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Integrated control of *Fusarium* damping-off in conifer seedlir

Integrierte Bekämpfung der durch *Fusarium* verursachten Umfallkrankheit an K feren-Keimpflanzen

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Summary

Interactions among three species of ectomycorrhizal fungi *(Hebeloma longicaudum, Laccaria bicolor Paxillus involutus)*, a litter decomposing fungus *(Clitocybe clavipes)*, two species of root pathc *(Fusarium moniliforme* and *F. oxysporum)*, and a fungicide (oxine benzoate) were studied. In dual cul *C. clavipes* and *P. involutus* reduced growth of *F. moniliforme* and *F. oxysporum*. Culture extrac *C. clavipes, L. bicolor*, and *P. involutus* reduced mycelial growth and spore germination of *F. monilij* and *F. oxysporum*. *Hebeloma longicaudum* showed no inhibitory effect against *F. moniliforme* and *F. sporum*. Two compounds, o-coumaric acid (isolated from *P. involutus*) and p-hydroxybenzoic (isolated from *L. bicolor*) reduced growth and spore germination of *F. moniliforme* and *F. oxysporum* a to 1000 µg/ml. Oxine benzoate at recommended dose reduced root rot severity and increased survi lodgepole pine and white spruce seedlings against *F. moniliforme* and *F. oxysporum*. *Clitocybe cla L. bicolor*, and *P. involutus* also improved seedling survival and reduced root rot severity when inocu onto the seedlings alone or in combination with recommended and reduced rates of oxine benzoa

Key words: Mycorrhizal fungi; biological control; integrated control; damping-off; conifer lings.

Zusammenfassung

Die Interaktionen zwischen drei Arten von Ektomykorrhiza-Pilzen *(Hebeloma longicaudum, Lac bicolor* und *Paxillus involutus)*, einem streuzersetzenden Pilz *(Clitocybe clavipes)*, zwei Arten von Wu pathogenen *(Fusarium moniliforme* und *F. oxysporum)* und einem Fungizid (Oxinbenzoat) wu untersucht. In Doppelkulturen reduzierten *C. clavipes* und *P. involutus* das Wachstum von *F. monili* und *F. oxysporum*. Kulturextrakte von *C. clavipes, L. bicolor* und *P. involutus* verminderten das M wachstum und die Sporenkeimung von *F. moniliforme* und *F. oxysporum*. *Hebeloma longicaudum* keine hemmende Wirkung gegen die beiden *Fusarium*-Arten auf. Zwei Verbindungen, nämlich o-Cu säure (isoliert von *P. involutus*) und p-Hydroxybenzoesäure (isliert von *L. bicolor*) reduzierte Konzentrationen von 100 bis 1000 µg/ml das Wachstum und die Sporenkeimung von *F. moni* und *F. oxysporum*. Oxinbenzoat verminderte in den empfohlenen Konzentrationen die Befallss der Wurzelfäule und erhöhte das Überleben der Keimpflanzen von Drehkiefern und Schimmelfic *Clitocybe clacipes, L. bicolor* und *F. Involutus* verbesserten ebenfalls das Überleben der Keimpflanze verminderten die Wurzelfäule, wenn die Keimpflanzen allein damit inokuliert wurden oder in Ko nation mit den empfohlenen und auch reduzierten Konzentrationen von Oxinbenzoat.

Stichwörter: Mykorrhizapilze; biologische Bekämpfung; integrierte Bekämpfung;Umfallkrankl
Koniferen-Keimpflanzen

1 Introduction

In North American conifer nurseries, species of *Fusarium* are responsible for causing damping-ol seedlings and result in considerable losses (BLOOMBERG 1973; SUTHERLAND et al. 1989; HIRATSUK al. 1995; PAIGE et al. 1995). Several fungicides are used to control the damping-off. Currently, tl fungicides have become less effective due to the development of pathogen resistance (OGAWA e 1981; ZHER 1982). It has been suggested by several authors that biological control, using fungi other micro-organisms as either an alternative or supplement to fungicides, may be a viable alterna for controlling damping-off (BAKER and COOK 1982; MARX 1972, 1973; PETERSON et al. 1984; BA 1987; DUCHESNE et al. 1989). Several species of ectomycorrhizal fungi are known to protect or parti protect conifer seedlings under laboratory conditions (DUCHESNE et al.1987, 1988a,b; CHAKRAVA et al. 1990, 1991; CHAKRAVARTY and HWANG 1991; HWANG et al. 1995). However, the rela effectiveness of ectomycorrhizal fungi in disease suppression varies with mycorrhizal species or isol host species, and soil conditions (SAMPAGNI et al. 1985).

