0971-4693/94 US $ 5.00
Allelopathy Journal 30 (1): 103-116 (2012) *International Allelopathy Foundation* 2012
Tables: 3, Figs : 6

Interactions of chemical additives, pH and temperature on conidia germination and virulence of *Colletotrichum truncatum*, a bioherbicide of *Sesbania exaltata*

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(Received in revised form: April 13, 2012)

ABSTRACT

We studied several factors influencing the conidial germination and pathogenicity of fungus *Colletotrichum truncatum*, a potential bioherbicide for hemp sesbania. The conidia were germinated on 2% water agar over pH (3.5 to 9.0) and the optimal pH for the germination depended on the specific buffer used. Citrate and acetate buffers of low pH inhibited the germination. Germination decreased as buffer concentrations increased from 1 to 100 mM and as conidial density increased. Washing the conidia improved the germination. Temperatures of 15 and 20°C proved more favourable for germination and infection of hemp sesbania than 35 and 40°C. However, the emergence of germ tubes from each cell of two-celled conidia and growth and branching of germ tubes were greater at 35°C than at lower temperatures. Increased germination and branching from both conidial cells did not increase the infectivity. Germination was similar under light or dark conditions. Additions of 10 mM amino acids (alanine, glycine, phenylalanine, and tryptophan), 10 mM sugars (glucose, galactose, and xylose) and extracts of various plants (hemp sesbania and pigweed) stimulated the germination, but cystine drastically inhibited the germination. The alanine or xylose amino acids enhanced the infectivity, when applied with conidia to hemp sesbania seedling tissues.

Keywords: Amino acids, bioherbicide, biological control, *Colletotrichum truncatum*, conidial germination, mycoherbicide, pH, sugars

INTRODUCTION

Colletotrichum truncatum (Schwein.) Andrus and W. D. Moore, an anthracnose pathogen, is a bioherbicidal fungus to control hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex A.W. Hill], a major weed in soybeans [*Glycine max* (L.) Merr.] (1,9,13). Hemp sesbania is leguminous weed in soybean, cotton (*Gossypium hirsutum* L.) and rice (*Oryza sativa* L.) (26). It ranks as one of the 10 most troublesome weeds in three southern U.S. states of Arkansas, Louisiana and Mississippi (19). It is a prolific seed producer and can produce ≤ 21,000 seeds per plant (33). Its emergence is quasi-simultaneous and thus, if dense crop canopy is not formed soon after post-emergence herbicide application (without residual weed control), re-infestation occurs (33).

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Colletotrichum truncatum effectively controls the hemp sesbania in laboratory, greenhouse, and field studies. However, its spores (conidia) requires a dew period for germination, establishing infection and causing disease (9,13). Free-moisture events are difficult to predict in the field, thus knowledge of the effects of several suboptimal dew events, as opposed to a single event, is important in predicting the bioherbicidal efficacy (40,41). Increasing the infectivity (ability to infect host) of a bioherbicidal fungus in any given dew period theoretically will increase the effectiveness of pathogen (46).

Numerous studies have shown that invert (water-in-oil) emulsions and various vegetable oil-in-water emulsions can trap water and retard the evaporation in bioherbicide spray mixtures, thereby decreasing the amount of additional free moisture required to initiate spore germination and infection (5,8,10,13,20,31,35,36,45). Hemp sesbania was effectively controlled in soybean by *C. truncatum* spores formulated in a water-in-oil invert emulsion applied using specialized spraying equipment (13). We have previously reported in greenhouse experiments that oil-in-water emulsions of unrefined corn oil and *C. truncatum* spore suspensions reduced the dew period requirements for maximum weed infection and mortality of hemp sesbania from 12 h to 2 h, and delayed the need for free moisture for greater than 72 h (10). We also demonstrated that unrefined corn oil stimulated the germination of *C. truncatum* spores, but refined corn oil and several other refined oils did not increase the germination (20). The surfactant, Silwet L-77 mixed with unrefined corn oil emulsion promoted the germination and infectivity of spores of *Alternaria helianthi* a bioherbicide of common cocklebur (*Xanthium strumarium* L.) (2,37). In greenhouse and field experiments, an oil-in-water emulsion of unrefined corn oil and Silwet L-77 also increased the biological weed control efficacy of *C. truncatum* on hemp sesbania (11). Conidia of *C. truncatum*, produced in a corn meal-soya flour medium and formulated in wheat flour-kaolin granules (`Pesta') improved efficacy and the storage shelf-life of this bioherbicide (12,14). Growing and/or storage of *C. truncatum* on solid substrates has shown that rice and corn are excellent solid substrates for increasing the activity of formulations of *C. truncatum* (1).

Prior to penetration and infection, a pathogen spore must germinate and grow on the surface of host plant. Many factors are involved in this process, including nutrients and pH requirements. Various sugars, amino acids and minerals secreted by plants at the leaf surface can trigger spore germination or provide nutrition for the pathogen, and some pathogenic spores will not germinate in the absence of these compounds (3). Pathogen development is influenced by temperature, moisture, light, aeration, nutrient availability and pH. The conditions necessary for survival and successful infection differ among pathogens (6). A range of carbohydrates and amino acids occur on leaf surfaces (17). These compounds may reach the leaf surface by permeation through the cuticle (38). Dew on plant surfaces comprises moisture from the atmosphere and guttation or exudation fluids from leaves that contain sugars, starches and amino acids which provide nutrients for pathogens (44). Some sugars and amino acids can act as fungal stimuli [22,39]. Exudates of some plant tissues (seeds) have also been reported to stimulate the fungal pathogen, *Pythium ultimum*, while exudates of other plant seeds did not (32). Furthermore, pH can affect permeation of amino acids through plant cuticles (7). Increases in plant xylem sap pH during soil drying and other stresses (24,42,43) and pH can mediate physiological

responses to stress (24) . Finally, an array of sugars and amino acids are present in flours and corn meal (e.g. wheat flour, 23) and these constituents could have influenced pathogen propagule germination, growth and efficacy in the studies cited above.

