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Biological Control of *Cannabis sativa*

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Abstract

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cannabis* is a suitable pathogen for use in the biological control of unwanted *Cannabis sativa*. Inoculum appropriate for large scale field use can be grown on a variety of media. One gram/m^2 (8.8 lb/A) of prepared inoculum results in 50% mortality of susceptible *C. sativa* the first year and increases in succeeding crops. The fungus can be seedborne. Italian hemp cultivars are resistant to the disease while all other cultivars are susceptible. The fungus causes disease only in *C. sativa*. No other kinds of plants have been infected. Because of the restricted host range, no special precautions are necessary for controlled use of the fungus.

Lutte Biologique contre *Cannabis sativa*

La fusariose provoquée par *Fusarium oxysporum* f. sp. *cannabis* constitue un agent pathogène approprié pour la lutte biologique contre la plant nuisible *Cannabis sativa*. Un inoculum s'adaptant à l'utilisation à grande échelle peut être cultivé dans toutes sortes de milieux. Une préparation de 1 g/m^2 (8.8 lb/A) permet de tuer 50% des plants sensibles de *C. sativa* au cours de la première année et ce pourcentage augmente au cours des années ultérieures. Le champignon peut se développer dans les graines. Les cultivars de chanvre italien sont résistants à la maladie tandis que tous les autres cultivars sont vulnérables. Le champignon ne nuit qu'à *C. sativa*. Aucune autre espèce de plantes n'a été infectée. En raison de la gamme restreinte d'hôtes, aucune précaution particulière ne doit être prise pour contrôler l'utilisation du champignon.

Introduction

The purpose of this study was to determine the feasibility of using fusarium wilt, caused by *Fusarium oxysporum* Schlecht. f. sp. *cannabis* Noviello & Snyder (Hyphomycetes) to control unwanted *Cannabis sativa* L. (Moraceae). The objectives of the research were to determine: (1) the genetic stability and host specificity of *F. oxysporum* f. sp. *cannabis*; (2) methods of producing and applying inoculum; (3) effectiveness of the fungus in the field; (4) survival of the fungus in soil and reinfection of host plants in succeeding seasons; and (5) special precautions or techniques for controlled use of the fungus as a pathogen directed to disease production in a specific host.

Fusarium wilt of hemp (*C. sativa*) causes serious losses in the cultivated crop in Italy (Noviello and Snyder 1962). The disease was observed in a field at Alvignano, Caserta, in 1959 and in some other localities in 1960. The foliage of infected plants yellows, wilts, dries up and hangs on the plants. A dark-brown discoloration of the vascular system attends the foliage symptoms. As a rule, infected plants are killed. In the field, the disease is first noted in June when the plants are three months old, and

by the end of June the disease is very evident. The disease is known to occur naturally only in Italy.

The disease is caused by *F. oxysporum* f. sp. *cannabis*. The form of the fungus that attacks *C. sativa* is highly specialized and is not known to infect other crop plants. *F. oxysporum* specialized forms are restricted in pathogenicity to very limited host ranges (Snyder and Hansen 1940). For example, the tomato form only attacks tomato. Crop plants such as watermelons, alfalfa, and beans (all susceptible to different specialized forms of the fungus) are not affected when grown in fields known to harbor the tomato form of the fungus, *F. oxysporum* Schl. f. sp. *lycopersici* (Sacc.) Snyder & Hansen. Hops (*Humulus lupulus* L.; Moraceae), one of the closest crop plants related to *Cannabis* spp., are not known to be susceptible to a fusarium wilt disease anywhere in the world.

Genetic Stability

The area devoted to the cultivation of hemp in Italy has declined from a high of 20,000 ha to the present level of approximately 80 ha in all of Italy. The crop is no longer grown in the Caserta area where fusarium wilt was severe in 1959. Most of the hemp in Italy is now grown in an area near Naples. The susceptible cultivars grown in 1959 and 1960 are no longer cultivated. The cultivar grown in 1972 through 1975 was 'Super Fibra' ('SF').

