BARD

FINAL REPORT

PROJECT NO. US-1021-85

Development of Plant Pathogens for Weed Control

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Standard BARD Cover Page for Scientific Reports

Date: April 5, 1991BARD
P.O. BOX 6
Bet Dagan, ISRAELBARD project No. U.S.-1021-85R

Title

Development of Plant Pathogens for Weed ControlInvestigators' Names
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Signature
Principal Investigator
Signature
Institution's Authorizing Official

630.72
BAR/QVI : 632.58 ; 589.2
2nd copy

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ABSTRACT

The objective of this research was to advance biological control technology in the use of plant pathogens (fungi) for weed control in crops important to both Israel and the United States. The focus was to be on characterization of pathogens already discovered in the U.S. and Israel as well as survey, discovery, identification, and characterization of other pathogens on weeds in each country. In Israel, methods were sought for obtaining germination of teliospores and basidiospores of *Puccinia xanthii* as a potential augmented weed pathogen of cocklebur. Germination of teliospores was unaffected by temperature; infection of cocklebur was attained by pretreating inoculum in 0.5% sucrose and then maintaining the host weeds under 100% RH. In the U.S., *Alternaria helianthi* was characterized as to its effects on cocklebur when applied in various spore concentrations on plants at various stages of growth. As the plants increased in size, resistance increased. Similar results were obtained for *Alternaria crassa* as applied to jimsonweed. Perhaps the greatest achievement of the entire project was the discovery and characterization of "new" pathogens: *Cercospora* nr. *caricis* against purple nutsedge in Israel and *Colletotrichum truncatum* against hemp sesbania in the United States. The *Cercospora* was found to infect nutsedge when applied as mycelia or as diseased straw to healthy plants. Leaves or plants at different ages and stages were susceptible. Apparently the fungus acts by accelerating abscission of leaves. The *Colletotrichum truncatum* isolate was compared with other isolates of the same species and was found to have a different host range, i.e., it was specific for hemp sesbania. Thus, evidence was presented for the designation of specialized forms of this species. Thus, a base was laid for biological control of weeds research in Israel and significant progress was met toward implementation of the use of weed pathogens for weed management systems in both countries.

d. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

Objectives of the original research proposal were: 1) to advance biocontrol technology for use of two pathogens, viz., *Alternaria helianthi* and *Puccinia xanthii*, as potentially useful weed pathogens for the main weed target, *Xanthium strumarium*; and 2) to conduct surveys to investigate the potential of other weed/pathogen combinations. These endemic fungi were to be used (investigated) in their respective countries. The *A. helianthi* research was to be focused in the USA while that on *P. xanthii* was to be centered in Israel. In both countries, objective 2 above (surveys and development of other weed/pathogen combinations) was pursued and greatly expanded the scope of the research. For example, research in the USA over the period of the BARD project included studies of *A. crassa* for control of *Datura stramonium* and *Colletotrichum truncatum* for control of *Sesbania exaltata*, while research in Israel included studies of *Cercospora near caricis* for control of *Cyperus rotundus*.

e. BODY OF THE REPORT

The report is divided into two main sections: Section A describes the research reported in Israel and Section B describes that reported in the USA.

I. FUNGI ATTACKING NOXIOUS WEEDS IN ISRAEL

Introduction

Israel, and apparently the entire Middle East region, has been until lately almost *terra incognita* as regards fungi pathogenic to noxious weeds. A cursory attempt (Kenneth and Eshel, unpublished) at testing *Cercospora molucellae* Bremer and Petrak against *Molucella laevis* L. was made ca 15 years ago. Lists of fungi on various wild plants has been published by Prof. T. Rayss at Jerusalem over a period of 30 years, but no attempt had been made to single out those on weeds. Over the years, fungi were found by Kenneth and by others on the following problematic weeds:

Cynodon dactylon (L.) Pers. (Bermudagrass)

Bipolaris cynodontis (Marign.) Shoem.

Phyllachora cynodontis (Sacc.) Niessl.

Ustilago cynodontis (Henn.) Henn.

Puccinia cynodontis Lacr. ex Desmaz.

Sorghum halepense (L.) Pers. (Johnsongrass)

Exserohilum turcicum (Pass.) Leonard & Suggs

Exserohilum rostratum (Drechs.) Leonard & Suggs

Puccinia purpurea Cooke

Peronoplasmodium sorghi (Weston & Uppal) C.G. Shaw

Hadrotrichum sorghi (Pass.) Ferraris & Massa

Various smuts

Echinochloa crusgalli (L.) P. Beauv. and *E. colonum* (L.) Link:

(Barnyardgrass and Junglericegrass)

Exserohilum monoceras (Drechs.) Leonard & Suggs

Pyricularia grisea (Cooke) Sacc.

The pathogens of bermudagrass did not seem to seriously affect the weeds. Furthermore, bermudagrass lawns are becoming popular in the country. Although Johnsongrass would seem to be a good contender as a subject, the fungi found here on it do not lend themselves for use, as they may attack our sorghum and (sometimes) maize crops.

The research in Israel during the first year of the project dwelt primarily on two weeds that are common and troublesome in both the USA and Israel: *Xanthium strumarium* (cocklebur) and *Cyperus rotundus* L. (purple nutsedge). Although the pathogen *Alternaria helianthi* was the principal fungus tested in the project in the USA against *Xanthium*, the danger to the important sunflower crop in Israel eliminated it from consideration; the monocyclic rust, *Puccinia xanthii* Schwein. was researched instead. Although interesting and potentially useful facts were obtained on the biology of this rust (see in following) its non-appearance at the end of the second year and during the third year forced us to desist in any field experiments with this obligate parasite. More stress was henceforth given to (1) Examination of *C. rotundus* pathogens, particularly a species of *Cercospora* (considered herein to be *C. near caricis*), thought to have the greatest potential. (2) to a *Cercospora* (actually *Pseudocercospora atramarginalis*) on *Solanum nigrum* (black nightshade). (3) Forays in the field to bring to light native fungal pathogens in Israel on various other weeds.

Fungi Found During This Project: Annotated

a. Listings

A list of fungi found and identified follows:

Cyperaceae:

Cyperus rotundus:

Cercospora m. caricis

Purple Nutsedge

leaf & inflorescence blotch

Pyricularia higginsii Luttrell

(= *Dactylaria higginsii* (Luttrell) M.B. Ellis leaf blotch

Puccinia romagnoliana Maire & Sacc. rust II & III

Curvularia senegalensis (Speg.) Subram. leaf spot

and *C. tuberculata* Jain leaf spot

Scirpus maritimus L.

Nimbya scirpicola (Lucas & Webster) Simmons leaf blotch

(= *Alternaria scirpicola* Lucas & Webster)

Uromyces lineolatus (Desm.) Schroet. leaf rust II,III

Leguminosae:

Prosopis farcata (Banks & Sol) Eig

Uromyces prosopidis Transzsch. rust

Solanaceae:

Solanum nigrum L. Black Nightshade

Pseudocercospora atro-marginalis (Atk.) Deighton leaf blotch

(= *Cercospora atro-marginalis* Atk.)

Colletotrichum sp. (falcate) stem lesions

Datura stramonium

Alternaria crassa (Sacc.) Rands zonate leaf blotch

Withania somnifera L.