Despite these important findings, little information is available about the specific factors or mechanisms involved in *C. truncatum* conidia germination. This information is important to maximize the rapid germination of conidia when applied to target weed, and to enhance disease initiation and increase weed control efficacy. Therefore, we investigated the effects of some specific chemicals (amino acids, sugars and plant extracts) and environmental factors (light, dark and temperature) on *C. truncatum* conidia germination and infection of hemp sesbania.

MATERIALS AND METHODS

I. Conidia Collection and Preparation

Conidia of *C. truncatum* (NRRL 18434) were collected from samples of infected hemp sesbania leaves and cultured on petri plates containing potato dextrose agar (PDA) as described previously (9,21). Subcultures of the fungus were obtained by transferring conidia to additional PDA plates without antibiotics and incubating them for 14 days at 25°C under an alternating 12 h fluorescent light (165 µmol m^{-2} s^{-1}) and12 h dark regime. During preparation for germination tests, a conidial mass was scraped from a plate and suspended in deionized water or test chemicals prepared in deionized water. Conidia were used in germination tests no later than 36 h after collection from a PDA plate. Conidial masses were either suspended in deionized water and washed thrice by centrifugation (900 g; 10 min) or used in germination tests without washing. Conidial densities were determined with a haemocytometer and adjusted to the desired concentration by dilution with deionized water or test solutions.

II. Sources of Chemicals

Mallinckrodt Chemical Company, St. Louis, MO was the source for most of the buffer components (glycine + HCl, potassium acid phthalate + HCl, boric acid + potassium phthalate, citric acid + sodium citrate, acetic acid + sodium acetate, sodium phosphate dibasic + sodium phosphate monobasic) used in these experiments. The buffer components Trisma HCl + trisma base, the amino acids (alanine, arginine, asparagine, cystine, dihydroxyphenylalanine, ethionine, glycine, leucine, lysine, isoleucine, methionine, norleucine, norvaline, phenylalanine, proline, serine, threonine, tyrosine, tryptophan, and valine), and the sugars [D (-) arabinose, D (-) fructose, D (+) galactose, β-D (+) glucose, α-lactose, D (+) maltose, D (+) raffinose, D (-) ribose, D (+) xylose] were products of Sigma Chemical Company, St. Louis, MO. All chemicals were of reagent grade quality.

III. Conidial Germination Tests

Suspended conidia (100 µl containing 5 x 10^5 conidia ml^{-1}) were evenly spread over the surface in the center of 6-cm petri plates containing 2% Difco-Bacto-Agar[1] in water. The conidia were incubated for 5 and 24 h. A drop of lactophenol cotton blue was added to the centre of plate and the first 200 conidia observed (under 400X magnification)

on each plate were scored for germination. A conidium was recorded as germinated, if a germ tube was visible as a projection under 400X magnification originated from either one or both cells of the two-celled conidium. In some experiments, germ tube lengths were determined with a calibrated eyepiece. Number of conidia with branched germ tubes and number of conidia with germ tubes originating from both cells were also determined.

Conidia were incubated at constant temperatures of 15, 20, 28, 35 or 40°C under either continuous fluorescent light (165 µmol m^{-2} s^{-1}) or continuous darkness or in an alternating 12 h 30°C light/12 h 20°C dark regime. To determine influence of pH upon conidia germination, buffers (1 to 100 mM) were incorporated into the 2% water agar plates prior to application of conidia to produce the desired pH range (3.5 to 9.0). The influence of nutrients was determined by adding 10 mM amino acids or sugars into the agar. The pH of un-buffered amino acids and sugars ranged from 6.1 to 7.6.

To determine the influence of plant material on germination and germ tube growth, the leaves and stems of 2 week-old, greenhouse- grown hemp sesbania seedlings were macerated with a mortar and pestle in 10 mM Tris buffer (pH 7.8.) and strained through two layers of cheese cloth. The extract was mixed with agar to give 2% agar containing plant extract concentrations of 0.8 to 8.0 mg/ml based on plant fresh weights. Similar extracts of 2-weeks-old, greenhouse-grown sicklepod (*Cassia obtusifolia* L.), velvetleaf (*Abutilon theophrasti* Medic.), redroot pigweed (*Amaranthus retroflexus* L.), cotton and soybean seedlings were also investigated for influences on conidial germination.

IV. Infectivity Tests

C. truncatum conidia (5 x 10^5 ml^{-1}) were suspended in water or test solutions and applied in 10 µl drops of water to leaves and stems of 10 to 14-day-old hemp sesbania seedlings. After 24 h in a dew chamber, the seedlings were placed in the greenhouse for up to 14 days. Detached stem sections of the hemp sesbania seedlings were also placed on moist filter paper in petri dishes, treated with 10 µl drops of water containing the conidia and then incubated in temperature-controlled chambers under alternating 12 h light/dark regimes. All treatments were visually evaluated for infection of the plants.

Experimental design and statistical analysis: All treatments of each experiment were replicated thrice. In germination and germ tube growth tests, 200 conidia were observed in each replication. In pathogenicity tests, each replication contained 10 leaves or stem sections each of seedlings. Each experiment was repeated at least twice. Results were subjected to analysis of variance. When significant differences were detected by the F test, means were separated with Fisher's protected LSD test at the 0.05 level of probability. All data were analyzed using SAS (Version 9.1, SAS Institute, Inc., Cary, NC) statistical software.

