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INFLUENCE OF EXOGENOUSLY APPLIED METHYL JASMONATE *CYLINDROCARPON DESTRUCTANS IN VITRO*

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Abstract

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Methyl jasmonate (MeJA), which is one of the plant lipid derivatives, is considered as a signaling substance during plant-microbe interactions. It is related to plant resistance to biotic and abiotic stress. To assess the influence of MeJA on soil pathogens, the direct effect of artificially applied MeJA on *Cylindrocarpon destructans* (a soilborne pathogen causing root rot of ginseng) was evaluated. MeJA strongly inhibited its growth and spore germination, whereas it stimulated phytopathogenic enzyme activities of this pathogen. The colony diameter of *C. destructans* on PDA decreased from 8.23 ± 0.15 cm (control) to 0.71 ± 0.00 cm (800 mg/L). The biomass was reduced by 65.3-100% and the percent of spore germination and germ-tube lengths were decreased by 100% at concentrations higher than 400 mg/L. Pectinase, cellulase, and amylase activities were stimulated at higher concentrations of MeJA, while the activity of protease was little changed. It was concluded that MeJA greatly inhibited *C. destructans* growth and spore germination, but stimulated activities of hydrolytic enzymes of *C. destructans* at higher doses.

Key words: Methyl jasmonate, biomass, Cylindrocarpon destructans, pathogenic enzyme

Introduction

Ginseng (*Panax ginseng* C.A. Meyer) widely cultivated, as a medicinal herb is an economically important cash crop in northeast China (Wang, 2001). The dried root is highly valued for its medicinal properties and is widely used in Chinese traditional medicine (Rahman and Punja, 2005a; Ali et al., 2006). *Cylindrocarpon destructans* (Zins) Scholten (teleomorph: *Nectria radicicola*), a pathogenic fungus responsible for Cylindrocarpon root rot of ginseng, is difficult to be eliminated from soil (Reeleder and Brammall, 1994; Punja, 1997). Cylindrocarpon root rot is one of the major threats to stable ginseng production (Reeleder and Brammall, 1994; Punja, 1997; Ahn and Lee, 2001; Rahman and Punja, 2005b; Kim et al., 2009), which can result in yield losses of up to 25-30% (Seifert et al., 2003; Kernaghan et al., 2007). The pathogen *C. destructans* is the most important soil-borne pathogen that caused root rot of ginseng, limiting the re-use of fields for successive ginseng crops (Reeleder and Brammall, 1994; Reeleder et al., 2002).

Much attention has been paid to the effect of *C. destructans* on ginseng and other plant hosts, but much less to the effect of host plants on the pathogen in the plant-microbe interactions. In fact, pathogen invasion is closely related to host aspects. Jasmonate (JA) is widely distributed in the plant kingdom with multiple physiological functions during plant development, growth, and defense responses (Creelman and Mullet, 1997). Methyl jasmonate (MeJA), one of the major physiological active forms of jasmonates, is a vital cellular regulator that mediates diverse developmental processes in plants. It has been demonstrated to alter defense responses against biotic and abiotic stresses in various plant species (Penninckx et al., 1998). Large amounts of work has been done on the ability of MeJA to elicit plant defenses against necrotrophic fungi. Previous results have shown that MeJA can protect spruce seedlings against the soil-borne pathogen *Pythium ultimum* (Kozlowski et al., 1999), and MeJA applied to potato leaves can induce systemic resistance against *Phytophthora infestans* (Cohen, 1993). Gaige (2010) suggested that MeJA and ethylene could induce partial resistance in *Medicago truncatula* against the charcoal rot pathogen *Macrophomina phaseolina*. The effect of MeJA on the control of Monosporascus root rot and vine decline of melon has also been studied (Aleandri et al., 2010). However, little research

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has been devoted to the direct effect of MeJA on specific pathogen itself e.g. on colony growth, spore germination, germ tube lengths, mycelial mass production, activities of pectinase, cellulase, amylase and protease of *in vitro* soil-borne pathogen, *C. destructans*.

The aim of this work is to assess the effect of MeJA on *C. destructans* and to investigate *in vitro* whether there is a relationship between MeJA and the pathogenic factors of *C. destructans*.

