EFFECT OF NUTRIENTS ON SPORE GERMINATION OF *GLIOCLADIUM ROSEUM* AND *OPHIOSTOMA PICEAE*

Benjamin E. Dawson-Andoh
Assistant Professor
Division of Forestry
West Virginia University
Morgantown, VA 26506-6125

and

Rawle Lovell
Technologist
Forintek Canada Corp
St. Foy Quebec, Canada GIP 4R4
(Received March 1999)

ABSTRACT

Biological control agents that are applied as spore suspensions must germinate to exert their desired effects. Nutrients are one possible factor that can be manipulated to enhance spore germination and subsequent microbial colonization of wood. Sugar alcohols, inorganic and organic nitrogen compounds (amino acids) were evaluated for their ability to selectively enhance germination of spores (conidia) of *Gliocladium roseum*. While sugar alcohols and inorganic nitrogen compounds showed no selective effect on spore germination of *G. roseum*, some of the amino acids, notably L-phenylalanine and β-alanine, selectively enhanced germination of *G. roseum* over *Ophiostoma piceae*.

Keywords: Biological control, biocontrol, spore germination, sapstain, *Gliocladium roseum*, *Ophiostoma piceae*, nutrients, sugar alcohols, inorganic nitrogen compounds, amino acids.

INTRODUCTION

One of the major causes of discoloration of freshly sawn softwood lumber in Canada is *Ophiostoma piceae* (Muench) H. & P. Syd. (Seifert 1993). Although sapstain is currently controlled by application of biocides, there is increasing public concern about the potential negative human and environmental impacts of these treatments (Dawson-Andoh and Morrell 1997). Alternative processes being evaluated include biological control or biocontrol. Biological control is currently less efficacious and more variable than biocides (Harman and Lumsden 1990).

Previous studies had identified *Gliocladium roseum* Bainier as a potential biological control of sapstain on wood (Seifert et al. 1988). Janisiewicz and Bors (1995) recommend that "once biocontrol agents have been identified and tested," further work should focus on improving efficacy and consistency by manipulating the physical, chemical, or biological environment to favor the biocontrol agent.

Microbial colonization of wood substrate is initiated by the arrival of propagules. Depending on biotic and abiotic factors, spores germinate to produce hyphae that colonize the substrate. Wood represents a fairly spartan regime for many fungi, particularly those that lack the ability to actively degrade cell-wall polymers (Dawson-Andoh and Morrell 1992). Identifying methods to selectively improve colonization will contribute to the successful application of biological control agents.

Colonization of wood by biological control agents can be enhanced by altering the wood substrate to stimulate spore germination and/or limit that of competing microflora. Germination of fungal spores on plant surfaces is stimulated by the quality of nutrients present. For example, nutrients affect populations of epiphytic microorganisms on plant surfaces

Wood and Fiber Science, 32(1), 2000, pp. 116-124

(Kosuge and Hewitt 1964; Blakeman 1975; Blakeman and Brodie 1977, Fokkema 1984). The inclusion of nutrients such as L-asparagine or L-proline and the nutrient analog 2-deoxy-D-glucose in the spore suspension of biocontrol agents enhanced the post-harvest control of blue mold on apple (Janisiewicz et al. 1992). Previously, Morris and Rouse (1982) demonstrated that the application of a single organic compound to the surface of snap beans altered the composition of the bacterial community enough to reduce bacterial brown spot. These examples suggest that altering the nutrients in the substrate may also enhance biocontrol efficacy.

In this paper, we report the effects of some carbon and nitrogen sources on spore (conidia) germination of a potential biocontrol fungus, *G. roseum* and a sapstain fungus, *O. piceae.*

MATERIALS AND METHODS

Fungi

Five isolates of *O. piceae* (Forintek isolates 387I, 387AA, 387AI, 387I, and 387J) and one isolate of *G. roseum* 784A (Forintek isolate) were used in this study. Isolate 387I was the most frequently used *O. piceae* in the study. All fungal cultures were maintained in 2% (wt/v) glycerol in cryo-preservation vials under liquid nitrogen.

