get infections in fruits, flowering stage was selected based on earlier observations[5]. Treatments were applied to flowers on March 7, March 27, April 29, May 29 and July 4, 2003. All treatments were sprayed on marked flowers one day before inoculation of *F. subglutinans*. Flowers on control plants were sprayed with sterile distilled water only. These treated flowers were inoculated with 0.1 mL spore suspension using a pipette after 24 h of treatment application. Each plant received the complete nutrient solution (described earlier) as needed. Growing conditions, maintenance of crop and control of insect pests were as recommended in the greenhouse vegetable production guide for commercial growers[6].

Experiment 2-2004: The experiment was conducted in 2004 in the same greenhouse used in 2003. Seeds of peppers (cv. Sympathy) were planted on December 15, 2002 and the crop was managed in the same manner as described for 2003 experiment. The concentrations for application of each treatment were: PlantShield® at 1.0 g L^{-1}, Rovral® at 1 g L^{-1}, BASF-516 at 1 g L^{-1}, Strain S-33 of *R. mucilaginosa* at $1x10^7$ CFU mL^{-1} , SoilGard® at 5 g L^{-1}, Prestop® at 10 g L^{-1}, Quadra-136 at 20 g L^{-1}, Quadra-137 at 10 g L^{-1}, Mycostop® at 1 g L^{-1} and inoculated control. Each treatment had 14 replicate plants and they were arranged in a Completely Randomized Design in the greenhouse. Treatments were applied to flowers on March 8, March 19, April 6, April 21 and May 5, 2004. Flowers were inoculated with *F. subglutinans* as described in experiment 1.

Disease rating and yield data: Ripe pepper fruits were assessed for disease incidence and fruit weight 60 days after inoculation of flowers. Diseased and symptomless pepper fruits that were produced from inoculated and treated flowers on each plant were recorded after fruits

were cut open and percent diseased fruits per plant were calculated. The weight of each pepper fruit that was produced from a *F. subglutinans*-inoculated flower was recorded. Mean disease incidence (%) and fruit weight for each treatment were determined.

Recovery of *F. subglutinans* from diseased pepper fruits: The presence of *F. subglutinans* in pepper fruits was assessed by fungal isolations made from the lesions of five randomly selected infected fruits from these trials. Infected tissues from the edge of lesions were surface sterilized in 1% sodium hypochlorite for 2 min, blotted dry on sterile filter paper and cultured on Potato Dextrose Agar (PDA). The plates were incubated at 22°C for 7 days.

Statistical analysis: Data were analysed by General Linear Model (GLM) procedure (SAS Institute Inc. Cary, N.C.). Internal fruit rot incidence data were arcsine transformed before statistical analysis[8]. The Waller Duncan K-ratio test was used to separate treatments at 5% level of significance.

RESULTS AND DISCUSSION

Fusarium subglutinans was isolated from all fruits exhibiting symptoms of fruit rot, but from none of the symptomless pepper fruits. The treatment x date interactions were significant for disease incidence and fruit yield for both years. Therefore, comparisons were made among treatments for individual dates and years. Significant differences were observed for percent infected fruits in various treatments for all inoculation times in both years (Table 1 and 3). Overall, most of the treatments significantly reduced percent infected fruits compared with the inoculated control (Table 1 and 3). Pepper fruits in PreStop®, Rovral®, BASF 516 and Q-137 treatments were significantly less infected than those observed in inoculated control treatment for 3 inoculations in both years (Table 1 and 3). Treatments with Mycostop® and PlantShield® showed significantly less infected fruits compared with the control for 2 inoculations in both years (Table 1 and 3).

All treatments showed significant differences for pepper fruits produced from *F. subglutinans* inoculated flowers in all inoculations except fourth inoculations in 2003 (Table 2 and 4). Flowers treated with Rovral® had significantly higher weight compared with the control for 4 inoculation dates in 2003 and for 3 inoculation dates in 2004 (Table 2 and 4). Treatments with PreStop® and Quadra-137 produced heavier fruit than the control treatment for 3 inoculation dates in both years (Table 2 and 4).