Hemp fields in Italy were visited in 1972, 1973, 1974 and 1975. Hemp plants exhibiting fusarium wilt were difficult to find but some infected plants were found and isolations were made from stem tissues. The fungus was present in most of the selected, infected plants. In 1972 the fungus was recovered from plants from three different fields. The incidence of the disease was very low; possibly $< 0.1\%$. *Sclerotium rolfsii* Sacc. (Agonomycetes), broomrape, and stem-boring insects made it difficult to identify plants with fusarium wilt. We now know that 'SF' is very resistant to fusarium wilt and this explains the low levels of the disease encountered. In retrospect it is surprising that it was even possible to find the disease.

The isolates of the fungus recovered from the hemp plants were similar in appearance on agar medium. Pathogenicity tests were conducted using four Italian hemp cultivars available: 'SF'; 'Carmagnola Originaria'; 'Eletta Campana'; and 'Carmagnola Selezionata' ('CS'). Roots of seedlings were planted in a sand-peat potting mixture and maintained in a growth chamber at 27°C. Very few plants exhibited fusarium wilt symptoms; however, the fungus was recovered from two plants of 'CS'. Twenty single-spore cultures were established from the re-isolated fungus. The single-spore isolates were all similar in appearance and did not differ from other isolates of the fungus. The characteristics of the fungus in culture were very stable. The fungus appears the same on agar medium today as it did when first isolated in 1972.

Pathogenicity tests using the four Italian hemp cultivars, 'Iowa' and 'Iran' did not reveal differences in pathogenicity among the fungus isolates; thus there appears to be a single race or pathotype of the fungus which is genetically stable.

Cultivar Susceptibility

Twenty-two *C. sativa* seed collections from different parts of the world were evaluated for disease susceptibility. We had determined that wounds were not necessary for infection and that plants became infected when seeds were sown in infested soil. The inoculum level was very high, in excess of 10,000 fungus propagules/g of soil. The tests were conducted in a growth chamber in continuous light and 8 h at 21°C and

16 h at 27°C. Seedlings emerged in 4–6 days depending upon the age and vigor of the seed. The first dead seedlings appeared 10–12 days after sowing the seed. Cultivars or seed collections from Mexico (three different collections), Pakistan, Turkey, Thailand, India, Nepal, South Africa, Czechoslovakia, Poland and Iran were all highly susceptible to the disease and in general there were no survivors or escapes in the growth chamber study. The four Italian hemp cultivars were resistant, and only 10–20% of the seedlings became infected. The survivors generally developed normally and were uninfected at the termination of the experiment. The fungus was consistently isolated from dead or diseased plants of the susceptible cultivars. Seeds obtained from Portugal were also resistant. Mr. Melisurgo of the Italian Hemp Growers Association believes that the Portugal seed is of Italian origin, that is grown in Italy and is most likely 'SF'.

Seeds from escaped hemp plants collected in Iowa ('Iowa') were intermediate in susceptibility. When 'Iowa', 'Iran' and 'SF' were compared, 100% of the 'Iran' plants were dead after 30 days, 70% of the 'Iowa' plants were dead, and 20% of the 'SF' plants were dead at the conclusion of the experiment. Fifty percent of the 'Iran' seedlings were dead in 14 days, while 19 days passed before 50% mortality occurred in the 'Iowa' seedlings. The 'SF' seedlings that died did so mostly (90%) in the first 20 days.

Host Specificity

A variety of plants were tested for susceptibility to the fungus. Seeds were either planted in heavily infested soil (excess of 20,000 propagules/g) or were transplanted into infested soil. Isolations were made from any plant that differed from the non-inoculated control plants. The fungus was never isolated from any plants other than *C. sativa* 'Iran' plants grown at the same time in infested soil. The 'Iran' plants always became infested. The plants were maintained in a greenhouse at 27°C. Tomatoes, cotton, and carnations were also grown in soil infested with their respective *F. oxysporum formae speciales* and became infected. Plants grown in infested soil are listed in Table 1.