Production of fungal spores

G. roseum and *O. piceae* were subcultured on 2% (wt/v) malt-agar (2%, wt/v) and oatmeal agar, respectively, and incubated at 27°C for 10 days. Six plugs were cut from the growing margin of each fungus and added to 200 ml of sterile 2% (wt/v) malt extract broth, which was incubated for 5 days at 25°C in total darkness on a liquid shaker at 100 rpm. At the end of this period, the contents of each flask were poured through sterile cheese cloth into a sterile 1-liter beaker to remove the mycelium. Filtered spores (conidia) were then transferred to sterile 500 ml round bottom centrifuge tubes and centrifuged at 6,000 rpm at 4°C for 20 min. The supernatant was poured off, and the compact spore sediment was twice washed with filtered sterilized deionized water. Washed spores were added to 50 ml of sterile 11% (wt/v) glycerol solution. A 1.8 ml aliquot of this spore suspension was transferred to each cryo-preservation vial, which was stored at −20°C for 1.5 h and then placed into liquid nitrogen for storage.

To retrieve spores from storage, the preserving vials were placed in a water bath at 37°C for 2–3 min to thaw. The thawed spores were then transferred to sterile micro-centrifuge tubes, spun down to pelletize, decanted, and washed with sterile deionized water. The concentration of the stock spore suspension was determined using a hemacytometer.

Preparation of western hemlock water leachates

Generally, fungal spores germinate within the moisture film on wood. To mimic this situation, a water leachate of western hemlock [*Tsuga heterophylla* (Ref.) Sarg.] wood at room temperature was used as the test media. Western hemlock was cut from two inches below the bark area. Western hemlock leachate was prepared by adding 150 g of unseasoned western hemlock wood (3 × 1 × 0.5 cm) to 1 liter of distilled water in a 5-liter flask. The latter was shaken continuously on a rotary shaker (100 rpm) for four days and the contents were filtered through a Whatman #1 filter paper; a 0.45-μm membrane filter and finally through a 0.22-μm membrane filter. The sterilized extract was refrigerated at 4°C prior to use.

Nutrients

A variety of carbon and nitrogen compounds was employed in this study. All carbon substrates tested were sugar alcohols, while nitrogen sources consisted of two subgroups: inorganic and organic (amino acids) nitrogenous compounds (Table 1).

Spore germination

Each nutrient was dissolved in sterilized western hemlock leachate to produce 0.11,

TABLE 1. *Nutrient sources evaluated in study.*

Carbon sources	Inorganic nitrogen compounds	Organic nitrogen compounds
Arabitol	Ammonium nitrate	Bacto-asparagine
Adonitol	Ammonium nitrate	Bacto-peptone
Mannitol	Sodium nitrate	β-Alanine
		L-Cysteine hydrochloride
		L-Proline
		DL-Methionine
		L-Threonine
		L-Lysine monochloride
		L-Hydroxyproline
		L-Glutamic acid
		L-Histidine
		Casein enzymatic hydrozylate
		Gallic acid
		L-Phenylalanine
		L-Leucine
		L-Isoleucine
		L-Glutamic acid
		DL-Aspartic acid

0.56, and 1.1% (wt/v) stock solutions. To 1.5 ml sterile micro-centrifuge tube containing 0.1 ml suspension of 10^6 spores/ml of each fungus, sterilized nutrient (0.9 ml) was added to produce nutrient concentrations of 0.1 (low), 0.5 (medium), and 1.0% (high, wt/v) and a desired spore concentration of 10^5 spores/ml of each fungus. Each treatment was replicated five times. The open-topped micro-centrifuge tubes were incubated at 15°C for 24 or 48 h in a desiccator with a saturated solution of sodium tartrate at the bottom to maintain a relative humidity of 94%. At the end of each incubation period, the contents of each centrifuge tube were shaken, and an aliquot was pipetted into a hemacytometer. Two hundred spores selected at random were counted and the number of spores that were swollen and germinated were recorded. Germination of spores in deionized water and western hemlock water leachate without added nutrients served as controls. However, the effect of added nutrients was compared only with western hemlock water leachate control, thus eliminating any effect of wood or microbial proteins carried over during the preparation of western hemlock leachate.