Hebeloma longicaudum Pers.: Fr. Kummer, *Laccaria bicolor* (R. Mre.) Orton, and *Paxillus invol* (Batsch) Fr. are the three most common species of ectomycorrhizal fungi in the prairie province Canada (NAGASAWA, unplublised report). They can form abundant ectomycorrhizae with contai grown conifer seedlings (MOLINA 1980, 1982; SHAW et al. 1982; BOYLE et al. 1987; BOYLE HELLENBRAND 1991) and have great potential for wide-scale nursery inoculations. *Clitocybe cla* (Fr.) Kummer is a saprophytic fungus that occurs both in mixed woods and coniferous forests (AR 1979) and is antagonistic to certain species of root pathogens. All these fungi also occur in diverse fo habitats throughout the temperate parts of the world and can be easily isolated from sporocarp tis

The objectives of this study were to investigate the protective effect of *C. clavipes, H. longicaud* *L. bicolor, P. involutus,* and a fungicide oxine benzoate (8-hydroxyquinoline benzoate) on lodge pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.) and white spruce (*Picea glauca* [Moench] V seedlings against *Fusarium moniliforme* Scheldon (= *Fusarium verticillioides* (Sacc.) Nirenberg) *F. oxysporum* Schlecht alone or in combination.

2 Materials and methods

2.1 *In vitro* antagonism of damping-off fungi and test fungi

Antagonism of *C. clavipes* (UAMH 5580), *H. longicaudum* (UAMH 9317), *L. bicolor* (NOF 2290), *P. involutus* (NOF 2340) against two species of root pathogens, *F. moniliforme* (BCRI-F45) and *F. oxy rum* (BCRI-F30), was studied on Modified Melin Norkrans (MMN) medium (MARX 1969) in 90- Petri plates. *Clitocybe clavipes, H. longicaudum, L. bicolor* and *P. involutus* were inoculated separatel placing 5-mm agar plugs at the margin of the plates. They were allowed to grow at 20 °C in the dark. A 7 days, 5-mm mycelial plugs of *F. moniliforme* and *F. oxysporum* were placed on the plate opposite to growing colonies of the test fungi. The antagonism was observed after 5 days and photographed.

2.2 Effect of culture extract of test fungi on mycelial growth and spore germination of *Fu rium* spp.

Clitocybe clavipes, H. longicaudum, L. laccata and *P. involutus* were grown in 250 ml liquid MI medium at 20 °C in the dark on a shaker. After 24 days, culture filtrate was collected by filte through Whatman No. 1 filter paper. The filtrate (200 ml) was concentrated under vacuo to 5 ml then extracted with ethyl acetate. Ethyl acetate extract was concentrated under vacuo to dryness. F concentrations (1, 10, 100, and 1000 µg/ml) of culture extracts were made using sterile distilled w and were stored at 2 °C for bioassay. p-hydroxybenzoic acid and o-coumaric acid were isolated from culture filtrate of *L. bicolor* and *P. involutus*, respectively. The culture filtrate (5 l) was concentr

watered with 300 ml of aqueous solution (100 %, 50 %, or 25 % of recommended dose) every 15 d
No fertilizer was used. Seedlings were harvested and evaluated 8 weeks later. Seedling survival, root severity, mycorrhizal short roots, and total dry weight of each seedling were determined. Root severity was based on a scale: 0 = no root rot symptoms, 4 = severely necrotic root system.

2.5 Data analysis

Data from all the experiments were subjected to analysis of variance. The individual means were compa using Scheffe's test for multiple comparison using SAS software (SAS INSTITUTE INC. 1990).

3 Results

3.1 Interactions of *F. moniliforme* and *F. oxysporum* and test fungi

In dual culture, *P. involutus* and *C. clavipes* reduced the growth of *F. moniliforme* and *F. oxyspor* (Fig. 1). Both *F. moniliforme* and *F. oxysporum* had mutual inhibition against *L. bicolor*, i. e., growth *F. moniliforme* and *F. oxysporum* and *L. bicolor* stopped when they came in contact with each oth Both *F. moniliforme* and *F. oxysporum* grew over *H. longicaudum*.