No crop plants, including tobacco, tomato, bean, wheat, grape, alfalfa, corn, potato, and squash, grown in rotations with hemp in Italy have become infected by the fungus.

Inoculum Production

Various animal feeds (including alfalfa meal, almond hulls, rolled barley, sugar beet pulp, cottonseed meal, rolled milo, safflower meal, soybean oil meal, malted barley, and barley straw) were evaluated as culture media for production of inoculum. Alfalfa meal, soybean meal and cotton seed meal were superior to the other feeds in the numbers of chlamydospores and conidia produced. The superiority of these materials may be related to the high protein content. Since these materials tended to cake and the fungus did not grow well in the autoclaved medium, they were diluted with barley straw to facilitate aeration and separation.

The fungus was grown on medium composed of barley straw (*Hordeum vulgare* L.; Gramineae) and soybean meal (*Glycine max* [L.] Merr.; Leguminosae) from which the oil had been extracted. The barley straw was milled so that the largest pieces were 30 mm but most pieces were smaller. Soybean meal, 160 g, was mixed with 800 g of barley straw and 2-*l* of water. The mixture was thoroughly stirred and placed in large glass flasks plugged with cotton for sterilization. It was necessary to autoclave the medium twice to eliminate heat-resistant bacteria. The first autoclaving was at 121°C for 1 h followed in 24 h by the second autoclaving for 1 h at 132°C.

Table 1. Plants grown in soil infested with *Fusarium oxysporum f. sp. cannabis* Noviello & Snyder.

Scientific Name	Common Name	Cultivar
Abelmoschus esculentus (L.) Moench	okra	Clemson Spineless
Allium cepa L.	onion	Tulelake 401
A. sativum L.	garlic	"Safeway"
Anethum graveolens L.	dill	-
Apium graveolens L. var. dulce (Mill.) Pers.	celery	Fordhook
Arachis hypogaea L.	peanut	Jumbo Virginia
Asparagus officianalis L.	asparagus	UC72
Avena sativa L.	oats	Montezuma
Beta vulgaris L.	beet	Ruby Queen
B. vulgaris	sugar beet	F56
B. vulgaris var. *cicla* L.	chard	Ocelga
Brassica juncea (L.) Czern. & Coss.	mustard	Fordhook Fancy
B. oleracea L. var. *botrytis* L.	cauliflower	Early Snowball
B. oleracea L. var. *capitata* L.	cabbage	Wisc. Golden Acre
B. rapa L.	turnip	Purple Top
Callistephus chinensis (L.) Nees	aster	Burpeana
Capsicum frutescens L.	pepper	Burpee Tasty Hybrid
Carthamus tinctorius L.	safflower	Gila
Chrysanthemum morifolium Ramat.	chrysanthemum	Iceberg
Cicer arientinum L.	chickpea	-
Citrullus lanatus (Thunb.) Matsum. & Nakai	watermelon	Klondike
Cucumis melo L.	muskmelon	Burpee 199
C. sativus L.	cucumber	Ashley
Cucurbita pepo L.	zucchini	Italian Marrow
Daucus carota L.	carrot	Danvers Half Long
Dianthus caryophyllus L.	carnation	Red Scania
Eschscholzia californica Cham.	California poppy	-
Glycine max (L.) Merr.	soybean	Kanrich
Gossypium hirsutum L.	cotton	SJ-1
Helianthus annuus L.	sunflower	Mammoth
Hordeum vulgare L.	barley	Calif. Mariout
Humulus lupulus L.	hop	California
Ipomea batatas (L.) Lam.	sweet potato	-
Lactuca sativa L.	lettuce	Great Lakes
Linum usitatissimum L.	flax	New River
Lycopersicon esculentum Mill.	tomato	Bonny Best
Matthiola incana (L.) R. Br.	stock	-
Medicago sativa L.	alfalfa	Moopa
Morus alba L.	white mulberry	-
M. nigra L.	black mulberry	-
Oryza sativa L.	rice	Colora
Papaver rhoeas L.	corn poppy	-
P. somniferum L.	opium poppy	Iran
Phaseolus aureus Roxbg.	mungbean	Yuba
P. vulgaris L.	bean	Calif. Red Kidney
Pisum sativum L.	pea	Thomas Laxton
Raphanus sativus L.	radish	Scarlet Globe
Solanum melongena L.	eggplant	Early Beauty

Table 1. Continued.