RESULTS AND DISCUSSION

Germination: Temperature and pH effects

The conidia germinated over a temperature range of 15 to 40°C in either the light or dark at pH 7.8 (10 mM Tris buffer) (Fig.1). A higher percentage of germination occurred at the lower temperatures, and germination was greatly reduced at 40°C.

Germination percentages were similar at 5 or 24 h of incubation at all temperatures in the dark. However germination was slightly higher at 24 h than at 5 h when it occurred in the light at the higher temperatures. No additional germination occurred when conidia were incubated for 48 h (data not shown).

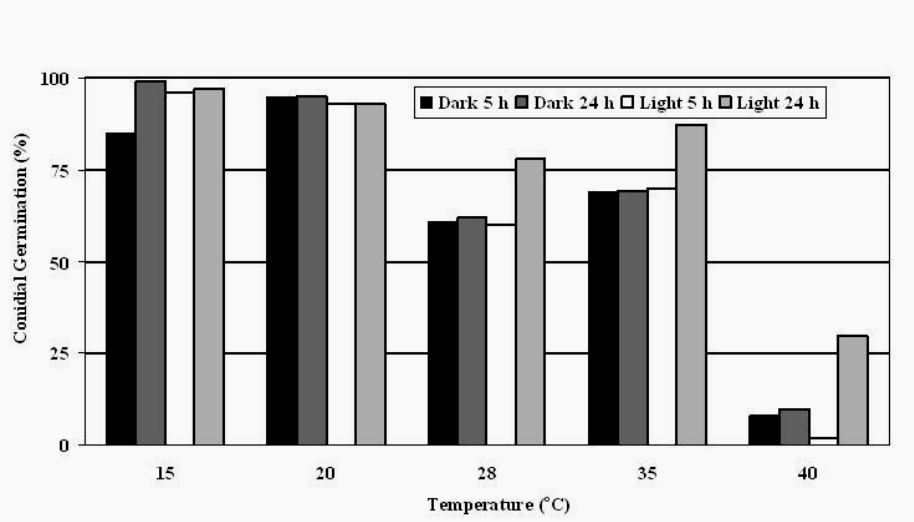

Figure 1. Effects of temperature on conidial germination percentage of *C. truncatum* under light and dark conditions.

Table 1. Conidia germination (%) of *C. truncatum* in 10 mM buffers of different pH.[a]

Buffer	pH							
	3.5	4.0	4.5	5.0	6.0	7.0	8.0	9.0
Glycine-HCl[b]	95	80	-	-	-	-	-	-
Phthalate-HCl	53	56	-	-	-	-	-	-
Boric Acid	-	71	-	-	-	-	-	-
Citrate	-	-	0	0	72	-	-	-
Acetate	-	-	0	-	-	-	-	-
Phosphate	-	-	-	-	75	80	78	-
Tris	-	-	-	-	-	58	81	87
HCl-KOH	-	63	70	32	73	69	38	96

Conidia (5×10^5) incubated 24 h in light at 28°C. [b]$LSD_{0.05} = 31$

Although the germination results varied considerably (LSD = 31%), germination occurred over the entire pH range of 3.5 to 9.0 and in some instances was over 80% at both ends of the pH spectrum (Table 1). The buffer type used to produce an acid pH influenced the results because conidia did not germinate in acetate buffer at pH 4.0 or citrate buffer at pH 4.5 and 5.0. Germination occurred in glycine-HCl, phthalate-HCl or boric acid buffers at pH 4.0 and also over the pH range of 4.0 to 9.0 when pH was adjusted with dilute HCl and KOH. The lack of germination at a low pH appeared to be due to inhibition by a

buffer component rather than due to pH (Table 1). Citrate buffer was not inhibitory at pH 6.0. Therefore the citric acid of the citrate buffer may have been responsible for the inhibition at the lower pH values.

Buffer concentration also influenced conidial germination (Figure 2). Progressively higher concentrations of citrate (pH 6.0) and Tris (pH 7.8) buffers correspondingly reduced conidia germination. In other germination trials where pH was regulated, 10 mM buffers were used. This concentration was sufficient to buffer pH and was not strongly inhibitory.

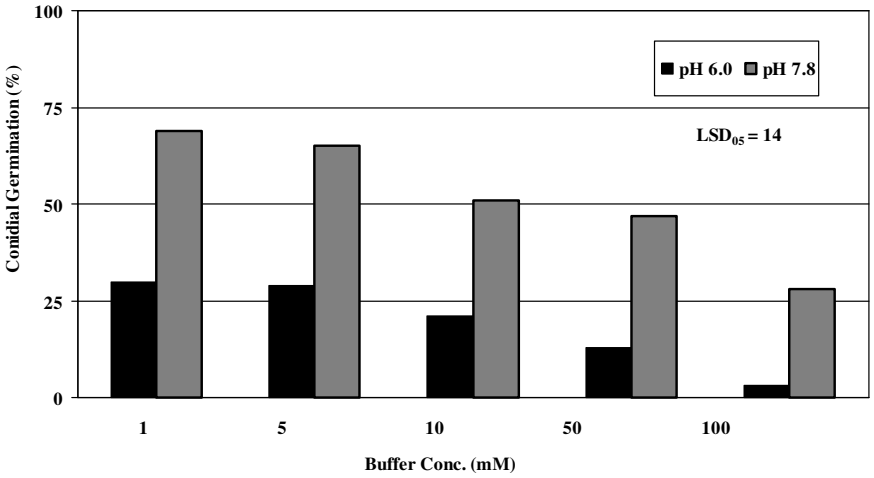

Figure 2. Effects of buffer concentration and pH on germination percentage of *C. truncatum* conidia.