Germination of a fungal spore is defined as the transformation of relatively inactive vegetative propagule to a highly active vegetative thallus. This process is characterized by physiological and morphological changes. The latter include spore swelling, germ-tube formation, and eventually thallus formation. Although swelling of spores was recorded, the data are not presented. A spore was considered germinated when the length of its germ-tube was at least three times its diameter.

Statistical analysis

For each nutrient type, means for the spore germination of each fungus were analyzed for significant difference using *t*-tests (SAS Institute 1990). All multiple comparisons of percent germination of *O. piceae* and *G. roseum* to selected nutrient amendments were first subjected to an analysis of variance (ANOVA and GLM), and significant differences between treatment means were determined using Duncan's Multiple Range Test at each concentration level. Experiments with nutrients that demonstrated some potential effects on germination were repeated.

TABLE 2. *Effects of carbon sources on spore germination of* Gliocladium roseum *784A and* Ophiostoma piceae *387I.*

Nutrient sources	Concentration % (weight/volume)	Incubation period (Hours)	Spore germination (%)[a]	
			G. roseum	*O. piceae*
Arabitol		24	N.D.	65.9 (5.1)
	0.1%	48	N.D.	79.5 (3.7)
		24	N.D.	61.1 (6.3)
	0.5%	48	N.D.	74.7 (3.9)
		24	N.D.	59.3 (3.4)
	1.0%	48	N.D.	81.1 (3.5)
Adonitol		24	56.3 (4.6)	29.8 (4.1)
	0.1%	48	57.1 (4.5)	74.7 (5.9)
		24	51.7 (3.5)	32.3 (4.5)
	0.5%	48	58.7 (8.3)	76.3 (4.8)
		24	55.5 (5.7)	41.2 (4.1)
	1.0%	48	57.2 (6.5)	82.6 (2.3)
Mannitol		24	50.5 (4.6)	31.2 (3.7)
	0.1%	48	88.6 (4.5)	82.2 (2.3)*
		24	46.5 (3.5)	30.1 (4.0)
	0.5%	48	70.8 (8.3)	69.9 (4.0)*
		24	54.1 (5.7)	30.5 (4.6)
	1.0%	48	92.4 (6.5)	70.9 (4.2)

[a] Values represent means of 5 replicates, while those in parentheses represent 1 standard deviation. ND = Not tested. * Indicates that spore germination of *G. roseum* did not differ significantly from that of *O. piceae* by *t*-test at $P = 0.001$.

RESULTS

Effect of sugar alcohols

Germination of *G. roseum* and *O. piceae* spores was significantly influenced by the type of sugar alcohol ($P = 0.001$) (Table 2). Mean percent germination of *O. piceae* in adonitol was significantly higher than for *G. roseum* at 24 h of incubation ($P = 0.001$). This effect was reversed at 48 h. This reversal implies

TABLE 3. *Effects of inorganic nitrogen sources on spore germination of* G. roseum *748A and* O. piceae *387I.*