Table 1: Effect of biological and chemical treatments on internal fruit rot disease incidence (%) caused by *F. subglutinanas* on greenhouse pepper (cv. Sympathy)-2003

		Inoculation				
Treatments	Concentration ($g\ L^{-1}$)	1st	2nd	3rd	4th	5th
Rovral	1	2.81c*	4.69d	36.87bc	17.31ab	28.75ab
BASF-516	1	11.25bc	6.56cd	32.37bc	21.50ab	35.50ab
Mycostop	1	19.06a-c	7.56b-d	38.75bc	21.25ab	29.00ab
PlantShield	1	8.75bc	9.06b-d	42.19a-c	18.56ab	40.75ab
PreStop	10	30.87ab	23.44bc	36.62bc	10.00b	21.75b
S-33	$1x10^7$ CFU mL^{-1}	19.06a-c	17.19b-d	44.19ab	17.87ab	30.81ab
Q-137	10	5.00c	23.12b-d	28.00c	30.5ab	36.62ab
Q-136	20	20.00a-c	19.06b-d	34.94bc	18.12ab	39.44ab
SoilGard	5	22.19a-c	25.62b	34.69bc	24.25ab	28.56ab
Inoculated control	Water	34.12a	55.06a	55.50a	35.81a	48.69a
SE		6.52	5.38	4.57	6.34	6.31

Table 2: Effect of biological and chemical treatments on weight (g) of pepper fmit (cv. Sympathy) produced from *F. subglutinanas* inoculated flowers-2003

		Inoculation				
Treatments	Concentration ($g\ L^{-1}$)	1st	2nd	3rd	4th	5th
Rovral	1	261.69a	202.83a	131.15a	168.59a	118.87a
BASF-516	1	201.78ab	182.89a	139.97a	126.82a	93.54ab
Mycostop	1	211.81ab	174.47a	126.66a	155.83a	80.42ab
PlantShield	1	188.21ab	176.73a	121.15ab	152.20a	92.30ab
PreStop	10	145.10b	152.15a	149.92a	178.83a	115.72a
S-33	$1x10^7$ CFU mL^{-1}	155.91b	162.42a	112.06ab	182.86a	99.93ab
Q-137	10	192.85ab	183.72a	150.80a	138.58a	113.46a
Q-136	20	211.40ab	179.96a	135.37a	149.21a	69.11b
SoilGard	5	167.92b	143.34a	122.34ab	154.64a	96.40ab
Inoculated control	Water	147.32b	80.04b	82.00b	117.31a	60.73b
SE		25.01	18.09	12.69	20.37	13.93

* Values within a column followed by the same latter(s) are not significantly (p=0.05) different according to the Waller Duncan K-ratio test

Table 3: Effect of biological and chemical treatments on internal fruit rot disease incidence (%) caused by *F. subglutinanas* on greenhouse pepper (cv. Sympathy)-2004

		Inoculation				
Treatments	Concentration ($g\ L^{-1}$)	1st	2nd	3rd	4th	5th
Rovral	1	29.64a-c*	7.14b	61.18ab	13.09b	23.81bc
BASF-516	1	18.45bc	21.42ab	39.39c	53.57a	27.37bc
Mycostop	1	34.51a-c	19.64ab	49.64bc	46.42a	32.14bc
PlantShield	1	23.21a-c	26.19ab	38.93c	44.64a	17.86c
PreStop	10	13.09bc	24.40ab	44.64c	46.43a	21.43bc
S-33	$1x10^7$ CFU mL^{-1}	27.38a-c	16.66ab	59.15ab	42.86a	17.86c
Q-137	10	9.52cd	27.37ab	52.01bc	42.86a	20.24bc
Q-136	20	26.19a-c	15.47ab	73.21a	48.81a	25.00bc
SoilGard	5	39.51ab	16.67ab	38.79c	49.40a	39.29ab
Inoculated control	Water	48.21a	36.90a	70.81a	44.03a	51.19a
SE		7.68	6.14	4.43	5.61	5.73

Table 4: Effect of biological and chemical treatments on weight (g) of pepper fmit (cv. Sympathy) produced from *F. subglutinanas* inoculated flowers-2004

		Inoculation				
Treatments	Concentration ($g\ L^{-1}$)	1st	2nd	3rd	4th	5th
Rovral	1	146.89a	172.06a	75.01a-c	45.80b	119.04a
BASF-516	1	130.68ab	149.79ab	112.91a	54.16b	78.12bc
Mycostop	1	121.94ab	163.41ab	51.12b-d	72.11ab	65.95bc
PlantShield	1	122.61ab	116.01ab	75.47a-c	113.49a	86.48a-c
PreStop	10	138.66a	122.52ab	88.14ab	53.32b	91.96ab
S-33	$1x10^7$ CFU mL^{-1}	81.29ab	167.96ab	66.34b-d	81.52ab	68.79bc
Q-137	10	136.98a	110.20b	109.24a	43.61b	88.15a-c
Q-136	20	135.68a	173.15a	25.12d	33.74b	88.66a-c
SoilGard	5	66.69ab	138.98ab	55.78b-d	56.28b	48.82cd
Inoculated control	Water	38.32b	127.24ab	39.72cd	41.52b	27.45d
SE		28.2	16.21	12.71	14.27	11.72

