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PRELIMINARY EVALUATION OF A DODDER ANTHRACNOSE FUNGUS FROM CHINA AS A MYCOHERBICIDE FOR DODDER CONTROL IN THE U.S.

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ABSTRACT

Dodder (*Cuscuta* spp.) is a noxious, parasitic, annual weed throughout most of the United States. A fungus used to control it in China was imported under permit for studies with U.S. dodder species in containment. The fungus, *Colletotrichum gloeosporioides*, sporulated on liquid and solid media at room temperature. Conidia from 7-12 day old cultures were diluted to 3.5 to 7 X 16^6 spores ml^{-1} for host range inoculations. Germination on water agar at 24 hrs was higher at 28 than 30 or 24 C. Inoculated plants were exposed to dew periods of 12-14 hrs at 24 or 28 C, then transferred to growth chambers with 12-hr photoperiods at constant temperatures of 24, 28, and 32C. Dodder species were severely diseased but rarely killed. Symptoms were most severe on native collections of *Cuscuta campestris* after 4 to 5 days incubation when this species on periwinkle seedlings was inoculated with 3.5 to 7 X 10^6 spores ml^{-1}. *Cuscuta cuspidata, C. pentagona,* and *C. campestris* from a California seedlot were also tested under optimum conditions for disease. The *C. campestris* from California was the most susceptible. Inoculation of 16 species in eight plant families revealed no other host except sweet potato which developed a necrotic fleck. This research indicates a need for strain improvement prior to field tests.

INTRODUCTION

Dodder (*Cuscuta* spp.) is a parasitic weed problem on many important crops throughout the world (Ashton and Santana, 1976; Kuijt, 1969). The genus is in the family Convolvulaceae and contains over 100 species. It appears as a tiny, yellow, orange, or green vine that entwines and attaches itself to a host plant. It eventually loses contact with the soil and becomes dependent upon the host for nutrients (Musselman and Sand, 1984). The primary problem of dodder infestations in crops is loss of vigor, resulting in yield loss. It also causes problems by transmission of certain systemic diseases, and by spread of seed into uninfested areas resulting in denial of seed certification (Ashton and Santana, 1976; Musselman and Sand, 1984; Woodham and Krake, 1983).

Control of dodder is achieved primarily by chemical and cultural means. Chemical control usually utilizes soil-applied herbicides such as chloropropham, DCPA, trifluralin, and fluometuron; while cultural control involves use of weed-free seed, roguing, and burning of infested areas (Ashton and Santana, 1976; Bewick *et al.*, 1988). These methods are often ineffective and chemicals may create environmental hazards from residues or contamination of food or groundwater.

Because of difficulties and risk of existing controls, alternative methods are needed. Some of the most promising means of alternative control are biocontrol; specifically, mycoherbicides. Mycoherbicides are fungal plant pathogens that are applied as inundative inoculum, as in standard herbicides, to control specific weeds (Templeton, 1985, 1986, 1987). In some cases, mycoherbicides have proven to be as effective or more effective than chemical herbicides (Daniel *et al.*, 1973). To date only two mycoherbicides have been commercialized: COLLEGO™, a formulation of the fungus *Colletotrichum gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* for control of northern jointvetch *(Aeschynomene virginica* [L.] B.S.P.) in rice and soybeans, and DeVine® , a fungus (*Phytophthora palmivora* Butler) for control of stranglervine (*Morrenia odorata* H. and A. Lindl.) in Florida citrus groves (Templeton, 1987).

There have been other attempts to control dodder with fungal pathogens in Russia and China. In the Soviet Union, an *Alternaria* species has been used to control dodder in certain crops (Ashton and Santana, 1976), and a *Colletotrichum* species has been used for control in China (Gao *et al.*, 1985; Li, 1985).

A *Colletotrichum species, C. gloeosporioides* (Penz.) Sacc. f. sp. *cuscutae* (Cgc), was obtained from China for use in this study. The purposes were: (a) to determine if the fungus would infect and cause disease on dodder species of the U.S. in controlled environments, and (b) to examine the host range of this fungus as a prelude to further tests of it as a mycoherbicide for indigenous species of dodder in the U.S.