Materials and Methods

Pathogen strains and chemicals

Cylindrocarpon destructans was isolated from infected ginseng roots from the major ginseng cultivation areas, by the laboratory of plant disease epidemiology, Shenyang Agricultural University, China. Colonies were cultured on potato dextrose agar (PDA) plates and grown at 20ºC in the dark in an incubator for 2 weeks (Rahman and Punja, 2005b, 2006). MeJA used in the experiment was obtained from the Sigma Co. (St. Louis, MO, USA).

Measurement of colony growth

To determine the effect of MeJA on colony growth, MeJA was added to PDA to achieve the desired concentrations. Petri dishes containing PDA were inoculated with a 7-mm-diameter mycelial plug from a 14-day-old culture of *C. destructans* and incubated at 20ºC for 2 weeks. Colony diameter was measured at 3-day intervals by taking two perpendicular measurements on each colony. Three replicate dishes of each treatment were carried out and the experiment conducted twice.

Determination of spore germination and germ tube lengths

Percent germination and germ tube lengths were determined for spores of *C. destructans* in MeJA solution, following methods descried by He and Wolyn (2005). Spores (1×10^6 spores/ml) were harvested from the plates by rubbing the surface mycelium gently with a rubber swab and collecting the spores in distilled water. Spore suspension (4 mL) was diluted with 4 mL MeJA solution for each of the treatments and the resulting suspensions were incubated at 20ºC for 8h. At least 100 spores per treatment replicate were measured microscopically for percent spore germination and germ tube length. The experiment was repeated twice with three replications and the data averaged.

Assessment of mycelial mass production and enzyme activity

The mycelial mass production was assessed by adapting the method of Rahman and Punja (2006) with minor modifications. Briefly, flasks containing 100 ml of potato dextrose broth were inoculated with a 5-mm-diameter mycelial plug from a 14-day-old colony of *C. destructans* and incubated on a rotary shaker (130 rpm) at 20ºC for 2 weeks. The mycelial mass (dry weight) from three replicate flasks was determined after filtration and drying at 80ºC for 12 h. The experiment was performed twice. Culture filtrate was centrifuged at 8 000 rpm for 10 min at 4ºC and the supernatant was used for enzyme assays.

Pectinase activity (mainly polygalacturonase) was determined described by Silva et al. (2005). One unit of enzyme activity was defined as the amount of β-galacturonic acid hydrolyzed from pectin per minute under the assay condition. Cellulase activity was assayed using the DNS (3, 5-dinitrosalicylic acid) method (Berlin et al., 2005). One unit of cellulase activity was defined as the amount of enzyme that produced 1 μmol reduced sugar per minute. Amylase activity was determined by the procedure according to Murado et al. (1997). One unit of amylase activity was defined as the amount of enzyme releasing 1 μmol of glucose per minute. The gelatin assay of Tseng and Mount (1974) was used to quantify protease activity. One unit of protease activity was defined as the amount of enzyme causing an increase in absorbance of 0.01 in 1 min at 280 nm. The protein concentration in enzyme preparations was measured by the method of Lowry et al. (1951) following precipitation with trichloroacetic acid.

Experimental design and analysis of data

Experiments were carried out using eight concentrations of MeJA: 0, 1, 10, 50, 100, 200, 400, and 800 mg/L. Data on the colony growth were analyzed by analysis of variance (ANOVA). Means of the treatments were compared by Duncan's multiple range tests at $p < 0.05$. All statistical analysis was conducted with SPSS Base Version 11.5 statistical software (SPSS Inc. Chicago, IL).

Results

Effect of MeJA on colony growth and mycelial mass production of *C. destructans*

The growth of *C.destructans* was strikingly suppressed by MeJA both in a potato dextrose liquid culture and on PDA plates. The dry weight of mycelia was decreased from 74.00 ± 9.54 mg (control) to 0 (800 mg/L MeJA) (Figure 1). A severe repression of colony growth on PDA was observed at high concentration of MeJA (Figure 2a), in which the colony diameter was found to be 5.54 ± 0.23 cm at a concentration of 400 mg/L and 0.71 ± 0.00 cm at a concentration of 800 mg/L, although the diameter was no difference compared with the untreated control (8.23 ± 0.15 cm) at lower concentrations (1-50 mg/L MeJA) (Figure 2b).