Incubation temperatures also influenced the numbers of germ tubes emerging from the two cells of each conidium and the degree of germ tube branching (Figure 3). At 15°C, only one cell per conidium produced a germ tube. However, incubation at 35 C resulted in germ tube emergence from both cells of many conidia. The total number of conidia that produced at least one germ tube was greater at the lower temperatures. The number of conidia with branched germ tubes increased as temperature increased. Regardless of incubation conditions, germ tubes of conidia incubated on agar did not form appressoria. Conidial germination and germ tube length of *Microsphaeropsis amaranthi* and *Phomopsis amaranthicola* (biological weed control fungi) varied on the leaf surfaces of several *Amaranthus* species, and temperature altered the number and length of germ tubes (34).

Germination decreased from 52% $\pm$ 15% to 21% $\pm$ 8% as conidial density was increased respectively from 3.0 x 10^5 to 2.0 x 10^7 conidia/ml on the water agar plates (data not shown). This reduction was possibly due to self-inhibition of germination caused by inhibitory substances in the conidia or conidia matrix. Washing the conidia with deionized water slightly improved germination from 0.3% $\pm$ 0.2% to 18.0% $\pm$ 6.1% (data not shown). Conidia were washed three times prior to all other germination tests.

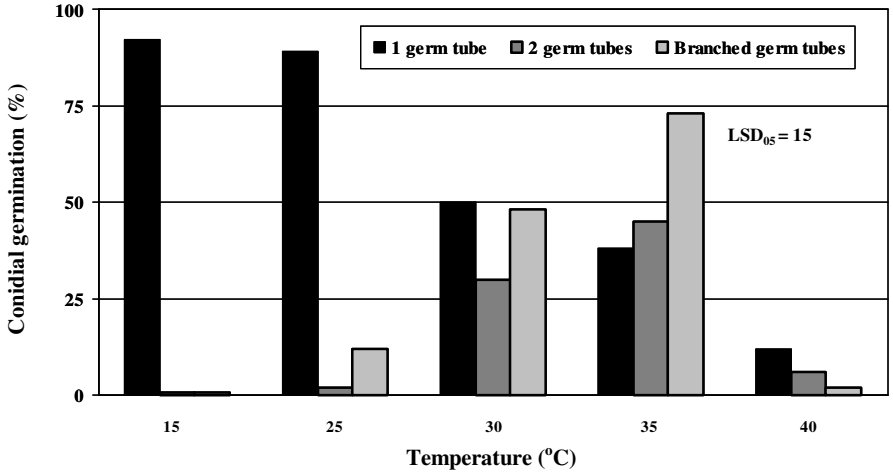

Figure 3. Effects of temperature on conidial germination (%) of *C. truncatum* producing one, two or branched germ tubes per conidium.

C. truncatum conidia germinated in the light or dark over the pH range of 3.5 to 9.0. Two buffers (citrate, acetate) in the acid range inhibited germination. In general, germination under acid conditions in other buffers was less rapid and reached lower total germination percentages than those obtained in neutral to slightly alkaline conditions. The buffer component as well as the pH influenced germination. The increased germination resulting from washing may have been due to the removal of self-inhibitors from the conidia from the conidial matrix (25,27). The reduction of germination by increased buffer concentrations indicated that increased ionic strength of the solution may be directly toxic to conidia or that higher osmotic properties of solutions may be inhibitory (18).

Germination: Amino acids, sugars, plant extracts effects

Addition of the amino acids, glycine, alanine, phenylalanine and tryptophan to water agar, significantly promoted *C. truncatum* conidia germination above the water controls (Table 2). Cystine totally inhibited conidial germination. The effects of the other amino acids were varied, and were not significantly different from the controls. Neither high percentages of germ tube emergence from either conidial cells, or increased branching of germ tubes, was associated with greater levels of germination.

Of the sugars that were added to the agar, xylose, galactose and glucose significantly stimulated germination as compared to the controls (Table 3). In other studies, sucrose was found to increase conidial germination in two distinct *Colletortrichum* spp. (*C. capsici* and *C. gloeosporioides*) (28).

We also found that germ tubes of germinated conidia in all sugar treatments had noticeably thicker walls than the germ tubes in the control. Germ tubes from conidia that were incubated with galactose, were noticeably shorter than those of the controls (data not

Table 2. Effects of DL-amino acids on conidia germination[a] (%) of *Colletotrichum truncatum*

DL Amino acid (10 mM)[c]	Conidia with		
	Germinated conidia	Germ tubes from both cells	Branched germ tubes
None (H_2O)	36	1	7
Tryptophan	85	1	0
Glycine	85	4	3
Alanine	83	1	3
Phenylalanine	72	10	8
Valine	66	35	15
Proline	61	15	12
Tyrosine	61	28	25
Asparagine	59	20	17
Dihydroxyphenylalanine	59	0	0
Methionine	52	0	3
Norleucine	51	16	18
Serine	50	14	16
Leucine	46	15	13
Norvaline	45	14	12
Lysine	42	6	1
Threonine	36	18	15
Ethionine	17	0	0
Arginine	9	0	0
Isoleucine	6	0	0
Cystine	0	0	0
LSD (0.05)	31	27	21

[a] Conidia were incubated on 2% water agar for 24 h at 28°C in the light., [b] Conidia with germ tubes originating from either one or both cells. [c] The pH of amino acid solutions ranged from 6.1 to 7.4.