* Values within a column followed by the same latter(s) are not significantly (p=0.05) different according to the Waller Duncan K-ratio test

This study has shown that the pepper fruits in PreStop® and Quadra-137 biological treatments were significantly less infected than those observed in inoculated control treatment. *In vitro* studies done by other researchers have shown that *Trichoderma* species inhibits the growth of *F. subglutinans* isolated from mango[9] and from sugarcane[10]. Species of *Arthrobacter* were shown to reduce conidial production of *F. subglutinans* on slash pines[11]. Similarly, *Streptomyces* sp. have reduced incidence of *F. subglutinans* on stored maize grains[12]. Most of these studies were *in vitro* or under greenhouse conditions. Results of this study are based on commercial biological products tested under near-commercial greenhouse conditions on greenhouse pepper crop.

Chemical treatments with Rovral® and BASF-516 were effective in controlling infection of *F. subglutinans* in pepper fruits. The other chemical treatments that showed some activity against *F. subglutinans* on corn seeds were thiram, mancozeb and captan[13], thiabendazole on long leaf pine seed[14] and on loblolly pine seedlings[15]. Captan, difenoconazole and fludioxonil were effective against twelve *Fusarium* species on maize seeds and seedlings when tested under laboratory and growth chamber conditions[16]. Results with Rovral® and BASF-516 are based on evaluation of these products under near-commercial greenhouse conditions on greenhouse pepper crop.

The biological treatments with Prestop® and chemical treatment with Rovral® and BASF-516 applied once as preventive spray on flowers controlled internal fruit rot of pepper in these experiments. This indicates preventive ability of these treatments to control internal fruit rot of pepper caused by *F. subglutinans*. The level of disease prevention provided by biological agent Prestop® was as effective as prevention provided by fungicide treatment Rovral® and BASF-516. Use of such biocontrol agent would help to avoid risk of fungicidal residue on the produce and environment. Only single application of Prestop® protected the crop from disease for a long period. It appears that the biological agent in Prestop®, *Gliocladium catenulatum* J1446, may be surviving well on flower/fruit or on pathogen to provide protection for 2-3 months till the fruit is mature and harvested. The main mode of action of *G. catenulatum* seems to be hyperparasitism; the antagonist slightly coils around the pathogenic hyphae causing lysis of the host by means of enzyme activity[17] . We have not determined the mode of action of this biocontrol agent in this study.

This study has shown that flower application of Quadra-137 (*Bacillus subtilis*) reduced the incidence of internal fruit rot of peppers caused by *F. subglutinans*. This suggests its ability to prevent the occurrence of *F. subglutinans* internal fruit rot disease of pepper under greenhouse conditions. Strains of *B. subtilis* have been reported to reduce the disease occurrence and promote growth in cereals and carrots[18], in corn[19], in pepper, snapdragon and tomato seedlings[20], in apple trees[21], in greenhouse tomatoes[22].

Solel and Bruck[23] have demonstrated that the higher nitrogen nutrient accelerated *F. subglutinans* disease development on inoculated pine shoots, compared to low nitrogen. Stem cankers were larger and rates of shoots exhibiting lesions or wilt were higher on pine plants. It was postulated by them that the disease enhancing effect associated with higher nitrogen content in stem tissues may resulted from an increased nitrogen availability to the pathogen. In commercial pepper production standard nutrient solution contains 632 and 568 g 1000 L^{-1} of $Ca(NO_3)_2$ and KNO_3, respectively. It would be interesting to evaluate the effect of low nitrogen content on the internal fruit of greenhouse pepper caused by *F. subglutinans*.

In summary, PreStop®, Rovral®, BASF-516 and Quadra-137 applied as a spray on flowers reduced percent infected pepper fruits and increased marketable fruit yield of peppers. This suggests that PreStop®, Rovral®, BASF-516 and Q-137 treatments have the potential to reduce infection on greenhouse peppers by *F. subglutinans*.

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