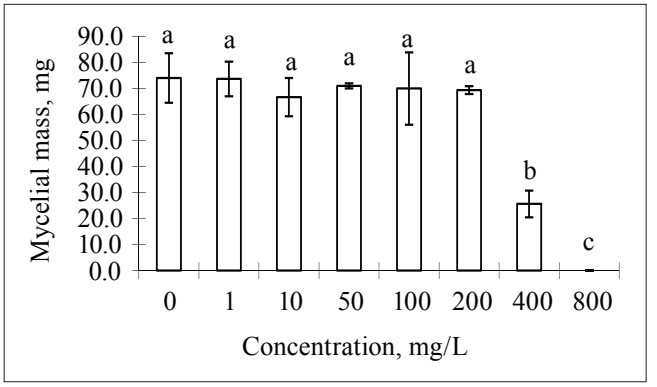
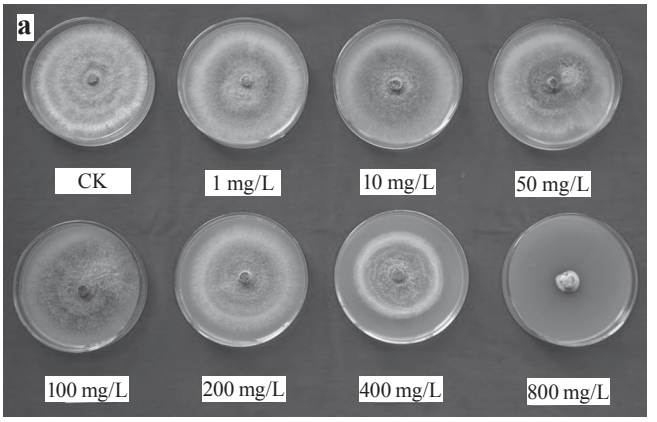


Fig. 1. Effect of MeJA on mycelial production by *Cylindrocarpon destructans*. Bars followed by different letters are significantly different according to Duncan's multiple range test (P <0.05). Vertical bars represent the standard errors of means from twice experiments each having three replicate flasks



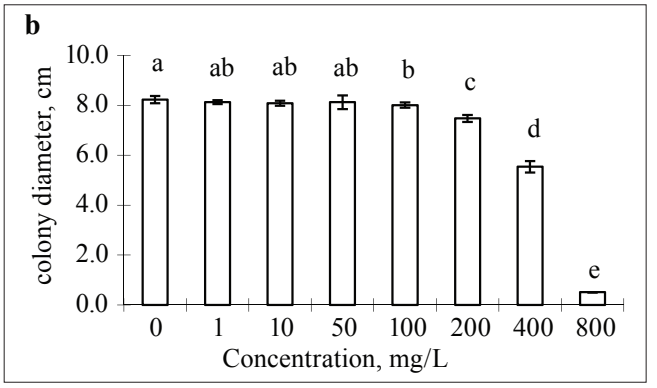


Fig. 2. Effect of MeJA on mycelial growth of *Cylindrocarpon destructans* on potato dextrose agar (after 12 d). Values followed by different letters are significantly different according to Duncan's multiple range test (P <0.05). Vertical bars on the top represent the standard errors of means from twice experiments each having three replicates

Effect of MeJA on spore germination and germ tube lengths

Dramatic inhibition of spore germination and germ tube growth by MeJA were obtained in a concentration-dependent manner. The percent of spore germination was strongly suppressed, with a reduction of 7.9-100.0% compared with the control (Figure 3). Potent suppression of the growth of germ-tubes was observed at all concentrations (1-800 mg/L), especially at 400-800 mg/L, where the growth of germ-tubes was inhibited completely (Figure 4).