Pseudocercospora withaniae (H. & P. Sydow) Deighton leaf blotch

(= *Cercospora withaniae* H. & P. Sydow)

Aecidium sp. leaf rust

Convolvulaceae:

Convolvulus arvensis L. Field Bindweed

Septoria convolvuli Desm. leaf blotch

(= *S. septulata* Beach)

Botrytis cinerea leaf blotch

Gramineae:

Imperata cylindrica (L.) Beauv.*Puccinia imperatae* Poirault. leaf rust

Polygonaceae:

Polygonum sp.*Uromyces polygoni-aviculariae* (Pers.) Schroet. leaf rust II (autoecious)

Chenopodiaceae

Chenopodium sp.*Cercospora dubia* (Riess.) Winter leaf spot(= ? *C. chenopodii* Fres.)

Asteraceae

Conyza (*Erigeron*) *canadensis* (L.) Cronquist Fleabane*Basidiophora entospora* Roze & Cornu Downy mildew*Xanthium strumarium* L. & *X. italicum* Moretti Cocklebur*Puccinia xanthii* Schwein Rust III

Euphorbiaceae

Ricinus communis L. Castor-bean*Alternaria ricini* (Yoshii) Hansford leaf blotch*Euphorbia geniculata* Ort. (= *E. heterophylla*)*Alternaria* sp. leaf blotch

Notes:

Nimbya scirpicola on *Scirpus maritimus*

This fungus has until lately been considered an *Alternaria* (Lucas & Webster, 1964). However, the large conidia have both distosepta and eusepta and did not appear to us to be a good *Alternaria*. Dr.E.M. Simmons (pers. comm.) agreed; he had erected a new genus to include a few species

with conidia of this sort. So-called *Cercospora scirpicola* is in fact this species.

In the field, under wet conditions, the heavily infected *Scirpus* plants grew in close contact with those of *Cyperus rotundus* (near Nitzanim), yet the ~~nut~~sedge remained free of the disease, in spite of the disease appearing on another *Cyperus*, *C. longus*, in Portugal (Lucas & Webster, 1964). The teleomorph, *Pleospora scirpicola*, was not found here in the field, or in the laboratory after holding culms wet for long periods.

In culture, #3461, Faculty of Agric., Rehovot.

Septoria convolvuli

A leaf blotch on field bindweed was found in March, 1989. Pycnidia were present, together with *Stemphylium* which prevented us from isolating the fungus. Pycnidia were epiphyllous within brown polygonal spots. They were thin-walled, darker around ostiole. 80–95 μm diam. Conidia were hyaline, filiform, widest near base or at mid-spore, attenuated to an acute apex. 1–3(4) septate; 23–45 μm . This fungus fits the description given by Jorstad (1965).

Botrytis cinerea on field bindweed. Although powdery mildew is very common on this very serious weed here, problems of handling such an obligate parasite caused us to look for more tractable pathogen causing leaf lesions (see above, *Septoria*).

On 2 occasions, small round to polygonal tan to brown blotches were found, but *B. cinerea* sporulated from the lesions, and in culture on PDA, sclerotia appeared. As the sclerotia seemed much smaller than those we associated with *B. cinerea*, we are keeping the isolate in culture, # 3483.

Basidiophora entospora causes downy mildew on plants of the tribe Astereae in the Composite family, and was unknown here until lately. (It is common in the winter on fleabane, a problematical weed present here

in all seasons. Damage to the weeds seems minimal, and as it attacks China aster (*Callistephus*) in cultivation, it cannot be used. Oospores were found here.

Uromyces lineolatus attacks *Scirpus maritimus* here, causing long striate uredosori (brown) and teliosori (blackish, pulvinate). Uredospores have 3-equatorial germ-pores. The description by Jorstad (1960) fits our rust. It is known to be heteroecious, with aecial stage on Umbelliferae but we did not find that stage here.

Pyricularia higginsii

This fungus incites small round to oval lesions (max. 10 mm diam) on leaves of *Cyperus rotundus* here. Although first found in Georgia, USA (Luttrell, 1954) it has since been found in Cuba, Venezuela, Sudan, Sarawak and Australia (Evans, 1987). The disease is very common on purple nutsedge here, appearing particularly in the warmer months. The geographical distribution in Israel is similar to that of *Cercospora* nr. *caricis*. Lesions on young and mature leaves are pale brown or beige with a dark border, the color turning gray after sporulation from the dense turf of conidiophores and tiny conidia produced in whorls on denticulations near apex. Sporulation is amphigenous. Although both the disease symptoms and the morphology of the fungus resemble those of *P. grisea* and other species of that genus on Gramineae, the shape of conidia is different enough to easily tell this species from the others. Conidia are hyaline to fuliginous, 1-3 septate, widest at basal cell, tapering to a narrowly rounded apex, and with a narrow blunt projection at the base. In all these, it differs little from the others (de Hoog and van Oorschot, 1985), but the conidia, ca 25-40 X 5-6 μm are much longer and somewhat narrower. *P. caricis* Arnaud appears to be the same but is a *nomen nudum* (See Matsushima, 1975 p. 192, 1-2). *P. higginsii* was identified by us from infected *Cyperus longus* L. sent to us from Adana, Turkey by Dr. A. Atac.

The disease may occur concurrently with blotch disease caused by *Cercospora* nr. *caricis*, on the same plants. The tendency, however, is for the disease to become noticeable only in hot weather, particularly in shady humid sites, e.g. in orchards and between rows of tall corn. It has been put into culture, # 3462, Faculty of Agriculture, Rehovot. It sporulates well on V-8 agar and PDA if temperature is more than 18°C, and well at 30°C, best under near UV light. It grows slowly on agar media and eventually stales.

When dead leaves from infected plants from the field were placed as a mulch at the base of potted nutsedge, and held in a moist chamber for 4 days (av. 27°C), and pots were then removed to a greenhouse bench, many typical lesions appeared by the eleventh day. In another experiment, inadvertant inoculation yielded lesions in 7 days (see under *Cercospora caricis* experiments).

Curvularia senegalensis and *C. tuberculata*

C. senegalensis had been found in Israel associated with a very common melting-out disease of lawns of St. Augustine grass (*Stenotaphrum secundatum*) during summer, but causality has not been proven (Kenneth, unpublished). *C. tuberculata*, unknown in Israel until now, has been isolated in many countries, including Egypt, from *Scirpus* sp. and many cultivated crops, such as peanuts, rapeseed, sugarcane, etc. (Sivanesan, 1985). Both fungi cause the same small, solid chocolate brown spots (max. 3 mm). Inoculations carried out with each on nutsedge following Koch postulates proved for pathogenicity. The diseases were found in the field in June and August at Rehovot and Huleh Valley. In culture, # 3211, *C. senegalensis* and # 3439, *C. tuberculata*, Faculty of Agriculture, Rehovot.

b. *Solanum nigrum* L. and *S. luteum* Mill.

Pseudocercospora atro-marginalis (Atk.) Deighton

(syn. *Cercospora atro-marginalis* Atk. 1892).