Nutrient sources	Concentration % (weight/volume)	Incubation period (Hours)	Spore germination (%)[a]	
			G. roseum	*O. piceae*
Ammonium nitrate		24	43.5 (3.9)	10.7 (1.0)
	0.1%	48	59.4 (9.1)	11.2 (3.1)
		24	41.9 (2.3)	19.1 (3.5)
	0.5%	48	48.8 (5.5)	14.2 (3.5)
		24	56.8 (4.3)	16.9 (6.1)
	1.0%	48	78.0 (4.5)	12.8 (2.9)
Ammonium sulfate		24	51.3 (4.8)	60.1 (2.9)
	0.1%	48	53.5 (5.8)	86.6 (4.5)
		24	49.5 (3.7)	66.7 (7.8)
	0.5%	48	53.7 (2.8)	88.5 (2.4)
		24	54.5 (5.5)*	61.6 (3.9)*
	1.0%	48	61.8 (5.4)	88.1 (208)
Sodium nitrate		24	69.2 (3.9)*	66.5 (3.2)*
	0.1%	48	80.8 (4.4)*	75.5 (2.7)*
		24	61.7 (4.9)*	69.3 (3.2)*
	0.5%	48	79.6 (3.8)*	73.5 (2.9)*
		24	64.8 (4.2)*	71.6 (4.5)*
	1.0%	48	85.9 (2.7)	71.2 (5.3)

[a] Values represent means of 5 replicates, while those in parentheses represent 1 standard deviation. ND = Not tested. * Indicates that spore germination of *G. roseum* did not differ significantly from that of *O. piceae* by *t*-test at $P = 0.001$.

TABLE 4. *Effects of organic nitrogen sources on spore germination of* G. roseum *748A and* O. piceae *387I.*

Nutrient sources	Concentration % (weight/volume)	Incubation period (Hours)	Spore germination (%)[a]	
			G. roseum	*O. piceae*
L-Leucine		24	6.1 (0.1)	1.6 (1.3)
	0.1%	48	14.4 (1.6)	9.5 (0.8)
		24	10.5 (1.3)	12.6 (2.9)
	0.5%	48	23.8 (3.7)	11.4 (2.1)
		24	11.9 (1.6)	7.5 (2.5)
	1.0%	48	21.2 (3.0)	9.5 (1.5)
L-Isoleucine		24	81.8 (3.5)	4.9 (0.7)
	0.01%	48	80.9 (2.9)	19.3 (32.0)
		24	80.4 (2.1)	9.1 (1.7)
	0.5%	48	89.2 (2.7)	25.0 (2.7)
		24	84.2 (3.2)	9.6 (1.7)
	1.0%	48	91.7 (2.4)	27.1 (5.0)
L-Hydroxyproline		24	84.4 (4.8)	32.0 (5.4)
	0.01%	48	83.2 (2.8)	35.9 (2.5)
		24	85.9 (3.2)	32.2 (4.5)
	0.5%	48	88.3 (2.4)	40.7 (5.0)
		24	89.4 (2.2)	40.9 (3.8)
	1.0%	48	91.0 (0.9)	34.3 (2.6)
L-Histidine		24	25.6 (6.0)	18.3 (2.2)
	0.1%	48	33.0 (8.4)	28.1 (4.5)
		24	25.7 (4.9)	12.9 (2.5)
	0.5%	48	34.0 (4.1)	27.0 (2.2)
		24	28.6 (3.2)	12.4 (3.5)
	1.0%	48	37.5 (6.6)	20.9 (2.7)
L-Cysteine hydrochloride		24	0.3 (0.4)	2.2 (0.4)
monohydrate	0.1%	48	7.2 (1.3)	33.5 (4.2)
		24	0.0 (0)	4.0 (1.4)
	0.5%	48	0.0 (0)	2.7 (1.5)
		24	0.0 (0)	5.5 (2.2)
	1.0%	48	0.0 (0)	6.7 (1.5)
L-Proline		24	77.0 (10.8)	29.15 (2.4)
	0.1%	48	91.9 (6.4)	64.4 (4.7)
		24	80.2 (3.1)	29.5 (3.0)
	0.5%	48	100 (0)	55.6 (10.6)
		24	70.1 (3.2)	23.2 (3.1)
	1.0%	48	98.9 (1.7)	75.0 (4.7)
DL-Methionine		24	8.3 (2.1)	4.2 (1.5)
	0.1%	48	11.4 (2.8)*	17.5 (5.1)*
		24	8.8 (1.0)	4.2 (1.2)
	0.5%	48	11.3 (1.9)*	14.8 (4.5)*
		24	12.3 (2.7)	5.9 (2.2)
	1.0%	48	13.3 (3.7)	20.5 (4.5)
L-Threonine		24	90.5 (3.0)	18.9 (2.6)
	0.1%	48	94.8 (2.1)	44.4 (3.0)
		24	91.5 (1.6)	23.4 (2.7)
	0.5%	48	94.9 (1.8)	46.2 (7.3)
		24	83.1 (3.6)	21.6 (3.1)
	1.0%	48	93.6 (2.4)	44.3 (3.3)
Bacto-Asparagine		24	0.0 (0.0)	4.0 (1.3)
	0.1%	48	0.0 (0.0)	8.0 (3.3)
		24	0.2 (0.3)	1.5 (1.1)
	0.5%	48	0.2 (0.3)	6.9 (2.8)
		24	0.0 (0.0)	1.0 (0.8)
	1.0%	48	0.0 (0.0)	1.6 (0.4)