LITERATURE REVIEW

Musselman and Sand (1984) describe dodder as a highly specialized parasite stripped of all but the essential parts, and appearing as a tiny yellow vine that smothers its host. Dodder is reported worldwide, with the largest number of species occurring in the Western Hemisphere. Dodder has little or no host specificity. It is a significant pest worldwide on tomatoes, alfalfa, sugar beets, raspberries, cranberries, onions, asparagus, carrots, potatoes, and tobacco (Ashton and Santana, 1976; Bewick *et al.*, 1988; Musselman and Sand, 1984).

Colletotrichum gloeosporioides (Penz.) Sacc. is the conidial form of the ascomycete, *Glomerella cingulata* (Stonem) Spauld & v. Schr. This species is responsible for a number of important anthracnose disease of *Citrus* and many other plant genera (Bessey, 1950).

An *Alternaria* species has been used to control dodder in the Soviet Union. Although this species (*A. cuscutacidae* Rudak) was less effective for control of dodder on sugar beets, other genera of fungi, including *(Cladosporium, Fusarium,* and *Rhizoctonia* improved control when they were added as secondary pathogens. A *Curvularia* species has also been used for dodder control in Russia (Ashton and Santana, 1976). A strain of *Colletotrichum gloeosporioides* specific to *Cuscuta* has traditionally been used for control in China (Li 1985; Gao *et al.*, 1985), and is reported to be very effective. The only problem reported was loss of virulence, which was promptly overcome by selection of isolates for improved virulence.

In this country, the mycoherbicide concept was initiated by researchers at the University of Arkansas in the early 1970's (Daniel *et al.*, 1973). A cooperative research and development effort led to commercialization of COLLEGO, a formulation of an indigenous strain of the fungus *Colletotrichum gloeosporioides.* It is marketed for the control of northern jointvetch (*Aeschynomene virginica* [L.] B.S.P.) in rice and soybeans in Arkansas by Ecogen Corporation, Langhorne, PA. Another mycoherbicide is DeVine, a formulation of *Phytophthora palmivora* (Butler) Butler for control of stranglervine (*Morrenia odorata* [H & A Lindl.] in Florida citrus groves. The persimmon wilt fungus used for control of persimmon, *Diospyros virginiana,* in rangeland in Oklahoma is *Acremonium diospyri* (Crandall) W. Gams and is provided free to ranchers by the Noble Foundation in Ardmore, Oklahoma.

Preliminary Evaluation of a Dodder Anthracnose Fungus from China

MATERIALS AND METHODS

The dodder strain of *Colletotrichum gloeosporioides* (Penz.) Sacc. was obtained in the summer of 1987 from Dr. Yang Han Li, Nanjing University, Peoples Republic of China. It was grown and maintained on torula yeast, maltrin (M-100), potassium phosphate (dibasic), and magnesium sulfate agar (TA); cornmeal, glucose, and yeast agar (CGY); and homemade potato, dextrose, and streptomycin agar (H – PDA + S) (Tuite, 1969). Torula agar was the preferred medium because of lush colony growth and spore production. Cultures for inoculation were grown at room temperature. Stock cultures were made by inoculating TA slants with mycelial plugs and storing the slants with or without mineral oil at 5C.

Seeds of two species of dodder (*C. campestris* Yunker and *C. cuspidata* Englem) were collected from local sites and germinated two ways; either seeds were soaked for one or five minutes in concentrated sulfuric acid, rinsed in water, and placed on Whatman No. 1 filter paper in a petri dish at room temperature, or seeds were planted directly in soil (prepared by mixing 3 parts potting soil and 1 part fine grade vermiculite) contained in 7.6cm plastic or 10.8cm clay pots. Seeds of *C. pentagona* Englem and *C. campestris* were obtained from Florida and California, respectively.

The primary host plant used for dodder was tall periwinkle (*Vinca rosea* L.). Periwinkle plants were propagated either by cuttings or by seeds. Cuttings were made from mature periwinkle plants by stripping the leaves up to a node, severing the stem just below the node, and placing the cutting in a vial of water for root growth. After rooting, the cuttings were planted in pots and placed in a greenhouse. Periwinkle seeds were planted directly in pots and placed in a greenhouse. All host range plants were started and maintained utilizing the same methods and conditions as the periwinkle plants. All plants were fertilized weekly with a commercial fertilizer.