Causal agent of leaf blotch. This disease was found along the entire coastal plain and in the Huleh Valley, particularly in summer and fall. The symptoms are usually pallid circular areas on the upper side of leaves of all ages, turning dark along with sporulation, and black discoloration on the obverse side of the same lesion (sporulation). The areas become necrotic, with centers of lesions on upper side usually gray, and remain grayish-black on underside. Lesions are till 5 mm across, but with necrosis, later reach 10–20 mm. Although sporulation is hypopyllous at first, it later becomes strongly amphigenous. Sporulation may extend beyond lesions into the green area, being dense but effuse, and arises from stomata. A substomatal stroma is present, bulging above leaf surface, and brown conidiophores (sometimes more than 20) arise, 1–3 septate, often divaricate, straight or crooked, with no noticeable geniculations. 12–48 x 3.6–4.8 μm , irregular in width. The apices are distinctly long or short obconical, ending in a truncate fashion, the scar of which is neither dark nor thickened. Occasional obconical truncate denticles may be seen near apex, where other conidia had been shed. Conidiophores are rarely branched.

Conidia are straight to slightly bent, subhyaline to straw-colored. All conidia have a short or long obconical truncate base, and most are cylindric, sometimes slightly attenuated toward apex which is always wide rounded. Occasionally, conidia have a more obclavate shape.

Septa may be distinct or indistinct, up to 7 cross-walls. 24–84 x 3.6–4.8 μm in nature.

At Karmia, South of Ashkelon, plants of *S. nigrum* in an avocado grove were growing intermingled with perennial *Withania somnifera*

Dun., a wild solanaceous plant, and both displayed the same symptoms as above, the plants being severely stricken. The fungus on *Withania* was determined to be *Pseudocercospora withaniae* (H. & P. Sydow) Deighton (syn. *Cercospora withaniae* H. & P. Sydow 1912). Disease symptoms and fungal morphology were similar for both fungi.

Both fungi have been put into axenic culture. Unlike some *Cercosporas* of the *C. apii* group, which can sporulate well in culture, although often losing that ability in time, the isolates of the above two species sporulate with great difficulty and furthermore grow very slowly, both in agar media or in shake culture on various broth media. Although growth ceases after a while, growth is slow from start to finish, presenting a problem for the future mass production of inoculum. Our isolate, from *S. nigrum* is # 3466, Faculty of Agriculture, Rehovot.

Taxonomy of *Cercospora*-like species on Solanaceae

There are over 3000 named species of *Cercospora* according to Flora Pollack (1987). The genus is heterogenous, to the extent that quite a number of new genera have been segregated from it, primarily by F.C. Deighton during the past few decades, on morphological grounds, and there still needs to be a complete revision of the entire group (Maung Mya Thauung, 1984). Furthermore, within each new genus, characters for separation into species are not well agreed upon, and indeed, most are not dependable, particularly as variability exists according to conditions. Traditionally, the host is a prime character for deciding on species, as these fungi appear to be limited to attacking within host families, with few exceptions.

Pollack (l.c.) lists 42 named species on the genus *Solanum*. The genus *Pseudocercospora* should be easy to recognize, on the strength of the

obconical truncate conidial base, the scar of which is not darker than the conidial wall, nor thicker than the conidial wall.

We carried out inoculations on various solanaceous plants, using inocula of *P. atromarqinalis* and *P. withaniae* of 2 sorts — comminuted mycelium of colonies growing on V-8 agar for 20 days, and leaf lesions ground up in water in an ultraturrox for one minute. Plants were sown from seed and held in a greenhouse for ca. one month, transferred to a growth chamber with 14:8 hr light and darkness, 25°–27°C day and 18°–20°C night. Plants were sprayed until run-off with the mycelial suspension and were covered with colorless plastic bags for 48 hr as a moist chamber, care being taken to prevent contact between bag and plant. The bags were removed, and presence of lesions noted daily. When found, leaves with symptoms were clipped and held in petri dish moist chambers for 24–48 hr and examined for sporulation. Microscope preparations in lactophenol with cotton blue were prepared. Other plants were first sprayed with tap-water, then dusted with ground-up infected leaves and treated as above. Control plants were sprayed with tap-water and treated as above.

Test plants were: *Solanum nigrum* and *Withania somnifera*, both of which were treated in all the above ways. *Hyoscyamus boveanus*, (Dun.) Asch. & Schwein, *H. aureus* L., *Nicotiana glauca* Graham, *Lycium europaeum* L., *Datura stramonium* L., *Datura innoxia*, and *Mandragora officinalis* L. were tested only to V-8 agar inoculum of *Solanum nigrum*.

Results

Control plants showed no disease symptoms. Typical lesions and amphigenous sporulation occurred in all plants of *S. nigrum* and *W. somnifera* inoculated with its own fungus or the 'opposite number'. First blotches appeared in 13–14 days. In moist chamber for a day, sporulation was observed. Leaves taken a week later displayed stronger symptoms. Of the other plants tested, *Hyoscyamus boveanus* and *Lycium europaeum*,

the former from eastern Sinai and the Arava, the latter from Israel, displayed noticeable dark round blotches, starting at 2 weeks after inoculation. The former showed a greater amount of sporulation than we have been accustomed to see in *S. nigrum* itself. It should be stressed that sporulation occurred on both sides of the leaf, even from the same blotch, in all the infected plants. This runs counter to the descriptions of those *Pseudocercospora* or *Cercospora* species on solanaceous hosts that bear any morphological resemblance to ours; in them, sporulation is 'hypophyllous' (M.A. Williams, 1987), 'chiefly epiphyllous' (Chupp, 1953; Govindu & Thirumalachar, 1954); or 'epiphyllous' (Ellis, 1976), etc. Although some species of *Cercospora* -like fungi are strictly hypophyllous or epiphyllous, e.g. *Cercospora* near *caricis* on *Cyperus rotundus* (see below), those we observed here on Solanaceae are not so, and sooner or later sporulate from both sides of the leaf, and thus, the manner of sporulation in the lit. depends on conditions obtained when collections were made.

Observations and measurements on all infected test plants showed:

- (1) Rather similar symptoms to those described above, regardless of plant and type of inoculum.
- (2) By and large, similar size and always the same shape of conidia among all test plants, all spores of which resemble those described above. This is true for conidia from either side of leaves. Some conidia on various plants reached 100 μm or even 114 μm in length, (rarely, on upper side of *Withania* leaves inoculated with V-8 culture from *Withania*), but width remained constant. Conidiophores were similar to those described above, but great variation was found even on the same leaves; length sometimes reached 71 μm ; the presence of long-obconically truncate apices with unthickened scar was exactly as described above.

As most named species on Solanaceae have conidia of entirely different shape from ours, e.g. *C. nicotianae*, *C. melongenae*, *C. physalidis*, *C. capsici*, *C. petuniae*, etc., we are left with only a few, probably all belonging to *Pseudocercospora*. *P. atromarginalis* (Deighton, 1976) fits our fungus from *S. nigrum* best. It is found in many countries of Africa, Asia, Australasia, and West Indies. It attacks *S. nodiflorum* and *S. aethiopicum*, but there is no information until now on physiologic specialization (Williams, C.M.I. Descriptions, No. 940, 1987). This is important, for M.B. Ellis' descriptions and illustrations (1976) show some morphological closeness of this species with *P. withaniae* on *Withania*, *P. fuligena* on tomato and *P. egenula* on *Solanum* spp., especially eggplant, and there is another possibly closely related species, *P. chengtzensis* (Tai) Deighton on *Lycium*.