TABLE 4. *Continued.*

Nutrient sources	Concentration % (weight/volume)	Incubation period (Hours)	Spore germination (%)[a]	
			G. roseum	*O. piceae*
Bacto-peptone		24	72.0 (5.1)	7.1 (2.9)
	0.1%	48	100 (0.0)	15.4 (2.7)
		24	87.5 (2.5)	7.5 (1.6)
	0.5%	48	100 (0.0)	17.9 (1.6)
		24	85.5 (3.0)	6.9 (1.3)
	1.0%	48	100 (0.0)	14.5 (2.7)
Gallic acid		24	50.2 (6.2)	45.2 (4.8)
	0.1%	48	36.6 (2.4)	39.3 (4.1)
		24	0.0 (0.0)	46.1 (4.7)
	0.5%	48	0.0 (0.0)	32.6 (3.0)
		24	0.0 (0.0)	29.7 (5.0)
	1.0%	48	0.0 (0.0)	15.5 (1.8)
L-Lysine monochloride		24	87.2 (3.6)	54.1 (2.2)
	0.1%	48	100 (0.0)	56.3 (4.7)
		24	87.3 (3.5)	35.0 (2.3)
	0.5%	48	100 (0.0)	55.4 (3.0)
		24	85.7 (3.2)	35.0 (2.9)
	0.1%	48	100 (0.0)	59.0 (2.5)
β-Alanine		24	82.5 (2.7)	6.6 (1.9)
	0.1%	48	91.4 (1.9)	11.9 (6.2)
		24	87.1 (2.6)	5.1 (0.7)
	0.5%	48	91.3 (1.7)*	10.2 (1.2)
		24	75.2 (7.4)	3.6 (0.4)
	1.0%	48	85.5 (10.6)	27.1 (5.1)
L-Glutamic acid		24	69.3 (4.9)	4.7 (4.7)
	0.1%	48	87.1 (9.3)	13.2 (2.3)
		24	52.1 (2.5)	6.1 (1.6)
	0.5%	48	78.8 (3.8)	13.4 (2.1)
		24	43.5 (4.2)	6.1 (1.8)
	1.0%	48	57.1 (4.4)	15.1 (1.4)
DL-Aspartic acid		24	86.5 (2.5)	89.7 (3.1)
	0.1%	48	93.8 (1.2)	75.8 (6.4)
		24	84.7 (3.8)	93.4 (2.9)
	0.5%	48	96.8 (1.6)	88.4 (5.9)
		24	89.5 (3.3)	ND
	1.0%	48	99.6 (0.9)	ND
L-Phenylalanine		24	82.2 (2.3)	10.1 (1.5)
	0.1%	48	89.2 (1.8)	19.1 (4.9)
		24	83.1 (4.4)	9.0 (2.1)
	0.5%	48	91.4 (2.7)	17.3 (1.9)
		24	85.3 (6.2)	6.8 (1.2)
	1.0%	48	92.5 (1.7)	11.7 (2.2)
Casein Enzymatic		24	25.1 (5.5)	10.5 (3.4)
Hydrozylate	0.1%	48	61.1 (20.8)	11.3 (1.9)
		24	20.8 (4.2)	11.4 (2.9)
	0.5%	48	52.6 (2.4)	13.1 (1.8)
		24	11.7 (5.0)	23.0 (6.1)
	1.0%	48	68.5 (10.7)	21.8 (6.3)