3.2 Effect of culture extract of test fungi on *F. moniliforme* and *F. oxysporum*

Mycelial growth of *F. moniliforme* and *F. oxysporum* was significantly reduced when treated w culture extracts of *C. clavipes*, *L. bicolor*, and *P. involutus* and o-coumaric acid at concentrations of 5 50 µg/ml (Table 1). For p-hydroxybenzoic acid, mycelial growth of *F. moniliforme* and *F. oxyspor* was reduced at 50 µg/ml. The culture extract of *H. longicaudum* had no inhibitory effect agai *F. moniliforme* and *F. oxysporum* (Table 1).

Spore germination of *F. moniliforme* and *F. oxysporum* was significantly reduced when treated w culture extracts of *C. clavipes*, *L. bicolor*, and *P. involutus* and o-coumaric acid and p-hydroxybenz acid at 100 to 1000 µg/ml (Table 2). The culture extract of *H. longicaudum* had no inhibitory effect spore germination of *F. moniliforme* and *F. oxysporum* (Table 2).

3.3 Effect of oxine benzoate on the *in vitro* growth of test fungi and *F. moniliforme* and *F. o: sporum*

Oxine benzoate reduced *in vitro* mycelial growth of *F. moniliforme* and *F. oxysporum* at 1.7 µg/ml. T growth of *C. clavipes*, *H. longicaudum*, *L. bicolor*, and *P. involutus* was reduced at 1700 µg/ml (Table

3.4 Effect of oxine benzoate on seedling mortality, growth, disease development, and ecton corrhiza formation of lodgepole pine and white spruce

When inoculated with *F. moniliforme* and *F. oxysporum*, seedling survival of lodgepole pine was 40 a 44 %, respectively (Table 4). For white spruce, seedling survival was 33 % and 35 % when inoculat with *F. moniliforme* and *F. oxysporum* (Table 5). When co-inoculated with *C. clavipes*, *L. bicolor*, *P. involutus* and oxine benzoate at recommended dose and 50 % of the recommended dose, seedli survival was increased and root rot severity was reduced (Tables 4 and 5). However, oxine benzoate one-quarter of the recommended dose (25 %) had no effect on seedling survival and root rot v similar to that of seedlings inoculated with the pathogens alone. Highest seedling survival and low root rot of lodgepole pine and white spruce were observed when seedlings were treated with t recommended dose of oxine benzoate alone or in combination with *C. clavipes*, *L. bicolor*, or *P. involu* (Tables 4 and 5). Mycorrhizal colonization was reduced when seedlings were inoculated with *F. mo liforme* and *F. oxysporum* or recommended rates and half rates of oxine benzoate. However, mycorrhi colonization in lodgepole pine was not decreased when treated with 25 % of the recommended rates oxine benzoate (Table 4). Total dry weight of seedlings was reduced when treated with *F. monilifor* and *F. oxysporum* alone. The growth of seedlings was improved when treated with recommended ra

Table 1. Effect of culture extracts of test fungi and their metabolites on the *in vitro* mycelial growth of *Fusari* *moniliforme* and *F. oxysporum*

Tab. 1. Wirkung von Kulturextrakten von den Testpilzen und ihren Metaboliten auf das *in vitro*-Wachstu von *F. moniliforme* und *F. oxysporum*

Concentration (µg/ml)	Mycelial dry wt. (mg)					
	Pi	Lb	Cc	Hl	OCA	PHI
F. moniliforme:						
0	185^a	182^a	184^a	185^a	182^a	18
0.05	186^a	182^a	183^a	185^a	183^a	18
0.5	186^a	182^a	182^a	186^a	182^a	18
5	173^b	182^a	162^b	184^a	169^b	18
50	163^c	164^b	148^c	185^a	161^c	16
F. oxysporum:						
0	186^a	180^a	181^a	185^a	181^a	18
0.05	185^a	183^a	182^a	185^a	181^a	18
0.5	185^a	182^a	182^a	185^a	181^a	18
5	172^b	175^b	174^b	170^b	170^b	18
50	164^c	166^c	165^c	185^a	161^c	16

Means within a column for a particular pathogen followed by the same letter are not significantly ($P \le 0.05$) different from each other by Scheffe's
Pi = *Paxillus involutus*, Lb = *Laccaria bicolor*, Cc = *Clitocybe clavipes*, Hl = *Hebeloma longicaudum*, OCA = o-coumaric acid, PHBA = p-hydroxyben acid.