Conclusions: (1) The results of our inoculations show that *P. atromarginalis* and *P. withaniae* are conspecific. Moreover, *Hyoscyamus*, a genus in which *Cercospora*-like fungal pathogens are unknown, was shown to be a good host, as was *Lycium*. The cogency of this finding is that there is reason to believe that some other solanaceous species might also be hosts. Tomatoes and eggplants, so important to the economy of Israel, have never been noticed to have symptoms of *Cercospora*-like diseases in Israel, and as they are grown so widely here, it is highly unlikely that it would have escaped notice. *P. atromarginalis*, if used for biocontrol of *S. nigrum*, would hardly constitute a hazard to these crops. It must be tested further, however. (2) *Hyoscyamus boveanus* is a much-disliked weed in the Arava, as it is a reservoir for viral diseases on solanaceous crops there. The dry, desert conditions obtained there are a challenge for this fungus, if it is to be used against that weed.

c. *Cyperus rotundus* L. Purple nutsedge

Cercospora nr. *caricis* Oudemans 1892

The disease incited by the fungus is the most common found in Israel on this weed, considered to be the most important weed on a world-wide basis (H.C. Evans, 1987; Holm et al., 1977). As *C. esculentus* L., a very problematical weed in the USA and elsewhere is also attacked by a *Cercospora*, which apparently is of keen interest as a potential biocontrol agent against that weed, it is imperative to clear up the relationship between the American isolate and the Israeli one i.e. are they possibly the same species? Farr et al. (1989) list two species of *Cercospora* on *Cyperus* species in the United States, but none on *C. rotundus*.

Geographical and Seasonal Distribution in Israel

Over a period of 3 years, surveys were frequently made to ascertain the limits, if any, of this disease. It was found attacking nutsedge in 47 locations, extending along the entire coastal plain from near Lebanon, as far south as Kissufim in the northern Negev, and also just east of that plain. The disease was present inland throughout the Jezreel Valley, Huleh Valley and Bet Shean Valley (the latter two being part of the Jordan Valley Rift). See Map.

The disease was observed during all months of the year. If the country were divided into north, center and south regions, the disease was found every month of the year within each region in at least one location. As a whole, the disease was most noticeable in summer and fall. It attacks nutsedge in cotton and corn fields, citrus and mango groves, in ornamental gardens, and does well in sunny, shady and half-shady sites.

At individual sites, the disease might be rampant for months and then disappear for a few months. The reason is considered (Kleifeld, based on observation) to be agrotechnical, e.g. in spring, there might be plowing and sowing of some crops, such as cotton and corn, thus disturbing the

stabilization of the weed, after which it sprouts again from tubers. In nearby field margins and other non-cultivated places, the weed together with the disease continues unimpeded, and eventually could be the source of infection for those within the crops.

Symptoms: Solid brown lesions on all above-ground parts of the plant, including leaves, inflorescence stalks and green parts of inflorescences. Young lesions are solid-brown, round spots only a few mm in diameter, later enlarging to diamond-shaped to ellipsoidal blotches, till 2 cm length. A yellow halo sometimes surrounds the blotch on both sides of leaf. Leaves may appear to be heavily spotted, and the disease may be severe on leaves of all ages — blotches coalescing, and often cutting across entire blade, causing death of the distal area. Stricken leaves tend to dry up from apex downward. Heavily-stricken plants completely dry up or 'burn' above ground, and this is noticeable particularly where there is a dense stand. During flowering, the blotched stalk often collapses. (In Israel, however, seeds are not germinable, and reproduction is vegetative and only by underground tubers). On leaves, the fungus sporulates only from the underside (hypophyllous). Sporulation has been seen only on the lesion itself. Dark round stomatic tissue is formed in substomatal cavities, dense fascicles extending through the stomata and immediately fanning out (divaricate) as olivaceous, thick-walled, 1-4-septate short conidiophores $37-50 \times 2.5-5.0 \mu\text{m}$. Rounded apices bear dark, thickened scars where conidia had been attached. Below tip, short geniculations with dark scars are seen in older conidiophores. Outer conidiophores in groups of up to 25 are bulged at their bases. Conidia are thin-walled, hyaline, straight to curved, cylindro-clavate, being approximately cylindric in proximal half and tapering to a narrow but rounded tail. There are 4-25 septa, either indistinct or easily discerned. The basal cell always narrows somewhat to a thick dark rounded or flattened scar. Conidial length is

very variable: 60–300 μm , being longest when sporulation from plants occurs in moist chambers. Width: 2.2–4.5 μm .

On various agar media, growth was always slow and sporulation sparse at best. Red pigment sometimes formed in colonies growing on PDA.

Taxonomy: Although the genus *Cercospora*, with >3000 names (Flora Pollack, l.c., 1987), has undergone revision in the last decades, transferring many species to many new genera, particularly by Deighton (see *Pseudocercospora* here on Solanaceae), all or most on Cyperaceae appear to fit the genus *Cercospora per se*, ruling out other genera. Although it is traditional to divide up this unwieldy and heterogenous genus into species primarily on the basis of host family (we think largely justified) or host genus (not always justified, see *P. atromarginalis* in this report), species may be named in the lit as inciters of a disease on a single host species, often on morphological grounds.

This situation holds for species on Cyperaceae, *Cyperus*, *C. rotundus* and other genera in Cyperaceae. It is doubtful if *Cercosporas* from Cyperaceae would infect plants of other families (although it might be worthwhile to test Juncaceae). Pollack (l.c.) lists 4 named species on *Cyperus*: *C. cyperi*, *C. cyperi-fusci*, *C. cyperi-rotundi*, *C. cyparicola*; 3 on *Scirpus*: *C. scirpi*, *C. scirpicola*, *C. uredinophila*; 3 on *Carex*, a genus phylogenetically more distant from *Cyperus*: *C. caricina*, *C. caricis*, *C. microstigma* and *C. ugandensis* on *Mariscus*. Chupp (l.c.) describes 3 others which can be discounted, "as conidia are narrowly linear-*Septoria*-like". *C. scirpicola* is not a *Cercospora* but is *Nimbya scirpicola*, acc. to Ellis (1976). *C. caricina* and *C. microstigma* are considered to be synonymous with *C. caricis* Oudemans as is *C. caricis* Dearness & House, 1916; Sawada's description of *C. cyperi* (In Chupp, l.c.) eliminates that species from consideration, as disease symptoms differ and sporulation is

amphigenous; *C. uredinophila* is associated with rust; *C. scirpi* (in Chupp) has acicular conidia with truncate base and acute apex and causes very small leaf spots, and is, according to Flora Pollack (l.c.) a *Cercospora*, and this does not fit symptoms and fungus from Israel.

In spite of the name, *C. cyperi-rotundi* Thirum. and Govindu (Thirumalachar and Govindu, 1953), this fungus from India and Indonesia is "chiefly epiphyllous", which runs counter to the behavior of the Israeli species, and the lesions are stripe-like. Blaney, van Dyke and Grand (Blaney et al., 1988) describe a species, *C. caricis*, attacking *Cyperus esculentus* L. (yellow nutsedge) in North Carolina. In comparing the description of *C. caricis* from Chupp, and herbarium material of supposed *C. caricis* on *Carex* spp. from Canada and the USA, as well as citing *C. caricis* Oud. previously reported, but not described, on yellow nutsedge in Illinois (Boewe, 1964), they concluded that their material "more closely resembles *C. caricis* than it resembles the 5 other species of *Cercospora* reported from *Cyperus* species". Other reports of so-called *C. caricis* on *Cyperus* species do not describe, but just name. However, we tend to agree with Blaney et al. on their choice of species, for 3 reasons: (1) Theirs does seem to be closer to *C. caricis* Oud. than to the others, according to their detailed description. (2) They demonstrated the variability of some characters among their own specimens and suggested that "characters traditionally used for species identification (in *Cercosporas*) such as width and shape of conidia and conidiophores, are not necessarily stable". (3) Our own fungus appear to be more closely related to theirs, and to *C. caricis*, than to any others mentioned above.