[a] Values represent means of 5 replicates, while those in parentheses represent 1 standard deviation. ND = Not tested. * Indicates that spore germination of *G. roseum* did not differ significantly from that of *O. piceae* by *t*-test at $P = 0.001$.

some potential for slower, but eventual, uptake by *G. roseum*. Of the two fungal spores, *G. roseum* exhibited significantly higher percent spore germination in mannitol at all concentrations and both incubation periods except for the medium concentration at 48 h.

Effect of inorganic nitrogenous sources on spore germination

The three inorganic nitrogen sources produced different effects on spore germination of *G. roseum* and *O. piceae* (Table 3). In sodium nitrate, mean percent spore germination for *G. roseum* was not significantly different from *O. piceae* at all concentrations and spore incubation periods except at 48 h for the high concentration of all three sources ($P = 0.001$). Mean percent spore germination for *O. piceae* in ammonium nitrate was consistently lower at all concentrations and both incubation periods. In contrast, mean percent spore germination for *O. piceae* in ammonium sulfate was significantly higher than that for *G. roseum* at all concentrations and both incubation periods except at the high concentration incubated for 24 h ($P = 0.001$).

Effect of amino acids on spore germination

Spore germination responses of the two fungal species in various amino acids (Table 4) could be divided into six groups, A, B, C, D, E, and F. Group A included L-leucine, L-histidine, L-cysteine hydrochloride, DL-methionine, and bacto-asparagine and produced mean percent spore germinations for *G. roseum* and *O. piceae* that were generally below 40%. Group B included L-isoleucine, bacto-peptone, β-alanine, L-theronine, L-glutamic acid, and L-phenylaline. In addition to the significant difference ($P = 0.001$) in the mean percent germination between *G. roseum* and *O. piceae*, germination of *O. piceae* spores in these sources was very poor. For Group C, which included only one amino acid (gallic acid), both fungi exhibited moderate mean percent spore germination (50.2–36.2%). However, no spore germination was observed for *G. roseum* spores at both the medium and high concentrations. Both fungi exhibited excellent spore germination in the Group D amino acid source, DL-aspartic acid. Group E included L-proline and casein enzymatic hydrozylate, and the response of fungal spores to these compounds was not well-defined. Each amino acid exerted different effects on spore germination. Spore germination of *O. piceae* in casein hydrozylate was poor at all concentrations for both incubation periods. Spore germination for *G. roseum* after 48 h was at least twice that at 24 h. Mean percent spore germination for *O. piceae* in proline after 24 h was low but increased 2–3 fold after 48 h. Mean spore germination for *G. roseum* in proline at both incubation periods was significantly higher than that for *O. piceae* ($P = 0.001$). For Group E, which included only L-lysine monochloride, mean percent spore germination for *G. roseum* was about twice that of *O. piceae* at all concentrations and incubation periods.

DISCUSSION

The observed low levels of spore germination of *G. roseum* and *O. piceae* in deionized water relative to western hemlock leachate may reflect the absence of nutrients present in the leachate. In previous studies, oospores of *Pythium hydnosporium* exhibited low germination in distilled water but show marked increases when nutrients were added (Al-Hassan and Fergus 1973).