Effect of MeJA on the activities of enzymes related to pathogenesis

MeJA observed increase of the pectinase activity with treatment. The activity of pectinase was increased by MeJA

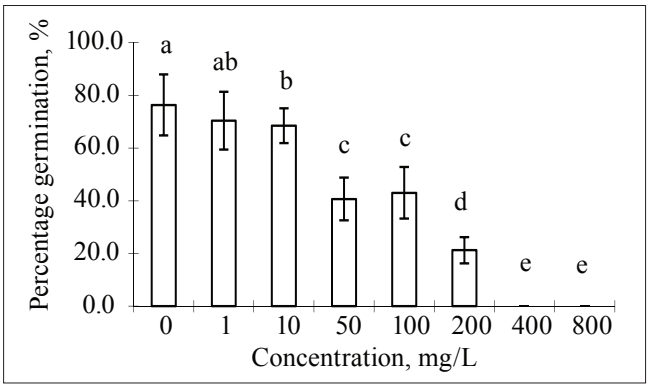


Fig. 3. Effect of MeJA on spore germination of *Cylindrocarpon destructans*. Values followed by different letters are significantly different according to Duncan's multiple range test (P <0.05). Vertical bars on the top represent the standard errors of means from twice experiments each having three replicates

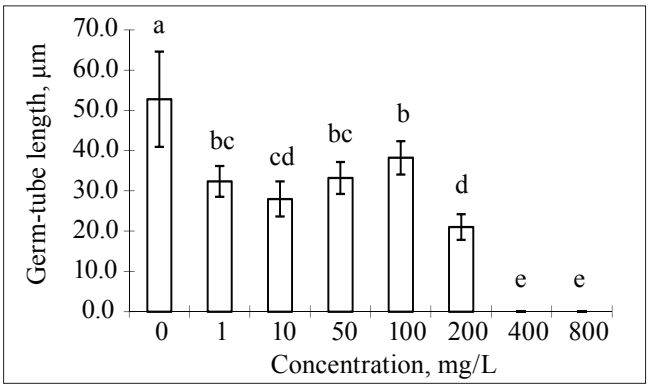


Fig. 4. Effect of MeJA on germ tube lengths of *Cylindrocarpon destructans*. Bars followed by different letters are significantly different according to Duncan's multiple range test (P <0.05). Vertical bars represent the standard errors of means from twice experiments each having three replicates

depending on its concentration, with the highest value of 0.613 ± 0.047 U ml^{-1} min^{-1} at the concentration of 800 mg/L (Figure 5a). The activity of cellulase was stimulated at high concentrations of MeJA (200-800 mg/L) in liquid culture, while it was suppressed at low concentrations (1-50 mg/L). The activity of cellulase was 0.306 ± 0.017 µmol min^{-1} at the highest concentration (800 mg/L) of MeJA (Figure 5b). At lower concentrations of MeJA (1-100 mg/L), amylase activity was little changed, but substantial increase of the activity was found at high concentrations of 200-800 mg/L, which was 0.450 ± 0.025 µmol min^{-1} at the concentration of 800 mg/L (Figure 5c). Protease activity by *C.destructans* was scarcely influenced by MeJA in liquid culture, although small amounts of fall tendency was observed, which the activity was almost no difference compared to control (Figure 5d).

Discussion

MeJA, a methyl ester of JA, plays an important role in the defence of plants against pathogens (Preston et al., 2001; Aleandri et al., 2010; Gaige et al., 2010). It can serve as a signal molecule bridging pathogen and plant host, particularly in the ginseng - *C. destructans* interactions. In the present study, the growth of *C.destructans* was strongly inhibited by MeJA in a liquid culture. The mycelial mass was inhibited completely at the concentration of 800 mg/L (Figure 1). On PDA plates, MeJA was not significantly inhibitory to *C. destructans* at lower concentrations, but a potent suppression of colony growth was observed at high concentrations of MeJA (Figure 2). This was in agreement with the report that MeJA inhibited mycelial growth of *Phytophthora infestans* in vitro (Cohen, 1993). Severe repression of spore germination and germ-tube growth were observed treated with MeJA at all concentrations, especially at concentrations higher than 400 mg/L, where germination and germ-tube growth were suppressed completely (Figures 3 and 4). It is well known that spore germination and mycelial growth of *C. destructans* play an important part in the infection process in plant diseases. We believed that decreased germination and mycelial growth of *C. destructans* by MeJA would be one of the mechanisms on plant resistance to pathogens. From the present study, MeJA not only enhances the plant resistance to pathogens but also directly inhibits the growth of the pathogens.