A very few differences are listed below, between the North Carolina material as described by Blaney et al. and Israeli material: Conidia were described as having truncate bases, and their Fig. 5 shows it clearly; we never observed truncate bases. Further into the description they mention

"base subtruncate to truncate" and their Fig. 4 shows rather indistinctly conidia resembling ours, with what seems to be wide rounded or perhaps subtruncate bases. Acicular conidia with width only 1.5–2.5 μm , produced in moist chamber conditions were not observed by us. The leaf spots on *C. rotundus* are not 'mottled yellow-orange at first', as they had found, but perhaps that is a reaction by a nutsedge species other than our own. The description of *C. caricis* Oud. (Chupp l.c.) fairly closely resembles ours; exceptions are: rarely septate; conidiophores 4–6 μm wide (only at the very base are ours sometimes as wide), conidial base truncate (to subtruncate). H.C. Evans (1987) has *C. ugandensis* Hansford 1943 from Uganda on *C. rotundus* and *Mariscus* as being synonymous with *C. caricis*, acc. to Herb, IMI, Kew. Evans listed *C. caricis* also on *C. rotundus* from Libya and Australia (with the specimen from Australia in culture at CMI, Kew), and *C. esculentus* from Nigeria.

As morphological characters for these *Cercosporas* on sedges are sometimes not well-defined or described in the literature or show variability, and as no characters in either the formal description of *C. caricis* or in the description of the North Carolina fungus are very different from ours, we consider it judicious not to add to the burden of having a new species described for the Israeli fungus, but to suffice in calling it *Cercospora* near *caricis*.

It would be worthwhile to test both the above fungi to some of the enormous number of *Carex* species to see whether the *Cyperus* fungi will attack *Carex*. Furthermore, it definitely would be worthwhile to test Israeli isolates to *Cyperus esculentus* at the USDA facility at Frederick, Md. and to test for cercosporin production, a diffusible toxin, as evidenced by results for *C. caricis* in North Carolina. Red pigment found in culture in Israel is evidence that cercosporin might be present.

d. *Puccinia romagnoliana* Maire & Sacc. 1903 on Purple Nutsedge (acc. to Evans (1987) and to Gjaerum (1984), this species is synonymous with *Puccinia conclusa* Thuem. 1878, which, if true, means that *P. conclusa* would have priority)

The rust here has been found only in the uredial and telial stages, and according to Gauemann (1959) the pycnial and aecial stages are unknown. There are many rusts on Cyperaceae. One, *Puccinia canaliculata* (Schwein.), Lagerheim, is being carefully research as a potentially good biocontrol agent on yellow nutsedge; although Castellani purportedly found this species in Eritrea on purple nutsedge in 1942, there are questions as to whether or not purple nutsedge is a good host (Phatak et al. 1987). Since its alternate (aecial) host, *Xanthium*, is a noxious weed, this rust may have advantages over others on sedges, but Callaway et al. (1985) demonstrated that sunflower is also an alternate host.

Taxonomy: The uredia, sometimes confluent with telia, are brown, very elongated, and pulverulent, on lower side of leaves. They may coalesce, so as to be as much as 3 cm long. Telia are blackish, elongate, narrow, at first covered with epidermis, tardily open and pulverulent.

Uredospores: light brown, ca. 1.6–2.5 μm wall, ovoid to elongate, very variable in outline, sometimes truncate, finely echinulate, germ pores 2, usually equatorial, sometimes not. Size: (17.4) 22.0–28.4 $\times$ 12.6–19.0 μm or 19.5–28.4 $\times$ 15.8–19.5 μm . (100 measurements each).

Teliospores: 2-celled, chestnut in apical cell, somewhat lighter in proximal cell. Often spores is pinched at septum; pedicel variable in length, sometimes till 39 μm long.

41.0–49.0 $\times$ 17.4–22.0 μm or 39.5–58.5 $\times$ 15.8–23 μm , with wall (1) 1.6–2.4 μm . At apex, often an umbel 4.7–8.0 μm . Our rust sori comprise a number of loci, with dense compressed paraphyses in between Rayss (1951)

mentions finding this rust in January 1938 at Bnei Brak on *C. rotundus* and stated that it was "extremely polymorphic".

Although there are numerous named species of *Puccinia* on Cyperaceae, most can be excluded from consideration because of one or two noticeable differences, e.g. *P. cyperi*. Arthur has uredospores with 3–5 pores, and has aecia on *Erigeron* (Savile 1973). This, a very common weed here in all seasons, does not have a rust on it. Arthur (1934) mentions that there are few or no paraphyses. *P. philippinensis* Syd. on *C. rotundus* has very small uredospores: 13–18 μm (Viennot-Bourgin, 1958); *P. cyperi cristati* Gjaerum on *C. cristatus* in Uganda often has curved teliospores with wall only 1 μm thick and short pedicel (Gjaerum, 1984). Aecia of *P. canaliculata*, whom Gauemann (1959) and Arthur (1934) contend is genetically related to the microcyclic *P. xanthii* (correlated species), have never been found in Israel on its alternate host, the very common cocklebur, or on sunflower, and has short sori and short pedicels. Some species, e.g. *P. abrepta*, have uredospore walls 1.5 μm or less thick and usually 3 germ-pores (Kern, 1919). *P. cyperi-tagetiformis* (P. Henn.) Kern resembles ours to an extent, but has very thin-walled teliospores (at their sides) and short pedicals (Kern, 1919). *P. romagnoliana* is notable for the dense mass of paraphyses within each teliosorus, dividing it into distinct locules (Gauemann, 1959, fig. 393 from Fragoso, 1924). Gauemann states that Thuemen stated categorically that *P. conclusa* Thuem. lacked paraphyses, so perhaps the 2 species should not be in synonymy.

This rust has been observed here and followed since 1984 (Kleifeld, unpublished). In cotton fields, it invariably appeared no earlier than mid-July. The crop is sown and sprinkle irrigated once for germination in April. From that time until first flowering in June, no irrigation is given, so that the weed is not present, and inter-row cultivation can be given during the time, also helping keep down nutsedge. In June, trickle

irrigation lines are laid down and used, and no cultivation can be given between rows, so that nutsedge now flourishes. At that time, the cotton and moisture in the crop is high, favoring foliage diseases on nutsedge. The rust disease can be very severe, but can also be sporadic. In the Huleh, in cotton, it was present in 1984, 1985, 1986, 1988, the last year being found throughout the Huleh Valley in October (Kleifeld and Quimby, observations) but in 1987 it could not be found there, but in the Esdraelon Valley and in Western Negev in mid-summer.

In irrigated orchards, e.g. avocado, the disease is not limited to mid-summer onward. It can appear on the same plant together with *Pyricularia higginsii* and *Cercospora* nr. *caricis*.