Wood is low in available nitrogen, and nitrogen becomes limiting for most microorganisms before carbohydrates (Blakeman and Brodie 1977). Simple sugars have been reported to have only limited effect on spore germination (Daigle and Cotty 1991). Spores of *G. roseum* and *O. piceae* responded differently to the three inorganic nitrogenous compounds and sugar alcohols and none exhibited any selective enhancement effect in the presence of added nitrogen.

From a practical point of view, the ideal nutrient additive should greatly enhance spore germination of the biological control agent rel-

TABLE 5. *Effect of L-Phenylalanine, L-alanine, L-isoleucine and L-Lysine on* G. roseum *784A and other isolates of* Ophiostoma piceae *at 15°C, 94% RH after 48-h incubation.*

Nutrient sources	Fungus	% Germination Nutrient concentration, % weight/volume		
		0.1	0.5	1.0
L-phenylalanine	*G. roseum* 784A	85.7a	86.9a	89.9a
	O. piceae 387I	14.5b	13.0b	9.2b
	O. piceae 387H	10.9c	11.1c	6.9c
	O. piceae 387J	6.8d	4.0c	5.6c
	O. piceae 387AA	3.1d	3.8d	0.7d
	O. piceae 387AI	2.9c	1.6d	0.6d
L-alanine	*G. roseum* 784A	86.9a	88.4a	82.1a
	O. piceae 387I	10.0c	6.7c	5.0c
	O. piceae 387J	11.9c	7.6c	7.0c
	O. piceae 387AA	6.3d	4.6d	5.0d
	O. piceae 387AI	38.0b	49.4b	47.2b
L-isoleucine	*G. roseum* 784A	85.3a	84.8a	87.9a
	O. piceae 387I	12.1d	3.4e	2.4d
	O. piceae 387H	17.1c	17.0c	18.3c
	O. piceae 387J	10.7d	2.3e	1.7d
	O. piceae 387AA	16.0c	10.2d	3.6d
	O. piceae 387AI	56.1b	34.1b	46.1b
L-lysine	*G. roseum* 784A	93.6a	96.3a	92.8a
	O. piceae 387I	55.2b	45.2b	46.7b
	O. piceae 387AA	22.0c	18.8c	13.2c
	O. piceae 387AI	23.6c	19.4c	21.1c

Means followed by the same letter indicates that spore germination of *G. roseum* did not differ significantly from that of *O. piceae* by Duncan's Multiple Range Test at $P = 0.001$.

ative to the sapstain fungus. Six of the amino acid sources (L-isoleucine, bacto-peptone, L-phenylalanine, L-hydroxyproline, L-threonine, and β-alanine) met this criteria. Janiesiewicz et al. (1992) reported that amino acids that strongly stimulated conidial germination also enhanced biological control.

Since nutritional requirements of strains of the same fungus may vary (Jackson et al. 1991), L-isoleucine, L-phenylalanine, and β-alanine together with L-lysine hydrochloride were screened for their effect on spore germination of other *O. piceae* isolates. Although significantly higher mean spore germination was recorded for *G. roseum* with respect to the other isolates of *O. piceae* (Table 5), low mean spore germination for other isolates of *O. piceae* and high mean spore germination for *G. roseum* was observed for only L-phenylalanine (Table 5). Mean spore germination for *O. piceae* 3871 in L-isoleucine was greater than 70% at both low and high concentrations.

Some organic nitrogenous sources clearly have the potential to selectively enhance spore germination of potential biological control agents over sapstain fungi such as *O. piceae.* These nitrogenous sources may be included in the deployment milieu to enhance the germination of applied propagules and thus contribute to the effectiveness of the biocontrol fungus. However, it is paramount that these amendments not simultaneously enhance germination and growth of the sapstain fungus.