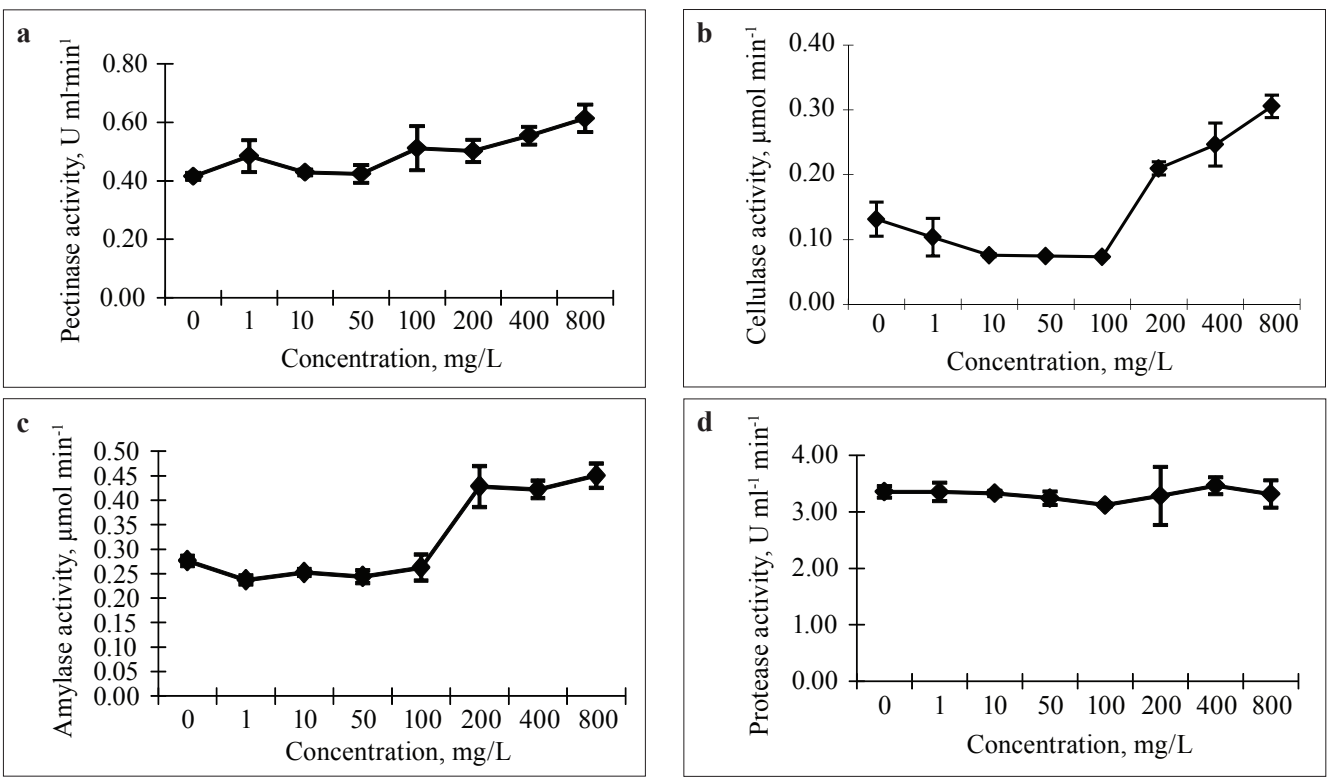


Fig. 5. Effect of MeJA at different concentrations on hydrolytic enzymes related to pathogenesis of *C.destructans* in a liquid culture. All data are mean values of two independent experiments each having three replicates, error bars indicate standard deviation. a pectinase activity; b cellulase activity; c amylase activity; d protease activity