At the end of July 1988, fresh infected nutsedge straw was brought from the Huleh Valley to Neve Ya'ar, where the disease had not been found before. After 5 days in paper sacks in the laboratory, the straw was placed directly on top of the drippers for trickle irrigation, in two rows, so that in each row alternate drippers did not have straw. Irrigation was given once a week, for 20 hr. After 2 weeks, at each dripper, for a radius of ca. 0.25–0.5 m, the nutsedge was completely covered with uredosori had largely dried up. There was no rust, whatever, around the neighboring drippers only 1 m away. At that time, the cotton was 1 m high, the canopy was closed, so that there was no wind, presumably to transport spores.

II. RESEARCH ON *PUCCINIA XANTHII* ON *XANTHIUM*.

Factors Affecting Teliospore and Basidiospore Germination of *Puccinia Xanthii*

Xanthium sp. is ~~an~~ annual weed of the Compositae family which, in Israel, occurs in the summer as a problem weed in cotton fields. Its natural habitat is that of wet places, being often found close to reservoirs, streams, etc. Because of the abundant watering of cotton fields these areas, too, provide an ideal habitat for the growth of this weed.

Puccinia xanthii (Schw.) Schr.: is a microcyclic, monoecious fungus appearing as light brown, becoming dark brown circular lesions of teliospores surrounded by chlorotic tissue on the underside of host leaves (Fungi Canadensis No. 99, Apr. 1977). In Israel it occurs naturally on *Xanthium* sp. and appears to be in balance with its host. Infection, in nature, of *Xanthium* sp. plants appears to be from plant litter infected with *P. xanthii* from the previous summer, that has undergone a winter period. It is thought that this winter period is important, as overwintering may be necessary for the successful germination of teliospores. Teliospores on leaves that have become dried appear to enter into a period of dormancy, and in order to break this dormancy several successive freezing/thawing regimes have been reported to be required (Van Dyke and Spurr, 1982). Teliospores from pustules on fresh leaves however, germinate readily in a moist environment at room temperature without the need of a dormancy-breaking treatment (Van Dyke and Spurr, 1982).

Experimental work was carried out *in vitro* in the laboratory and in growth chambers, in order to examine the influence of the following factors on the germination rate of *P. xanthii* teliospores and basidiospores.

- 1) A hot/cold dormancy-breaking treatment of teliospores as inoculum material.

2) Temperature

3) Light

Results

Hot-cold Dormancy-Breaking Treatment of Teliospores as Inoculum Material

A minimal difference was seen between hot/cold treated cultures (48 h -20°C; 18 h -27°C): 38% at 28°C and 21% at 20°C, and non-hot/cold-treated cultures 37% at 28°C and 20% at 20°C. In terms of promycelium formation, however, hot/cold teliospores produced on average, nearly twice that recorded for non-hot/cold treated teliospores, 63 and 32% respectively, at 28°C Basidiospore germination was enhanced by the hot/cold treatment, recording 89% as compared to non-hot/cold treated of 72%.

The Effect of Temperature and Light on Teliospore and Basidiospore Germination of *P. xanthii*.

In these experiments, two temperature regimes were used (1) 15–20°C, (2) 15–35°C and also two sources for teliospores (a) stressed leaves from plants grown in winter conditions (b) non-stressed leaves from plants grown in summer conditions. The data shows that temperature has no real effect on teliospore germination; however, on basidiospore germination the influence of temperature is distinct. In the temperature range 15–30°C, basidiospore germination decreased as temperature increased; percent germination recorded being 93%, 72%, 14% and 12% for the control when temperatures were 15°C, 20°C, 25°C and 30°C.

In the temperature range 15–35°C, drastic reduction in basidiospore germination was seen only from 25°C, disappearing completely at 30°C. Light appeared to have little effect on teliospore germination, regardless of temperature or incubation conditions. However, basidiospore

germination was highly affected by light; at 30°C light totally inhibited basidiospore germination.

Conclusion

The hot/cold treatment, which was an approximate mimicking of natural winter conditions as received by *P. xanthii* sori-bearing leaves, definitely appears to encourage both teliospore and basidiospore germination. This is particularly so where teliospore sori are on dried brown leaves. On dried green leaves, however, good germination of teliospores can take place without a hot/cold treatment. This is supportive of that reported by Van Dyke and Spurr (1982) concerning germination of *P. xanthii* teliospores from fresh green leaves.

Temperature is not a critical factor in teliospore germination for the range of 15°C–30°C. Similar data has recently been reported by Anikster for several rust fungi (Anikster, 1986), and is also supportive of that reported by Hasan (1973). In terms of basidiospore germination, however, its influence is very pronounced. This can be equated with the probability of basidiospore germination, in nature, taking place at night when temperatures are low. Further evidence to support this can be found when considering the effect of light on basidiospore germination. Light completely inhibits basidiospore germination at high temperatures but has little effect when temperature is low. Light, as reported by Anikster (1986), has little effect on teliospore germination.

Storage of Teliospores of *P. xanthii*

Xanthium leaves having lesions of teliospores of *P. xanthii* that had been stored in various conditions for varying lengths of time, were checked for their continuing germination potential. Teliospores were prepared from five material sources, these being:

- 1) Green and brown dried leaves stored in brown paper bags in normal room conditions from June 1987.
- 2) As for 1) except stored in a refrigerator at: +4°C from June 1987.
- 3) Brown dried leaves stored in a glass beaker sealed with parafilm in a freezer at: -20°C from September 1987.
- 4) Green and brown dried leaves stored in a plastic bottle sealed with parafilm in a freezer at: -20°C from July 1985.
- 5) Green fresh (moist) leaves stored in paper within transparent plastic bags in a freezer at -20°C from May 1988 (used as control).

Two experiments were carried out: in one, source material underwent a surface sterilizing technique: 3 min. in 1% sodium hypochlorite solution, followed by three rinses in de-ionised water prior to the production of teliospores from all sources were sown onto 0.5% water agar (Difco) and incubated at 20°C 16 h day. Cultures were checked every 24 h until 96 h.

At 96 h it was seen that germination was low in all treatments, both where surface sterilization had and had not been carried out. No germination was seen in treatment (1) and 1% in (4). Highest germination was seen in (5) - 16%. Treatment (2) germination was approximately 10%. Surface sterilization appeared to depress germination.

Freeze-drying could be a more successful method of storing material as could ready-prepared teliospores.

Weakening of *Xanthium occidentale* Plants with Commercial Herbicides in Order to Facilitate Improved Infection by *P. xanthii*

Three commercial herbicides: Bazagran, Glyphosate (Round Up), and Lentogan, were applied at various application rates to plants of *X. occidentale* in order to establish an optimum application rate that would weaken, but not kill plants.

1987-1988
1987-1988
1987-1988

Concentration of herbicides tested were: 200 ml; 100 ml; 50 ml; 25 ml and 12.5 ml per dunam.

After application of herbicides, plants were placed in a growth chamber at 27°C constant day. Plants were sprayed at 4–6 true leaf stage.

Two experiments were carried out (1) using concentrations of 200 ml, 100 ml and 50 ml per dunam. (2) 50 ml, 25 ml, and 12.5 ml per dunam. The first experiment was repeated as control and treated plants became infected by a micro-organism that caused severe wilting and eventual plant death. Experiment (2) in terms of height of plants and number of leaves, Lentogran had negligible effects at all three concentrations. Glyphosate at 25 ml weakened plants perceptibly without causing over-extensive damage to leaves. At 50 ml, damage was severe, and at 12.5 ml, negligible. Bazagran at 50 ml, caused severe damage, at 25 ml, plant height and number of leaves was unaffected. However, chlorosis was severe for the bottom half of the plants. At 12.5 ml, plant height and leaf number was again not affected, with chlorosis being less severe for the bottom half of plants than at 25 ml per dunam.