Scientific Name	Common Name	Cultivar
S. tuberosum L.	potato	Russet Burbank
Sorghum bicolor (L.) Moench.	milo	Double Dwarf
Spinacia oleracea L.	spinach	America
Trifolium repens L.	white clover	-
Triticum aestivum L.	wheat	Pitic 62
Vicia sativa L.	vetch	Lana
V. faba L.	fava bean	-
Vigna unguiculata (L.) Walp.	cowpea	California 3
Zea mays L.	sweet corn	Golden Bantam
Zinnia elegans Jacq.	zinnia	Bodger

After cooling, the medium was inoculated using conidial suspensions obtained from cultures of the fungus grown on potato-dextrose agar. After 2–4 wks at 20–22°C the inoculum was removed from the flasks and dried for 5–7 days in a laboratory room at 20–22°C, r.h. 40–50%. After drying, the inoculum was broken up by hand, placed in polyethylene plastic bags and stored in the laboratory or in a freezer at −10°C. No attempt was made to maintain the inoculum in a sterile condition after removal from the culture flasks.

The inoculum was composed of particles of the straw and soybean meal colonized by the fungus — mycelium, conidia, and chlamydospores.

The inoculum used in the 1974 field trials in Portici, Italy, was 3–4 months old. No appreciable loss of viability has been detected in inoculum kept at 20–22°C for 1 yr or at −10°C, although one might expect a slow loss of viability of inoculum kept at 20–22°C. A more detailed report has been published (Hildebrand and McCain 1978).

Application of Inoculum

In greenhouse and growth chamber studies the prepared inoculum was incorporated into soil at various levels. The soil was moistened and left undisturbed for 2 wks. The number of propagules of the fungus/g of soil was determined by soil dilution plating (Nash and Snyder 1962). Three hundred propagules/g of soil were present when 0.025 g of inoculum was mixed with 1000 g of air-dry sandy loam soil, 2900 propagules/g at 0.050 g/1000 g soil; 11,300 propagules/g at 0.250 g/1000 g soil; 18,500 propagules/g at 0.500 g/1000 g soil; 67,000 propagules at 2.50 g/1000 g soil; and 95,000 propagules/g at 5.00 g/1000 g soil.

When susceptible 'Iran' was sown in soil containing different levels of the fungus, the time required for total kill of the seedlings was proportional to the inoculum level. At 7000 propagules/g of soil, 100% mortality was reached in 9 days; at 1400 propagules/g, 15 days elapsed before 100% mortality was reached; 22 days at 700 propagules/g; and 47 days at 70 propagules/g. At 7 propagules/g, 25% mortality occurred after 47 days (when the experiment was terminated).

Effectiveness of fungus in the field. In April 1974, four fields in Italy were inoculated using three levels of air-dry straw-soybean inoculum. The inoculum was distributed by hand and mixed into the top 10 cm of soil with a hoe to prevent loss by blowing and so that the hemp plants would be certain to make contact with the inoculum.

Ten g/m^2 were distributed in the plot at Vitulazio, Italy. The soil at Vitulazio is a loamy clay. The inoculated plot was seeded to 'CS' and 'SF'. No *Fusarium*-infected plants were observed at harvest in July in this field.

A field near Alvignano, Italy, was inoculated with 10 g/m^2 and seeded with 'SF' and seeds from 'Iran'. There was insufficient seed of 'Iran' to plant the Marcianise and Vitulazio fields in 1974. When final disease counts were made in August, 71% of the 'Iran' plants had died while only two plants of 'SF' in the inoculated plots were found infected. The surviving 'Iran' plants in the inoculated plots were 1.45 m in height while those in the non-inoculated control plots were 3.60 m in height. The surviving plants were probably infected but isolations were not made from these plants. Isolations confirmed that the dead or dying plants were infected by the fungus. By June 14, 29% of the 'Iran' plants had died. It was clear that the inoculum was effective and that 'SF' was highly resistant to the disease.