Table 3. Effects of sugars on conidia germination[a] of *Colletotrichum truncatum*.

Sugar (10 mM)[c]	Conidia with		
	Germinated conidia	Germ tubes from both cells	Branched germ tubes
None (H_2O)	45	< 1	5
D (+) Xylose	90	33	23
D (+) Galactose	84	21	2
β-D (+) Glucose	66	0	32
Maltose	64	19	33
D(-) Ribose	62	5	17
D(-) Arabinose	52	10	10
D(-)Fructose	50	23	17
α-Lactose	37	25	30
D (+) Raffinose	19	17	15
LSD (0.05)	20	21	18

[a]Conidia were incubated on 2% water agar for 24 h at 28°C in the light, [b]The pH of solutions ranged from 6.2 to 7.6. [c]Conidia with germ tubes originating from either one or both cells

shown). Production of germ tubes from both cells of the conidia and germ tube branching varied among the sugar treatments.

Addition of water extracts of hemp sesbania seedling to the water agar significantly stimulated *C. truncatum* conidia germination (Figure 4). One or more substrates in the extracts were stimulatory to conidial germination. The extract concentration (8 mg/ml) that produced the highest percentage of germinated conidia also produced the highest percentage of germ tube production from both conidial cells and greatest incidence of germ tube branching. The stimulatory substance was not exclusive to host plant material extracts, since extracts of soybean, cotton, velvetleaf, sicklepod and redroot pigweed significantly stimulated conidia germination (data not shown). In other studies, conidial germination and appressoria formation of *Colletotrichum capsici* and *C. gloeosporioides* were greater on immature green or ripe red fruits of pepper (*Capsicum annuum*) than in water droplets on glass slides (28). These results indicate that plants contain constituents that have regulatory activity on germination/infectivity of some pathogens.

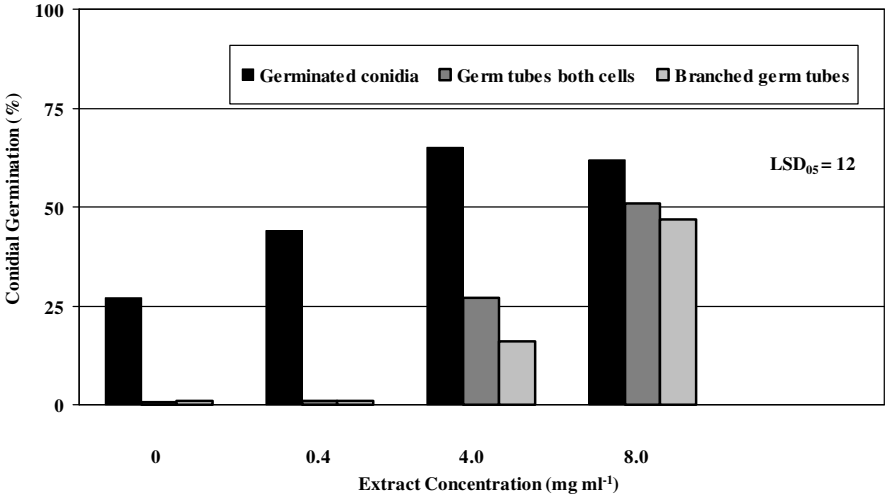

Figure 4. Effects of water extracts of hemp sesbania seedlings on conidial germination percentage of *C. truncatum.*

However, additions of 10 mM amino acids (alanine, glycine, phenylalanine, tryptophan) or sugars (xylose, galactose, glucose) enhanced germination, which indicated that if 10 mM was osmoticaly inhibitory, then the inhibition could be overcome by nutrients. The high level of germination at pH 3.5 and 4.0 with the glycine HCl buffer indicates that glycine, which was a stimulatory amino acid at pH 6.5, will also stimulate germination at a low pH. Specific nutrients may benefit germination under different conditions. Cystine strongly inhibited germination, thus inhibition or stimulation depended upon the specific amino acid. Cysteine (reduced form of cystine) has been reported to

strongly inhibit germination of *Alternaria* species (15). Daigle and Cotty (16) suggested that cysteine might be used to alleviate diseases caused by *Alternaria*. Evidently these two closely related amino acids are possible anti-fungal agents. The stimulation of conidia germination by extracts of several plants, including non-host, indicated that the stimulation by the plant extract was not due to host-specific materials. In contrast to our findings here that many compounds stimulated conidia germination, research on another *Colletotrichum* spp., *C. coccodes* showed that α-aminooxy-acetic acid, 2-deoxy-d-glucose, mannose, and oxalic acid reduced *C. coccodes* conidia germination and appressorial formation (4). But even though germination was reduced by these chemicals, the virulence of *C. coccodes* was enhanced when applied to velvetleaf (*Abutilon theophrasti*) after mannose and oxalic acid had been vacuum-infiltrated into leaf tissue.

C. truncatum: **Infectivity**

Temperatures of 15 to 28°C were more favorable than 35 and 40°C for infection of hemp sesbania tissue when conidia were applied to the stem segments (Figure 5). Only additions of alanine or xylose to the conidial formulation significantly enhanced infection severity (Figure 6).