From these results, Glyphosate at the concentration of 25 ml and Bazagran at 12.5 ml, appear to sufficiently weaken *Xanthium* plants to enable a possible enhancing of infection by *P. xanthii* to take place.

Conditions Necessary for the Successful Infecting of *Xanthium* sp. Seedlings With *Puccinia xanthii* using Teliospores or Basidiospores as the Inoculum

Introduction

Many attempts at inoculating *Xanthium* sp. seedlings with teliospores of *Puccinia xanthii* using various inoculum sources, pre-treatments for teliospore-bearing-leaf-particles (TLP) and various post-

inoculation incubation conditions, have been made. From many experiments carried out, certain criteria for successful inoculation have emerged, these are:

- 1) A pre-soaking (by floating) period for TLPs prior to use as inoculum; 24–48 h at 20–25°C has given good results.
- 2) A pre-wetting of host leaves with water and wetting agent before application of inoculum.
- 3) During the infection period a very high level of humidity 100%+ to ensure that host leaves remain wet; approximately 27 h at a temperature between 20–23°C has proved successful.
- 4) After the initial infection period, temperatures of between 28–35°C appear to encourage good production of teliospore-bearing sori.

The following outlines the work to date, current data concerning inoculation of *Xanthium* sp. with *P. xanthii*, and comparison of inoculum sources/pre-treatments and also the possibility of using *P. xanthii* basidiospores as an inoculum.

Teliospores as inoculum source were produced from:

1. Fresh green leaves stored at –20°C non-hot/cold treated
2. Dried brown leaves stored at –20°C for one month
3. Dried green leaves hot/cold treated
4. Dried green leaves non-hot/cold treated
5. Dried brown leaves hot/cold treated
6. Dried brown leaves non-hot/cold treated.

Teliospores were distributed onto 0.5% water agar + 0.5% sugar with chloramphenicol at 350 mg/1 tr and placed at 20°C constant dark for 72 h. Cultures were then examined under the microscope and the first 100 teliospores counted. The number of those 100 with germinated basidiospores surrounding them was also noted. Four replicas for each treatment were used.

Five mg teliospores from each of the six treatments were floated on either (1) 5 ml distilled water or (2) 5 ml 0.5% sugar/distilled water solution within plastic cups and inoculated at 25°C constant dark for 48 h.

Teliospores were then inoculated onto *Xanthium* sp. seedlings at 3rd, ~~true~~ leaf stage, that had been ~~grown~~ in an open-sided greenhouse. Inoculation was effected by first misting the plants with distilled water plus Triton 6, then applying the teliospores and their solutions to leaf surfaces with a fine horse-hair brush. Plants were then placed within clear nylon sacks and put into a growth chamber at 20°C for 72 h. After 72 h, the nylon sacks were removed and the plants returned to their open-sided glasshouse, where they were misted each morning. Lesions were counted 15 days after inoculation and the numbers noted.

This experiment was repeated using teliospores produced from: 1) hot/cold treated room stored brown leaves; 2) fresh-picked infected leaves, green/yellow; and 3) room-stored brown leaves that received a period of -20°C for one month.

P. xanthii basidiospores were also used as inoculum and were taken from the following sources: teliospores produced from: 1) fresh green infected leaves kept at -20°C. 2) Brown dried leaves kept at normal room conditions, then stored at -20°C for one month+. 3) Dry green room-stored leaves hot/cold treated. 4) Brown room-stored leaves hot/cold treated. 5) Fresh *P. xanthii* infected leaves, green-yellow in color.

Basidiospore inoculum was produced in the following way: 5 mg teliospores from each inoculum source floated on 5 ml 0.5% sugar solution within plastic cups for either 1) 48 h or 2) 72 hours at 25°C constant dark. After 48 h, solutions were then strained through a fine mesh strainer to remove TLPs but allowed basidiospores to remain in the solution. Solution volume was then increased to 15 ml per treatment.

Inoculum was applied using a De Vilbiss automatic spray gun, each spray duration being approximately 1 second.

On another occasion, materials (2), (4) and (5) were applied to plants using a horse-hair brush, with identical inoculum preparation and inoculating techniques being used as well as identical incubation conditions.

Three pots containing three plants per pot at third-leaf stage were used per inoculum source. Where inoculation was via the spray gun 6 sprays were given per pot of plants, 3 to the upper and 3 to the lower leaf surfaces. Inoculation by brushing was carried out to upper and lower leaf surfaces. All plants were misted immediately before inoculation, and immediately after inoculation all plants were placed in clear nylon sacks, one sack per inoculum source, and the sacks closed. Plants were then placed in a growth chamber at 23°C for 72 h after which they were placed in an open-sided glasshouse. Here they received shade for the first 48 h and after this time full sun. Lesions were counted after 15 days and noted.

Results.

The data show that materials (2) and (5) gave best germination (52% and 65% respectively). All the green leaf material germinated less well than the brown leaf material. Basidiospore germination was also much higher in brown leaf material than in green leaf, percent germination being almost equal in (1) (5) and (6) (95%, 89% and 96% respectively). Basidiospore germination was particularly poor in (4) - 4%.

Lesions on plant leaves were obtained at a higher rate in all materials except (1) when sugar was used in the teliospore-soaking solution. As stated. Best results were obtained with material (2) and (5) where sugar was used. Material (3) also gave reasonable infection. Where sugar was not used materials (1) and (2) produced the most lesions with the remaining materials giving very poor infection generally.

Using basidiospores as an inoculum source was not effective at all when using a De Vilbiss spray gun. No infection sites were seen either where the inoculum material received 48 or 72 h soaking periods. When brushing was used in inoculation, some lesions did occur. The number of lesions obtained could be increased, possibly, with a shorter teliospore-soaking period and a higher concentration of basidiospores per inoculum being used.

In this part, the research concentrated on the phenomenon that artificial inoculations with *P. xanthii* teliospores resulted in water-soaked lesions which rapidly developed into necrotic lesions, rather than teliosori, which previously had appeared after inoculum. Such symptoms developed from different sources of teliospores as inoculum for the artificial inoculations; laboratory storage 4°C and -20°C storage. In an intensive re-isolation study, we isolated several strains of the yeast *Rhodotorula glutinis* and the bacterium *Pseudomonas* sp. A mixture of the inoculum source which has been obtained from the symptom-bearing leaves showed infectivity potential. However, each component separately failed to produce the same symptoms. It seems that under certain conditions, which are unknown yet, the *Rhodotorula* and *Pseudomonas* populations which might be part of the leaf microorganism population, were involved in the loss of viability of the *P. xanthii* teliospores. The *R. glutinis* is in axenic culture #3467, Faculty of Agriculture, Rehovot. *Rhodotorula glutinis* var. *Rubescens* has been shown (Kilpatrick, 1959) to cause a disease resembling that mentioned here, but on various genera of legumes, when placed on leaf surfaces. Although it cannot itself penetrate into the plant, it induces a breakdown of cellular structure. Kilpatrick suggests that it produces a toxin. We think that pursuing this course would be worthwhile.

At this point we decided to put most of our efforts on the biology and epidemiology of several fungal pathogens of *Cyperus rotundus*.