Attachment of the dodder to the host plants was achieved four ways: (1) Germinated dodder seedlings were placed in 1.5ml micro vials filled with water, attached to the upper stem of a host plant, and placed in a growth chamber or greenhouse. (2) Germinated dodder seedlings were placed in the soil next to periwinkle plants. (3) Established dodder stems were clipped and placed in vials of water implanted in the soil next to a host plant. (4) At different times, young periwinkles were infected by placing them among dodder infested periwinkle plants.

Spore suspensions were prepared by rinsing the spores with distilled water directly from 7 to 12 day old cultures grown on TA at room temperature. Mycelium was removed by filtration through cheesecloth, and desired concentrations were obtained by dilution with distilled water.

Germination of the fungal spores at different concentrations was determined by spreading 0.1ml of spore suspensions containing either 0.1, 1.0, or 10 million spores per ml on water agar plates and holding at room temperature for 24 hours. Germination percentages were determined by observing 200 spores at 10x magnification with a compound microscope and counting the germinated spores.

Spore germination at different temperatures was tested by spreading 0.1ml spore suspension containing 1 million spores per ml on water agar plates, wrapping the plates in foil, and holding at 24, 28, or 30 C for 24 hours.

C. campestris was used for most tests. It was grown in the greenhouse, trimmed to the point of attachment on the host, then moved to growth chambers about seven days before each test to provide more uniform plants.

Inoculations were made with an aerosol sprayer and applied until runoff occurred. Spore concentrations for tests ranged from 3.5 to 7 million spores per ml. A 0.5% concentration of a surfactant (Soydex) was added to the spore suspension for one test. Control plants were sprayed with distilled water only or with Soydex only. At least three replications were utilized for each test. Immediately following inoculation, plants were placed in a dew chamber. Dew period temperatures for tests were 24 or 28 C; the temperature chosen for most tests was 28 C, based on good spore germination at this temperature. Length of dew periods ranged from 12 to 14 hours. After the dew period, plants were placed in growth chambers at 24, 28 or 32 C.

Quantification of disease for the initial tests was achieved by stripping the entire dodder plant from the host plant, usually 7-8 days after inoculation, and measuring dodder stems from each host plant. Necrotic tissue (Any tissue completely shrunken and discolored and all tissues terminal to a necrotic lesion were designated as necrotic tissues.) of dodder stems was then measured. Total necrotic length was then divided by total stem length and multiplied by 100 for percent diseased tissue.

Quantification of disease for the later tests was achieved similarly, except inoculated tissues *only* was measured and all new growth of the dodder during incubation was excluded. This was done by marking certain stems of the dodder with ink before inoculation and retrieving those stems *only* for the measurements. These data were also taken 7-8 days after inoculation.

Quantification of disease for the host range tests was achieved by rating as follows: (–) = no infection or reaction, (+) = positive reaction.

Confirmation of pathogenicity of the fungus was checked by surface-washing infected tissue in a 10% clorox solution for 1 minute and placing the tissue on TA agar. All water checks were excluded from tabulated data.

RESULTS

Spores germinated best at 1 million spores per ml (40%) as compared to concentrations of 10 million (11%) or 0.1 million (37%) over a 24 hour period. The optimum temperature for spore germination over a 24 hour period was 24 to 28 C (33 and 34% respectively) compared to 17% at 30 C.

Disease symptoms began to appear on parts of the dodder stems 24 to 36 hours after inoculation. Stems first sagged, then developed small flecks, and finally collapsed, leaving only remnants of tips or midsection areas (Fig. 1). The most severe symptoms were observed during the fourth or fifth day after inoculation. After this period, severity did not increase under the test conditions.

Figure 1. Disease development on *Cuscuta campestris* four days after inoculation with *Colletotrichum gloeosporioides* f. sp. *cuscuta*.

The amount of disease (using figures which included all adventitious growth) appeared to be the greatest at 28 C, following the inoculation including soydex. With this surfactant, the average percent kill was 21.7%, compared to 12.4% at 24 C and 15% at 28 C.

In the last three temperature tests (using figures which did not include adventitious growth after inoculation), the amount of disease was greatest at 32 C. At 32 C, an average of 69% of the inoculated tissue was killed. In contrast, 40% to 47% was killed at both 28 C and 24 C. These data are summarized in Table 1.

Table 1. Kill of dodder tissue at three temperatures.