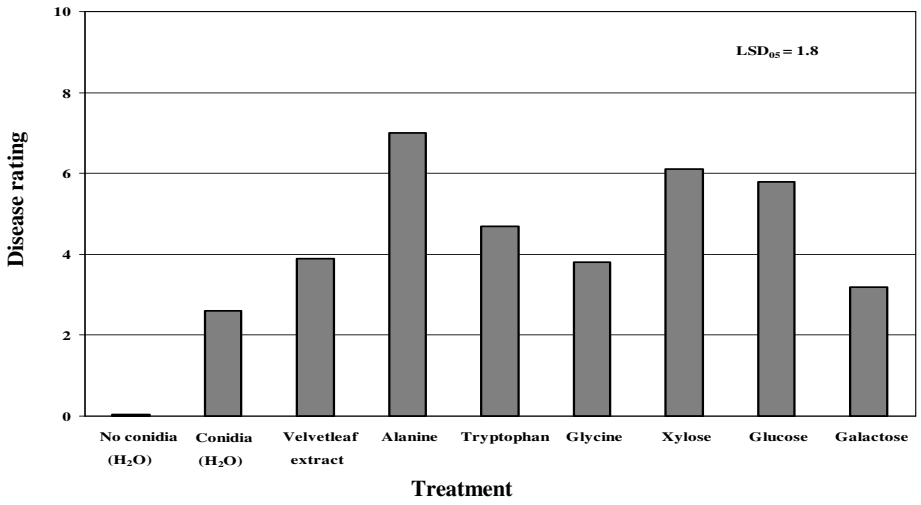

Figure 5. Conidial infectivity of excised hemp sesbania stems under constant temperatures of 15, 20, 28, 35 and 40°C and exposure to light. Plants were rated for disease severity after 14 days. A visual disease severity rating scale was used to estimate disease progression where: 0 represents unaffected, and 0.2, 0.4, 0.6, 0.8 represent 20, 40, 60, and 80 % leaf and stem lesion coverage/injury, respectively, and 1.0 = plant mortality.

Several factors influenced germ tube development. The production of two germ tubes per conidium (one for each cell) at 35°C, a temperature that reduced germination, may be an adaptive response by the pathogen to stress. The higher temperatures were less

favorable for infection of hemp sesbania by *C. truncatum*, and the response to high temperature by conidia, may be an attempt for attachment to the host by the stressed conidia. Conidia that produce two germ tubes may have an increased opportunity for achieving infection.

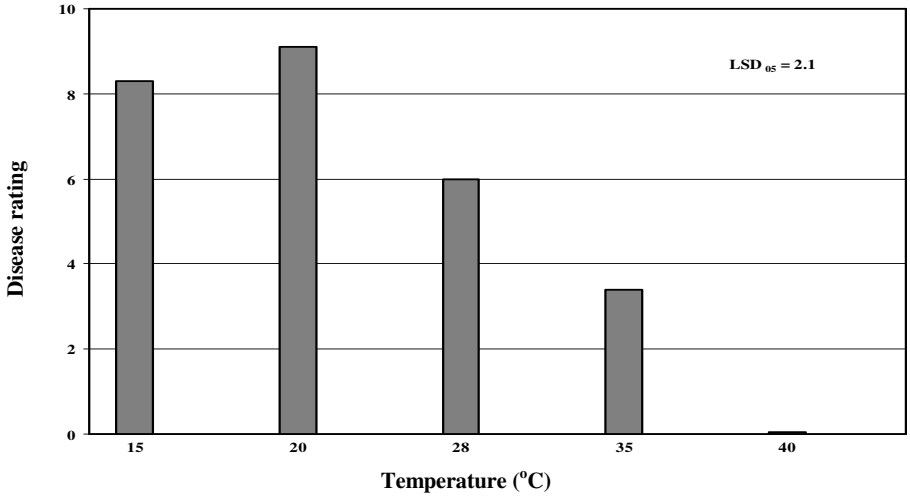

Figure 6. Influence of amino acids and sugars (10 mM) on infectivity of *C. truncatum* ~~to~~ against hemp sesbania seedlings.

The promotion of increased branching of germ tubes by high temperature should also result in additional opportunities for the pathogen to locate sites for establishment of infection. However, completion of the infection process may be hindered at some point. Regardless, increased germ tube branching, increased germ tube growth or increased germination of both conidial cells, did not consistently result in increased infectivity. We noted that when conidia were combined with hemp sesbania extract and applied to plant leaves, germination and tube growth were extensive whereas infectivity was not significantly greater than that obtained with conidia alone (Figure 6). The plant extract enhanced germination and germ tube growth on the plant surface but did not enhance the infection process. Others have shown that appressorium formation was more adversely affected by high temperature than was germination (30). Thus, infection of the plant by conidia could be reduced because of appressoria inhibition. Furthermore, McMeekin (29) found that low molecular weight alcohols initiated branching of *Peronospora parasitica* germ tubes but also reduced total germination. Two possible reasons for increased branching included disturbance of vesicle membranes sufficient to start branching but insufficient to prevent tube development, and reduced viscosity of protoplasm within the tube (29). We suggest that high temperature may also reduce viscosity of protoplasm within the tube and contribute to initiation of branching. Also, increased membrane permeability or weakening of specific membrane locations due to high temperature may be

involved. Thus branching may be a response by several fungi to stresses such as high temperature or toxic chemicals.

Sugars and amino acids that were most effective in stimulating germination were not necessarily most effective in enhancing germ tube production from both cells of a conidium or inducing germ tube branching (Figure 2 and Table 3). However, the hemp sesbania extract (4 and 5 mg/ml) increased all of these responses (Figure 4). It appeared that the plant extract promoted *C. truncatum* conidia germination and germ tube development, whereas specific sugars, amino acids and high temperature did not promote all of these processes. Without addition of nutrients or plant extract, typically only one cell per conidium produced a germ tube and germ tube branching regardless of germination percentages. The finding that adding alanine or xylose to the conidia formulation stimulated both conidia germination and increased infectivity, indicated that the addition of some germination stimulants might increase the mycoherbicidal activity of *C. truncatum*. Because several treatments that enhanced germination and germ tube growth did not cause comparable enhanced infectivity, further research is necessary to determine if this approach will be successful.