Table 2. Effect of culture extracts of test fungi and their metabolites on spore germination of *Fusarium monilifo* and *F.oxyxporum*

Tab. 2. Wirkung der Kulturextrakte von den Testpilzen und ihren Metaboliten auf die Sporenkeimung v *F. moniliforme* und *F. oxysporum*

Concentration (µg/ml)	Spore germination (%)					
	Pi	Lb	Cc	Hl	OCA	PHI
F. moniliforme:						
0	96^a	90^a	91^a	95^a	95^a	94
1	95^a	90^a	90^a	95^a	96^a	95
10	95^a	90^a	90^a	95^a	95^a	95
100	75^b	90^a	83^b	96^a	70^b	81
1000	49^c	66^b	73^c	95^a	44^c	63
F. oxysporum:						
0	92^a	90^a	91^a	91^a	90^a	9C
1	92^a	90^a	91^a	92^a	91^a	91
10	92^a	91^a	91^a	91^a	91^a	9C
100	75^b	84^b	84^b	91^a	70^b	83
1000	39^c	74^c	75^c	91^a	35^c	72

Means within a column for a particular pathogen followed by the same letter are not significantly ($P \le 0.05$) different from each other by Scheffe's
Pi = *Paxillus involutus*, Lb = *Laccaria bicolor*, Cc = *Clitocybe clavipes*, Hl = *Hebeloma longicaudum*, OCA = o-coumaric acid, PHBA = p-hydroxyben acid.

use of ectomycorrhizal fungi in the biological control of root pathogenic fungi has been discussed ZAK (1964), MARX (1972, 1973), DUCHESNE et al. (1989), CHAKRAVARTY and UNESTAM (1987a, and CHAKRAVARTY et al. (1991). Several authors have proposed a mechanism of disease suppression ectomycorrhizal fungi. MARX (1969) and MARX and DAVEY (1969) suggested that the introduction disease resistance by ectomycorrhizal fungi is associated with either the formation of a physical bar

Table 4. Interactions of test fungi and *Fusarium moniliforme* and *F. oxysporum* on seedling survival, growth, r rot severity, and ectomycorrhizal development on lodgepole pine seedlings

Tab. 4. Interaktionen der Testpilze und *F. moniliforme* und *F. oxysporum* auf das Überleben der Keimpflanz auf das Wachstum, auf die Schwere der Wurzelfäule und auf die Ektomykorrhiza-Entwicklung Keimlingen der Drehkiefer