There were two inoculum experiments conducted at Portici, Italy, in 1974. One was in a field of fine sandy loam and the other in large ceramic pots containing the same field soil. Three levels of inoculum were applied: 1, 10 and 30 g/m^2. Three cultivars of hemp ('CS', 'SF' and 'Iran') were seeded on 20 April 1974. There were four replications, and diseased (dead) counts were made periodically and the infected (dead) plants removed to facilitate counting. Removal of the dead plants of course reduced inoculum for succeeding crops. The first infected plants were removed from the plots on 4 June. Final counts were made on 3 August. Dead 'Iran' plants for the three levels of inoculum (1, 10 and 30 g/m^2) were 50, 94.2 and 93.7%, respectively. No plants in the non-inoculated plots became infected. The level of disease in the Italian hemp cultivars 'CS' and 'SF' was low: only 4% of the 'CS' cultivar died in the plots receiving the highest inoculum level of 30 g/m^2 and 14% of the 'SF' variety died at this inoculum level.

'Iran' plants that did not die in the Portici field were stunted. Plants in the non-inoculated plots averaged 1.3 m in height; those in the 1 g/m^2 plots were 0.96 m, and 0.71 m in the 10 g/m^2 plots. When height measurements were made, there were insufficient plants to measure in the 30 g/m^2 plots. Results from the experiment in the ceramic pots were almost identical to the field trial.

Downy mildew, *Pseudoperonospora cannabina* Otth (Hoerner) (Peronosporales), was severe on 'Iran' cultivar and zineb fungicide was applied to control the disease. Downy mildew was not severe on the Italian hemp cultivars and did not require sprays which were, however, applied.

Survival of the fungus in soil. An experiment was conducted in a sandy loam field at the University of California San Joaquin Valley Agricultural Research and Extension Center at Parlier. Two levels of straw-soybean inoculum, 1 g/m^2 and 10 g/m^2, were incorporated into the top 7.5 cm of soil on 16 April 1974. A crop of corn was planted which was harvested in October. The inoculated plots were sampled periodically and the number of propagules of *F. oxysporum* f. sp. *cannabis* determined. *F. oxysporum* f. sp. *cannabis* was readily distinguishable from the native *F. oxysporum* present.

The average number of propagules in the inoculated plots declined from the initial levels of 1500/g of soil in the 1 g/m^2 plots and 90,000 g of soil in the 10 g/m^2 plots to a just detectable level (19 propagules/g) in the 1 g/m^2 plots and 250 propagules/g in the 10 g/m^2 plots 200 days after incorporation. Monitoring of the fungus level was discontinued in the 1 g/m^2 plots after 200 days. After 460 days the propagule level had declined to 40/g in the plots that had received 10 g/m^2 of the straw-soybean inoculum.

Barley was planted in the spring of 1975. *F. oxysporum* f. sp. *cannabis* in the inoculated plots increased in numbers in senescent barley roots along with other *F. oxysporum* forms, and then declined again as the soil became dry. The last propagule count was made in August 1975, 16 months after inoculation. The fungus was still detectable at 125 propagules/g of soil. Survival of the artificial, straw-soybean inoculum in the absence of host plants was adequate to ensure the presence of the fungus in the soil for at least one growing season.

The experimental work area was fumigated with methyl bromide gas beneath a polyethylene cover in November 1975. The fungus is most likely no longer present in this field.

Reinfection of host plants. Three of the same fields in Italy inoculated and planted with hemp in 1974 were replanted in 1975. The susceptible 'Iran' cultivar was planted in each field in addition to seeds which were purchased in Portugal.

In the Vitulazio field, where no disease was detected in the resistant Italian hemp cultivars in 1974, there was 6.2% fusarium wilt in the 'Iran' in late July 1974 and little or no disease in 'Portugal'. The fungus was able to survive or maintain itself on the resistant cultivars.