ACKNOWLEDGEMENTS

We deeply appreciate and thank Robin H. Jordan for her expertise related to the graphics and editing of this manuscript.

REFERENCES

1. Abbas, H.K. and Boyette, C.D. (2000). Solid substrate formulations of the mycoherbicide *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) control. *Biocontrol Science and Technology* **10:** 291-300.
2. Abbas, H.K. and Egley, G.H. (1996). Influence of unrefined corn oil and surface active agents on the germination and infectivity of *Alternaria helianthi*. *Biocontrol Science and Technology* **6:** 531-538.
3. Agrios, G.N. (1988). *Plant Pathology*, Third edition, Academic Press, New York, NY, USA. p 45.
4. Ahn, B., Paulitz, T., Jabaji-Hare, S. and Watson, A. (2005). Enhancement of *Colletotrichum coccodes* virulence by inhibitors of plant defense mechanisms. *Biocontrol Science and Technol*ogy **15:** 299-308.
5. Amsellem, Z., Sharon, A. and Gressel, J. (1991). Abolition of selectivity of two mycoherbicidal organisms and enhanced virulence of avirulent fungi by an invert emulsion. *Phytopathology* **81:** 985-988.
6. Anonymous (2003). Plant Pathology. Copyright © University of Sydney. Last updated December, 2003. kerem.daldal@bio.usyd.edu.au
7. Arand, K., Stock, D., Burghardt, M. and Riederer, M. (2010). pH-dependent permeation of amino acids through isolated ivy cuticles is affected by cuticular water sorption and hydration shell size of the solute. *Journal of Experimental Botany* **61:** 3865–3873.
8. Auld, B.A. (1993). Vegetable oil suspension emulsions reduce dew dependence of a mycoherbicide. *Crop Protection* **12:** 477-479.
9. Boyette, C.D. (1991). Host range and virulence of *Colletotrichum truncatum*, a potential mycoherbicide for hemp sesbania (*Sesbania exaltata*). *Plant Disease* **75:** 62-64.
10. Boyette, C.D. (1994). Unrefined corn oil improves the mycoherbicidal activity of *Colletotrichum* for hemp sesbania (*Sesbania exaltata*) control. *Weed Technology* **8:** 526–529.

11. Boyette, C.D., Hoagland, R.E. and Weaver, M.A. (2007). Biocontrol efficacy of *Colletotrichum truncatum* for hemp sesbania (*Sesbania exaltata*) is enhanced with unrefined corn oil and surfactant. *Weed Biology and Management* **7:** 70-76.
12. Boyette, C.D., Jackson, M.A., Bryson, C.T., Hoagland, R.E., Connick, W.J., Jr. and Daigle, D.J. (2006). *Sesbania exaltata* biocontrol with *Colletotrichum truncatum* microsclerotia formulated in 'Pesta' granules. *BioControl* **52:** 413-426.
13. Boyette, C.D., Quimby, P.C., Jr., Bryson, C.T., Egley, G.H. and Fulgham, F.E. (1993). Biological control of hemp sesbania (*Sesbania exaltata*) under field conditions with *Colletotrichum truncatum* formulated in an invert emulsion. *Weed Science* **41:** 497-500.
14. Connick, W.J., Jr., Daigle, D.J., Boyette, C.D., Williams, K.S., Vinyard, B.T. and Quimby, P.C., Jr. (1996). Water activity and other factors that affect the viability of *Colletotrichum truncatum* conidia in wheat flour-kaolin granules ('Pesta'). *Biocontrol Science and Technology* **6:** 277-284.
15. Daigle, D.J. and Cotty, P.J. (1991a). Factors that influence germination and mycoherbicidal activity of *Alternaria cassiae*. *Weed Technology* **5:** 82-86.
16. Diagle, D.J. and Cotty, P.J. (1991b). The influence of cysteine, cysteine analogs, and other amino acids on spore germination of *Alternaria* species. *Canadian Journal of Botany* **69:** 2353-2356.
17. Derridj, S. (1996). Nutrients on the leaf surface. In: *Aerial Plant Surface Microbiology* (Eds., C. E. Morris, P. C., Nicot and C. Nguyen-The) pp 25-42. Plenum Press, New York, USA.
18. Dillard, H.R. (1988). Influence of temperature, pH, osmotic potential and fungicide sensitivity on germination of conidia and growth from sclerotia of *Colletotrichum coccodes in vitro*. *Phytopathology* **78:** 1357-1361.
19. Dowler, C.C. (1992). Weed survey – Southern states. *Proceedings of the Southern Weed Science Society* **45:** 392-407.
20. Egley, G.E. and Boyette, C.D. (1995). Water-unrefined corn oil emulsions enhance conidia germination and mycoherbicidal activity of *Colletotrichum truncatum*. *Weed Science* **43:** 312-317.
21. Egley, G.H., Hanks, J. and Boyette, C.D. (1993). Invert emulsion droplet size and mycoherbicidal activity of *Colletotrichum truncatum*. *Weed Technology* **7:** 417-424.
22. Ellis, R.J., Timms-Wilson, T.M., Beringer, J.E., Rhodes, D., Renwick, A., Stevenson, L. and Bailey, M. J. (1999). Ecological basis for biocontrol of damping-off disease by *Pseudomonas fluorescens* 54/96. *Journal of Applied Microbiology* **87:** 454-463.
23. Gruppen, H., Marseille. J.P., Voragen, A.G.J., Hamer, R.J. and Pilnik, W. (1989). Mild isolation of water-insoluble cell wall material from wheat flour: Composition of fractions obtained with emphasis on non-starch polysaccharides. *Journal of Cereal Science* **9:** 247-260.
24. Hartung, W, and Radin, J.W. (1989). Abscisic acid in the mesophyll apoplast and in the root xylem sap of water-stressed plants: the significance of pH gradients. *Current Topics in Plant Biochemistry and Physiology* **8:**110–124.
25. Lax, A.R., Templeton, G.E. and Meyer, W.L. (1985). Isolation, purification, and biological activity of a self-inhibitor from conidia of *Colletotrichum gloeosporiodes*. *Phytopathology* **75:** 386-390.
26. Lorenzi, H.J. and Jeffery, L.S. (1987). *Weeds of the United States and Their Control.* Van Nostrand Reinhold, New York, USA.
27. Louis, I., Chew, A. and Lim, G. (1988). Influence of spore density and extracellular conidial matrix on spore germination in *Colletotrichum capsici. Transactions of the British Mycological Society* **91:** 694-697.
28. Manandhar, J.B., Hartman, G.L. and Wang, T.C. (1995). Conidial germination and appressorial formation of *Colletotrichum capsici and C. gloeosporioides* isolates from pepper. *Plant Disease* **79:** 361-366.
29. McMeekin, D. (1981). Initiation of branching in the germ tubes of *Peronospora parasitica* by alcohol. *Mycologia* **73:** 252-262.
30. Miehle, B.R. and Lukezic, F.L. (1972). Studies on conidial germination and appressorium formation by *Colletotrichum trifolii* Bain and Essay. *Canadian Journal of Microbiology* **18**: 1263-1269.
31. Mintz, A.S., Heiny, D.K. and Weidemann, G.J. (1992). Factors influencing the biocontrol of tumble pigweed (*Amaranthus albus*) with *Aposphaeria amaranthi*. *Plant Disease* **76**: 267-269.