Enzymes related to pathogenesis secreted by *C. destructans*, such as pectinase, cellulase, amylase and protease, were important pathogenic factors in the progression of the infection. Pectinases and cellulases facilitate the penetration of the fungus into the plant by the hydrolytic cleavage of polymers (pectic substances, cellulose) which constitute the plant cell walls (Fuchs et al. 1965). It has been proposed that proteases may be required for nutritional purposes or to degrade protein in the plant cell wall to allow spread of the pathogens or overcome host defenses (Dow et al., 1990). Increase of amylase activity from the fungi contributes to the deposition and utilization of host carbon source. In the current study, pectinase, cellulase and amylase activity of *C. destructans* was stimulated by MeJA. Pectinase activity at the highest concentration of MeJA was increased by 47.7%. Cellulase activity was repressed by MeJA at concentrations lower than 100 mg/L, while was stimulated at high concentrations (200-800 mg/L). A great increase of amylase activity was obtained treated with MeJA at concentrations higher than 200 mg/L, which was increased by 63% at the concentration of 800 mg/L. Little effect of MeJA on *C. destructans* protease activity was found (Figure 5). The findings meant that excessive MeJA artificially added in practice would have adverse effect on the plant, which needs to be further studied in the future.

In conclusion, MeJA inhibited the colony growth and spore germination of *C. destructans*, while at the same time stimulated the production of phytopathogenic enzymes.

Acknowledgements

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References

Ahn, I. P. and Y. H. Lee, 2001. A viral double-stranded RNA up regulates the fungal virulence of *Nectria radicicola*. *Molecular Plant-Microbe Interactions*, **14:** 496-507.

Aleandri, M. P., R. Reda, V. Tagliavento, P. Magro and G. Chilosi, 2010. Effect of Chemical Resistance Inducers on the Control of Monosporascus Root Rot and Vine Decline of Melon. *Phytopathol. Mediterr.*, **49:** 18-26.

Ali, M. B., K. W. Yu, E. J. Hahn and K. Y. Paek, 2006. Methyl Jasmonate and Salicylic Acid Elicitation Induces Ginsenosides Accumulation, Enzymatic and Non-enzymatic Antioxidant in Suspension Culture *Panax ginseng* Roots in Bioreactors. *Plant Cell Rep.*, **25:** 613-620.

Berlin, A., N. Gilkes, D. Kilburn, R. Bura, A. Markov, A. Skomarovsky, O. Okunev, A. Gusakov, V. Maximenko, D. Gregg, A. Sinitsyn and J. Saddler, 2005. Evaluation of Novel Fungal Cellulase Preparations for Ability to Hydrolyze Softwood Substrates-Evidence for the Role of Accessory Enzymes. *Enzyme Microb. Technol.*, **37:** 175-184.

Cohen, Y. G. U. and T. Niederman, 1993. Local and Systemic Protection Against *Phytopthora infestans* Induced in Potato and Tomato Plants by Jasmonic Acid and Jasmonic Methyl Ester. *Phytopathology,* **83:** 1054-1062.

Creelman, R. A. and J. E. Mullet, 1997. Biosynthesis and Action of Jasmonates in Plants. *Annu. Rev. Plant Physiol. Plant Molec. Biol.*, **48:** 355-381.

Dow, J. M., B. R. Clarke, D. E. Milligan, J. L. Tang and M. J. Daniels, 1990. Extracellular Proteases From *Xanthomonas campestris* pv. *campestris*, the Black Rot Pathogen. *Appl. Environ. Microbiol.*, **56:** 2994-2998.

Fuchs, A., J. A. Jobsen and W. Wouts, 1965. Arabanases in Phytopathogenic Fungi. *Nature*, **206:** 714-715.

Gaige, A. R., A. Ayella and B. Shuai, 2010. Methyl Jasmonate and Ethylene Induce Partial Resistance in *Medicago truncatula* Against the Charcoal Rot Pathogen *Macrophomina phaseolina*. *Physiol. Mol. Plant Pathol.*, **74:** 412-418.

He, C. Y. and D. J. Wolyn, 2005. Potential Role for Salicylic Acid in Induced Resistance of Asparagus Roots to *Fusarium oxysporum* f.sp *asparagi*. *Plant Pathol.*, **54:** 227-232.