In the Marcianise field, where in 1974 there were scattered infections in the resistant Italian cultivars without correlation to inoculum level, there was severe disease in 'Iran' and the amount of disease (mortality) was correlated with the 1974 inoculum levels. The percent mortality in July 1975 in the 1 g/m^2, 10 g/m^2 and 30 g/m^2 rates of inoculum (applied in April 1974) were 36, 87 and 93%, respectively.

The ceramic containers were also replanted using 'Iran', 'Portugal' and 'SF' seeds. Percent of disease were: 97.5% for 'Iran', 5.9% for 'Portugal', and 3.9% for 'SF'. There was no infection in the noninoculated pots since there was no contamination of these pots from soil movement.

Seed-borne inoculum. Susceptible 'Iran' plants that became infected in field plots in Italy did not produce seed. However, in most fusarium wilt diseases viable seeds do not become infected, but the fungus contaminates the seed as bits of infected tissue mixed with the seed or as spores that cling to the seed.

To demonstrate that *F. oxysporum* f. sp. *cannabis* could also be seed-borne, moistened 'Iran' seeds were mixed with straw-soybean inoculum and large visible pieces of inoculum removed. The inoculated seed was indistinguishable from the non-inoculated seed. After air-drying the seeds were planted in pasteurized soil. Fifty percent of the inoculated seeds became infected and died within 30 days, when the experiment was terminated.

Safety of Inoculum

Inoculum composed of straw-soybean meal colonized by the fungus was tested for mycotoxins and mutagens. Chloroform extracts of the inoculum were injected into mice with no adverse effects. The chloroform extracts were also used in culture medium of bacteria strains sensitive to mutagens. These tests were conducted by Dr. Leonard Bjeldanes, Nutrutional Sciences, University of California, Berkeley. Professor Chester J. Mirocha, Department of Plant Pathology, University of Minnesota, analyzed a sample of inoculum and found no T-2 tricothecene toxin nor diacetoxyscirpenol.

Discussion

In absence of host plants *F. oxysporum* f. sp. *cannabis* slowly declines in the soil. A relatively healthy crop of *C. sativa* might be grown in a previously infested field after

a number of years, possibly five or more, free of *C. sativa*. But the disease would build up again on succeeding crops. Where *C. sativa* is a weed in other crops, susceptible *C. sativa* plants would be eliminated. Although 'Iowa' (from escaped plants) was not as susceptible as 'Iran', it is not known if the surviving plants grown in inoculated soil were escapes or were genetically resistant. If they were genetically resistant, then the effectiveness of the fungus as a weed control agent would be diminished.

Since resistant cultivars are known, it would always be possible to grow *C. sativa* in areas where the fungus had been introduced. The presence of resistant cultivars might limit the length of time that the fungus would be useful in the control of illicit marijuana.

For biological control purposes, inoculum of the fungus could be introduced by air. It would not be necessary for complete coverage of a field since natural spread would occur by soil and plant debris movement and through the use of contaminated seed.

The biological control of *C. sativa* utilizing the fungus should be tested in fields where *C. sativa* is a weed and in fields where *C. sativa* is an illicit crop. The introduction of straw-soybean inoculum of the fungus by airplane would be considerably less expensive than overland travel and burning or otherwise disposing of the illicit crop. It would also have the advantage of not being necessary to return to the same area in succeeding years.

Downy mildew was severe on 'Iran' cultivar in the Italian field trials. This fungus might also be used in a biological control program, and might even be used together with *F. oxysporum* f. sp. *cannabis*. Hemp downy mildew is not known to infect other plants but cross-inoculation studies with hemp and hop downy mildew (*P. humuli*) should be conducted since hop downy mildew has been reported from *C. sativa*. Production of inoculum of *P. cannabina* would be difficult since it would be necessary to grow the parasite on *C. sativa* plants and the sporangia are not long-lived. Plantings of infected *C. sativa* might be maintained upwind of areas where illicit *C. sativa* is grown.

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