32. Nelson, E.B. and Hsu, J.S.T. (1999). Nutritional factors affecting responses of sporangia of *Pythium ultimum* to germination stimulants. *Phytopathology* **84**: 677-683.
33. Norsworthy, J.K. and Oliver, L.R. (2000). Hemp sesbania interference in drill-seeded glyphosate-resistant soybean. *Weed Science* **50:** 34–41.
34. Ortiz-Ribbing, L. and Williams, M.M., II. (2006). Conidial germination and germ tube elongation of *Phomopsis amaranthicola* and *Microsphaeropsis amaranthi* on leaf surfaces of seven *Amaranthus* species: Implications for biological control. *Biological Control* **38:** 356–362.
35. Quimby, P.C., Jr, Fulgham, F.E., Boyette, C.D. and Connick, W.J., Jr. (1989). An invert emulsion replaces dew in biocontrol of sicklepod - A preliminary study. In*: Pesticide Formulations and Application Systems* (Eds., D. Hovde and G.B. Beestman) pp 247-270. American Society for Testing Materials, West Conshohocken, PA, USA.
36. Sandrin, T.R., TeBeest, D.O. and Weidemann, G.J. (2003). Soybean and sunflower oils increase the infectivity of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* to northern jointvetch. *Biological Control* **26:** 244-252.
37. Sanyal, D., Bhowmik. P.C. and Abbas, H.K. (2008). Effect of surfactants on bioherbicidal activity of *Alternaria helianthi* on multiple-seeded cocklebur. *Plant Pathology* Journal **7:** 104-108.
38. Singh, P., Piotrowski, M., Kloppstech, K. and Gau, A.E. (2004). Investigations on epiphytic living *Pseudomonas* species from *Malus domestica* with an antagonistic effect to *Venturia inaequalis* on isolated plant cuticle membranes. *Environmental Microbiology* **6**: 1149–1158.
39. Soloman, P.S., Tan, K.C. and Oliver, R.P. (2003). The nutrient supply of pathogenic fungi; a fertile field for study. *Molecular Plant Pathology* **4**: 203–210.
40. Walker, H.L. and Boyette, C.D. (1986). Influence of sequential dew periods on biocontrol of hemp sesbania (*Cassia obtusifolia*) by *Alternaria cassiae*. *Plant Disease* **70:** 962–963.
41. Weidemann, G.J., Boyette, C.D. and Templeton, G.E. (1995). Utilization criteria for mycoherbicides. In: *Biorational Pest Control Agents: Formulation and Delivery,* (Eds., F.R. Hall and J.W. Barry) pp 238-251. American Chemical Society, Washington, DC, USA.
42. Wilkinson, S, Corlett, J.E., Oger, L. and Davies, W.J. (1998). Effects of xylem sap pH on transpiration from wild-type and *flacca* mutant tomato leaves: A vital role for abscisic acid in preventing excessive water loss from well-watered plants. *Plant Physiology* **117:** 703–709.
43. Wilkinson, S. (1999). pH as a stress signal. *Plant Growth Regulation* **29:** 87-99.
44. Williams, D.W., Powell, A.J., Jr., Dougherty, C.T. and Vincelli, P. (1998). Separation and quantification of the sources of dew on creeping bentgrass. *Crop Science* **28:** 1613-1617.
45. Winder, R.S. and Van Dyke, C.G. (1990). The pathogenicity, virulence, and biocontrol potential of two *Bipolaris* species on johnsongrass (*Sorghum halepense*). *Weed Science* **38:** 89-94.
46. Yang, X.B. and TeBeest, D.O. (1993). Epidemiological mechanisms of mycoherbicide effectiveness. *Phytopathology* **83:** 891-893.