Centigrade	Regrowth Included	Percent
24	YES	13.8
	NO	44.0
28	YES	13.9
	NO	43.0
32	--	--
	NO	69.0

The most disease was observed on the California collection of *C. campestris,* with almost complete control obtained. Disease on the other three dodders appeared to be about equal with a moderate control level observed for all.

For the host range test of species other than dodder, all plants seemed unaffected by the fungus except for sweet potato (*Ipomoea batatas* [L.] Lam.). Spots or lesions appeared on the leaves a few days after inoculation, and the fungus was reisolated from infected tissue. All other species were resistant in this test (Table 2).

Table 2. Reaction of plants other than *Cuscuta* spp. to Cgc.

Family/species	Common name	Reaction
Apocynaceae		
Vinca rosea	Tall periwinkle	(-)
Chenopodiaceae		
Chenopodium album L.	Lambsquarters	(-)
Convolvulaceae		
Ipomoea coccinea L.	Red morningglory	(-)
Ipomoea lacunosa L.	Pitted morningglory	(-)
Ipomoea hederacea (var. integriuscula gray)	Entireleaaf morningglory	(-)
Ipomoea batatas (L.) Lam.	Sweet potato	(+)
Fagaceae		
Quercus palustris muenchh.	Pin oak	(-)
Poaceae		
Setaria viridis L.	Green foxtail	(-)
Lauraceae		
Cassytha sp.	cassytha	(-)
Fabaceae		
Aeschynomene virginica	Northern jointvetch	(-)
Glycine max (L.) Merr.	Soybean (var. Lee74)	
Lespedeza stipalacea Maxim.	Korean lespedeza	(-)
Medicago sativa L.	Alfalfa	(-)
Solanaceae		
Lycopersicon esculentum Mill.	Tomato (var. big boy)	(-)
Nicotiana glutinosa	"Tobacco"	(-)
Nicotiana tabacum L.	Tobacco(var. Kentucky 16)	(-)

Rating by observation only (+)=reaction, (-)=no reaction

DISCUSSION

These experiments demonstrated that the Chinese strain of Cgc would infect species of dodder indigenous to the United States. Though degree of control varied and complete control was rare, the fungus never failed to cause disease on all of the dodder plants in these test conditions.

It appears that this fungus strain may do better at warmer temperatures. Maximum disease development occurred at 30-32 C. Infection occurred at a dew period and temperature (24 to 28 C for 12 to 14 hr) that would be typical of much of the southern U.S. The fungus grew well in culture and sporulated abundantly. The host range test needs additional study due to the reaction of sweet potato. Since Cgc is an exotic organism, it should be intensively tested for any indication of risk to economic crops where it might be used.

The primary limitation of the fungus was its inability to control the adventitious growth of the dodder plant. If we take into consideration the inoculated tissue only, the percent of control was much greater than if the entire dodder plant (including adventitious growth) was considered in calculating the control percentages. Since the nature of this weed demands that new growth be considered, the lower percentages are probably more realistic. The use of a surfactant improved control but did not destroy adventitious stems. Dodder haustorial coils, from which all new stems erupt, were rarely infected, but when they were, the entire dodder plant died. Environmental or other conditions that would optimize haustorial infections were not found in these tests.

The biology of the dodder plant makes it a difficult weed to control because the host-parasite relationship is complicated, and the dodder seems to follow closely its host's biology. Dodder may be more easily controlled on some hosts than on others. For instance, the *Alternaria* species used in the U.S.S.R. controlled dodder quite well on alfalfa (90%), but was much less effective on sugar beets (Ashton and Santana, 1976). It may be possible to tailor fungal strains to be host-specific on certain dodder-crop associations and suggest areas of investigation that should be studied, if this control measure is to be feasible on a commercial scale.

The potential for this fungus as a mycoherbicide for dodder in this country, as a result of these tests, appears to be low. Additional work, however, is justified because these tests proved that the fungus could be an aggressive pathogen. Research is needed to determine optimum pre- and post-inoculation conditions, better application procedures, and the possibility for the use of surfactants or other enhancement compounds. It should be possible to select more virulent strains of the fungus. In China, loss of virulence in culture was overcome by repeated strain selection (Gao *et al.,* 1985). Selection of strains more virulent to U.S. species of dodder would seem necessary to enhance the commercial feasibility of this fungus in this country.

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