Kernaghan, G., R. D. Reeleder and S. M. T. Hoke, 2007. Quantification of *Cylindrocarpon destructans* f. sp *panacis* in Soils by Real-Time PCR. *Plant Pathol.*, **56:** 508-516.

Kim, J. H., S. G. Kim, M. S. Kim, Y. H. Jeon, D. H. Cho and Y. H. Kim, 2009. Different Structural Modifications Associated with Development of Ginseng Root Rot Caused by *Cylindrocarpon destructans*. *Plant Pathol. J.*, **25:** 1-5.

Kozlowski, G., A. Buchala and J. P. Metraux, 1999. Methyl Jasmonate Protects Norway spruce [*Picea abies* (L.) Karst.] Seedlings Against *Pythium ultimum* Trow. *Physiol. Mol. Plant Pathol.*, **55:** 53-58.

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall, 1951. Protein Measurement with the Folin Phenol Reagent. *J. Bio. Chem.*, **193:** 265-275.

Murado, M. A., M. P. Gonzalez, A. Torrado and L. M. Pastrana, 1997. Amylase Production by Solid State Culture of *Aspergillus oryzae* on Polyurethane Foams. Some Mechanistic Approaches From an Empirical Model. *Process Biochem.*, **32:** 35-42.

Penninckx, I., B. Thomma, A. Buchala, J. P. Metraux and W. F. Broekaert, 1998. Concomitant Activation of Jasmonate and Ethylene Response Pathways is Required for Induction of a Plant Defensin Gene in Arabidopsis. *Plant Cell*, **10:** 2103-2113.

Preston, C. A., G. Laue and I. T. Baldwin, 2001. Methyl Jasmonate is Blowing in the Wind, But Can It Act as a Plant-Plant Airborne Signal? *Biochem. System. Ecol.*, **29:** 1007-1023.

Punja, Z. K., 1997. Fungal Pathogens of American Ginseng (*Panax quinquefolium*) in British Columbia. *Can. J. Plant Pathol.*, **19:** 301-306.

Rahman, M. and Z. K. Punja, 2005a. Biochemistry of Ginseng Root Tissues Affected by Rusty Root Symptoms. *Plant Physiol. Biochem.*, **43:** 1103-1114.

Rahman, M. and Z. K. Punja, 2005b. Factors Influencing Development of Root Rot on Ginseng Caused by *Cylindrocarpon*

destructans. *Phytopathology*, **95:** 1381-1390.

Rahman, M. and Z. K. Punja, 2006. Influence of Iron on Cylindrocarpon Root Rot Development on Ginseng. *Phytopathology*, **96:** 1179-1187.

Reeleder, R. D. and R. A. Brammall, 1994. Pathogenicity of *Pythium* Species, *Cylindrocarpon destructans*, and *Rhizoctonia solani* to Ginseng Seedlings in Ontario. *Can. J. Plant Pathol.*, **16:** 311-316.

Reeleder, R. D., R. Roy and B. Capell, 2002. Seed and Root Rots of Ginseng (*Panax quinquefolius*) Caused by *Cylindrocarpon destructans* and *Fusarium* spp.. *J. Ginseng Res.*, **26:** 151-158.

Seifert, K. A., C. R. McMullen, D. Yee, R. D. Reeleder and K. F. Dobinson, 2003. Molecular Differentiation and Detection of Ginseng-Adapted Isolates of the Root Rot Fungus *Cylindrocarpon destructans*. *Phytopathology*, **93:** 1533-1542.

Silva, D., K. Tokuioshi, E. D. Martins, R. Da Silva and E. Gomes, 2005. Production of Pectinase by Solid-State Fermentation with *Penicillium viridicatum* RFC3. *Process Biochem.*, **40:** 2885-2889.

Tseng, T. C. and M. S. Mount, 1974. Toxicity of Endopolygalacturonate Trans-Eliminase, Phosphatidase and Protease to Potato and Cucumber Tissue. *Phytopathology*, **64:** 229-236.

Wang, T. S., 2001. China Ginseng. *Liaoning Science and Technology Press*, Shenyang, pp. 372(Ch).

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