Treatment	Seedling Survival (%)	Root Rot Index	Mycorrhizal short roots (%)	Total dry wt. (n
Control	100 ± 0	0 ± 0	0 ± 0	851 ± 5.
Pi	100 ± 0	0 ± 0	90 ± 1.5	840 ± 4.
Lb	100 ± 0	0 ± 0	85 ± 2.0	865 ± 2.
Cc	100 ± 0	0 ± 0	0 ± 0	844 ± 4.
Fm	40 ± 2.0	2.6 ± 0.7	0 ± 0	114 ± 3.
Fo	44 ± 2.0	3.4 ± 0.7	0 ± 0	212 ± 2.
Pi + Fm	69 ± 2.3	2.0 ± 0.8	61 ± 1.2	454 ± 5.
Lb + Fm	70 ± 2.3	2.6 ± 0.8	63 ± 1.5	390 ± 3.
Cc + Fm	68 ± 1.8	1.8 ± 0.6	0 ± 0	410 ± 2.
Pi + Fo	72 ± 1.8	1.6 ± 0.7	50 ± 1.3	472 ± 3.
Lb + Fo	70 ± 1.6	1.8 ± 0.9	54 ± 1.7	453 ± 5.
Cc + Fo	77 ± 1.8	1.4 ± 0.7	0 ± 0	421 ± 5.
Fm + Ob (100 %)	95 ± 1.3	1.0 ± 0.8	0 ± 0	549 ± 5.
Fm + Ob (50 %)	63 ± 1.5	2.5 ± 1.0	0 ± 0	512 ± 3.
Fm + Ob (25 %)	35 ± 1.8	3.0 ± 1.0	0 ± 0	233 ± 3.
Fo + Ob (100 %)	98 ± 1.1	0.5 ± 0.8	0 ± 0	535 ± 4.
Fo + Ob (50 %)	60 ± 1.3	2.5 ± 1.0	0 ± 0	499 ± 2.
Fo + Ob (25 %)	38 ± 1.6	3.0 ± 1.0	0 ± 0	250 ± 3.
Fm + Pi + Ob (100 %)	95 ± 1.3	0.5 ± 0.7	70 ± 2.0	720 ± 4.
Fm + Lb + Ob (100 %)	96 ± 1.0	0.1 ± 0.8	64 ± 2.2	710 ± 2.
Fm + Cc + Ob (100 %)	93 ± 1.3	0.5 ± 0.8	0 ± 0	700 ± 2.
Fm + Pi + Ob (50 %)	77 ± 1.6	1.5 ± 0.8	85 ± 1.7	610 ± 3.
Fm + Lb + Ob (50 %)	81 ± 1.1	1.5 ± 1.0	77 ± 1.8	593 ± 2.
Fm + Cc + Ob (50 %)	83 ± 1.5	0.5 ± 0.8	0 ± 0	630 ± 3.
Fm + Pi + Ob (25 %)	45 ± 1.7	2.0 ± 0.9	90 ± 1.7	550 ± 3.
Fm + Lb + Ob (25 %)	50 ± 1.5	2.5 ± 1.0	85 ± 1.7	534 ± 3.
Fm + Cc + Ob (25 %)	48 ± 1.7	2.0 ± 1.0	0 ± 0	570 ± 3.
Fo + Pi + Ob (100 %)	97 ± 1.2	0.5 ± 0.8	66 ± 1.9	709 ± 2.
Fo + Lb + Ob (100 %)	98 ± 0.9	0.3 ± 0.6	65 ± 2.0	697 ± 1.
Fo + Cc + Ob (100 %)	95 ± 1.8	0.2 ± 0.6	0 ± 0	700 ± 1.
Fo + Pi + Ob (50 %)	60 ± 1.2	1.0 ± 1.0	75 ± 2.0	591 ± 1.
Fo + Lb + Ob (50 %)	71 ± 1.5	1.0 ± 0.9	70 ± 1.8	618 ± 3.
Fo + Cc + Ob (50 %)	76 ± 1.7	0.5 ± 0.7	0 ± 0	627 ± 3.
Fo + Pi + Ob (25 %)	51 ± 1.4	2.0 ± 0.8	87 ± 1.7	510 ± 2.
Fo + Lb + Ob (25 %)	48 ± 1.4	2.0 ± 1.0	82 ± 1.8	540 ± 1.
Fo + Cc + Ob (25 %)	46 ± 1.8	1.5 ± 0.7	0 ± 0	575 ± 2.

Values are the means (± SE) of 10 seedlings. Pi = *Paxillus involutus*, Lb = *Laccaria bicolor*, Cc = *Clitocybe clavipes*, Fm = *Fusarium moniliforme*, Fo = *oxysporum*, Ob = Oxine benzoate.

liforme and *F. oxysporum;* however, chemical protection was better than biological control alone. Tl synergistic effect involving integrated control using chemical and biological control may be mo effective and longer lasting than the control achieved through either alone (Ohr et al. 1973; Elad et 1980; Schroth and Hancock 1981; Chakravarty et al. 1991). For successful integration of biolog cal and chemical control of plant pathogens, the systems must be compatible. The potential of integrated system is shown in our study, oxine benzoate at 170 µg/ml was not toxic to test fungi but w highly toxic to *F. moniliforme* and *F. oxysporum* at 1.7 µg/ml. Half and quarter rates of application oxine benzoate plus *C. clavipes*, *L. bicolor*, or *P. involutus* increased seedling survival more than d oxine benzoate applied alone. However, the highest seedling survival was recorded when the recon treated with fungicide alone. Rigorous testing in the field is necessary to determine the interactions specific strains of beneficial fungi, pathogens, fungicides, and host plants and to determine t economic benefit of integrated biological and chemical control.

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