CONCLUSIONS

The sugar alcohols and inorganic sources studied exhibited no selective enhancement of spore germination of the potential biological control fungus, *G. roseum* 784A over that of the sapstain fungus. *O. piceae.* Amino acids produced the greatest differential effect on spore germination of the biological control, *G. roseum,* relative to the sapstain fungus, *O. pi-*

ceae. L-phenylalanine selectively enhanced germination of *G. roseum* species relative to the sapstain fungus, *O. piceae,* β-alanine showed some potential.

ACKNOWLEDGMENT

This work was supported by the Canadian Forestry Service.

REFERENCES

AL-HASSAN K. K., AND C. L. FERGUS. 1973. The effects of nutrients and environment on germination and longevity of oospores of *Pythium hydnosporum.* Mycol. Appl. 51:283–293.

BLAKEMAN, J. P. 1975. Germination of *Botrytis cinerea* conidia in vitro in relation to nutrient conditions on leaf surfaces. Trans. Br. Mycol. Soc. 65:239–247.

———, AND I. D. BRODIE. 1977. Competition for nutrients between epiphytic micro-organisms and germination of spores of plant pathogens on beet root leaves. Physiol. Plant. Pathol. 10:29–42.

DAIGLE, J. D., AND P. COTTY. 1991. The influence of cysteine, cysteine analogs, and other amino acids on spore germination of *Alternaria* species. Can. J. Bot. 69: 2353–2356.

DAWSON-ANDOH, B. E., AND J. J. MORRELL. 1992. Enhancing the performance of bioprotection agents by treatment with waterborne salts. Wood Fiber Sci. 24(3): 347–352.

———, AND ———. 1997. Biological protection of freshly sawn sapwood from biological discolorations. *In* Prevention of discolorations in hardwoods and softwood logs and lumber. Forest Products Society Pub No. 7283: 3–9.

FOKKEMA, N. J. 1984. Competition for endogenous and exogenous nutrients between *Sporobolomyces roseus* and *Cochliobolus sativus.* Can. J. Bot. 62:2463–2468.

HARMAN, G., AND R. D. LUMSDEN. 1990. Biological disease control. Pages 259–279 *in* J. M. Lynch, ed. The rhizosphere. John Wiley and Sons, Chichester, NY.

JACKSON, A. M., J. M. WHIPPS, AND M. J. BAZIN. 1991. Effects of same carbon and nitrogen sources on spores germination, production of biomass and antifungal metabolites by species of *Trichoderma* and *Gliocladium virens* antagonistic to *Sclerotium cepivorium.* Biocontrol Sci. Technol. 1:43–51.

JANIESIEWICZ, W. J., AND B. BORS. 1995. Development of a microbial community of bacterial and yeast antagonists to control wound-invading postharvest pathogens of fruits. Appl. Environ. Microbiol. 61(9):3261–3267.

———, J. USTALL, AND B. BORS. 1992. Nutritional enhancement of biocontrol of blue mold on apples. Phytopathol. 82:1364–1370.

KOSUGE, T., AND W. B. HEWITT. 1964. Exudates of grape berries and their effect on germination of conidia of *Botrytis cinerea.* Phytopathol. 54:167–172.

MORRIS, C. E., AND D. I. ROUSE. 1982. Diversity of epiphytic bacterial communities on bean (*Phaseolus vulgaris*) leaves and pods based on nutrient utilization. Abtrs. Phytopathol. 72:936.

SAS INSTITUTE INC. 1990. SAS/STAT Users Guide. Version 6. 4th Edition. Cary. NC.

SEIFERT, K. A. 1993. Sapstain of commercial lumber by species of *Ophiostoma* and *Ceratocystis.* Pages 145–155 *in* M. J. Wingfield, K. A. Seifert, and J. M. Webber, eds. Ceratocystis and ophiostoma: Taxonomy ecology and pathogenicity. American Phytopathological Society.

———, C. BREUIL, L. ROSSIGNOL, M. BEST, AND J. N. SADDLER. 1988. Screening microorganisms with the potential for biological control of sapstain on unseasoned lumber. Mat. Und Org. 23:81–95.