III. RESEARCH ON CERCOSPORA NR. CARICIS FROM CYPERUS ROTUNDUS

Much of the following research was carried out by or with Mrs. Elinoar Bonne Nitzani, as an M.Sc. thesis at the Faculty of Agriculture, the Hebrew University of Jerusalem, Rehovot (under the direction of R. Kenneth and Y. Kleifeld): "Studies on *Cercospora* nr. *caricis* and Evaluation of its Use as a Biocontrol Agent of Purple Nutsedge (*Cyperus rotundus*)".

The fungus was isolated from leaves of purple nutsedge by way of conidia or by surface-sterilized leaf blotches placed on agar media in petri dishes. Sub-cultures were made by transfer of mycelium, or mycelium with agar, because conidia on such media was never plentiful and served primarily to prove that colonies were indeed of *Cercospora*. Today we have in our collection cultures of 4 isolates from various parts of Israel (# 3453, #3481, #3482 and #3483). Tests were made on the effect of various agar media on growth and sporulation of the fungi (leaf parts with lesions were also employed for sporulation studies) and as inocula for pathogenicity studies. Viability of the fungus in leaf straw was tested. Growth in liquid shake culture in different media was tested, as was infectivity of mycelial suspensions from liquid culture. Germination of conidia under various conditions, primarily temperature and light regime, was undertaken on distilled-water agar.

1. Vegetative Growth on Artificial Media

a. **Solid Media.** The following were tested: Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Corn Meal Agar (CMA), Water Agar (WA), Malt Agar (MA), Czapek Agar, V-8 Agar, *Urtica* Agar (UA) and *Cyperus rotundus* Agar (CRA). These were tested with or without the addition of Chloramphenicol, useful for preventing growth of many bacteria in culture. In most experiments there were 6 replications for each treatment. The fungus was grown in 3 ways: 1. Addition of agar discs with mycelium to petri dishes containing the agar medium in question. 2. Mycelium first comminuted in water and poured into petri dishes containing agar media. 3. Transfer of agar discs with mycelium obtained from method 2.

The dishes were held at various temperatures in darkness, or continuous fluorescent light or in alternating light and darkness (natural light from north window). For sporulation experiments, continuous near-UV light was used, in addition to the above. The diameter of colonies over time was the criterion used.

Results:

1. The fungus grew very slowly under all conditions, and furthermore was of staling type.
2. Growth occurred from 10°C to 34°C, (temperature lower or higher were not tested), with optimum at 25°C among all temperatures tried.
3. Growth after 9 days in total darkness was better than under alternating light and darkness or continuous light, on all the media tested. Eventually (after 21 days) this difference narrowed greatly (Fig.). Possibly light resulted in a longer lag period in mycelium emerging from the agar discs.

4. In experiments using all the above media, growth was best on PDA and CMA and slowest on MA and YEA. Yeast extract added to PDA did not enhance growth.
5. Method #3 of sowing the fungus proved the most effective for rapid growth.
6. Chloramphenicol had no effect on vegetative growth.
7. Sporulation was, at best, sparse in all treatments.

Conidia usually resembled those formed on plants, in all ways, but true conidiophores could not be recognized. Conidia were of typical size and shape, down to the dark scar at the base. A globose, enlarged, thickened cell was sometimes noticed near the apex or middle of spores produced on various agar media, bringing to mind a chlamydospore (such cells are known to occur in stressed conidia such as in *Exserohilum turcicum*), and if produced in nature might prove to be a structure allowing persistence under difficult conditions.

The poor sporulation or lack of it was disappointing but not unexpected. Although a fair amount of sporulation occurs in culture in some species of *Cercospora* and its allies, many species are intractable, and some media favor sporulation, e.g. *Urtica* agar, according to Dr. O. Constantinescu, Curator of Mycological Herb., University of Uppsala (pers. comm.). V-8 agar is much in use for inducing sporulation in dematiaceous fungi, including *Cercospora* spp.

8. Much variation occurred in the shape, texture and color of colonies, and light sectors sometimes appeared.
9. Hyphae of many sorts were found. Dark, thick-walled filaments or hyphae with bloated thick-walled chains of cells resembling arthrospores were common at the center or in the depths of colonies. (Fig.)

10. On PDA, reddish-pink pigment sometimes appeared within the colonies themselves. Cercosporin, a reddish non-host-specific toxin in plant disease had been found in *C. caricis* by Blaney et al (1988). We did not pursue the subject further.

b. Growth in Liquid Media

Agar discs with mycelium were added to 50 ml liquid broth (V-8 or Corn Meal) (the corn meal medium was unlike the prepared Difco CMA used in culture as solid media) in 100 ml Erlenmeyer flasks, and held in reciprocal shaker with water bath at a constant 25°C. Day and night conditions obtained in the laboratory, with light from north windows. Speed of shaker: 70, 100, 140 rpm. As growth was slow, amount of mycelium recovered was weighed (dry weight) only after 14 days. V-8 broth was henceforth used for shake culture.

Results

1. Growth was best at 100 rpm at this temperature.
2. Growth was much better in V-8 medium than in CM.
3. Conidia were never found in this or other treatment in liquid culture.
4. Growth consisted of globular masses around the agar-medium discs introduced. They were 5-20 mm diam. after 7 days. Most were grayish-white and some totally black, some with smooth surface, others with projections.
5. When the agar-mycelium discs were used as inoculum, results were poorer than if mycelium scrapings alone were used.
6. The use of comminuted mycelium from shake culture to seed agar medium did not increase speed of growth on agar over that of mycelium from agar media.
7. Mycelium produced in shake culture resembled, microscopically, the various kinds found in solid media.

2. Effect of Media, Light Regime and Temperature on Sporulation in Solid Media

Among the many hundreds of petri dish cultures examined, sporulation was noticed only in 34. These were in isolates from 6 localities out of 10, and on 6 media.* Sporulation, never profuse, occurred in continuous fluorescent or in near-UV light; alternating light and darkness, and in darkness; at various constant or fluctuating temperatures, e.g. 18°C, 25°C, 30°C. First appearance of conidiation was on the 3rd day from transfer of discs or comminuted mycelium or as late as the 37th day. No sporulation was ever found when growth was on CMA, MA, YEA, WA or Czapek Agar.

In conclusion, no particular factor or combination of factors seemed to favor sporulation over others tested. A possible exception is that substrates giving the best growth, e.g. CMA, allowed no sporulation, whereas those supporting slow growth e.g. CRA, UA generated the highest degree of sporulation, when it did occur.

3. Sporulation from Lesions

Freshly collected infected leaves were tested for sporulation at 10, 18, 20, 25, 27, 30 and 34°C in darkness, at 13–22°C on a laboratory bench under day:night conditions and at 30°C and 34°C also under continuous fluorescent light. Leaves had been wiped clean of any previous sporulative structures, and were held in moist chamber petri dishes.

Results

After 24 hr, sporulation had occurred at all temperatures except 10°C and 34°C with densest sporulation at 25°C. At 34°C, sporulation occurred in 2 days, and at 10°C in 4 days, but was sparse in both. In a repeat experiment, sporulation occurred within 15 hr at all but 10° and 34°C. At

30° and 34°C, conidia and conidiophores were distinctly shorter when produced in light than in darkness, but were produced to the same extent.

Infected leaves dried in a plant press kept in the herbarium for 3, 5, 7, 8, 11, 13, 18, 20 and 23 months and tested as above at room temperature gave fairly sparse sporulation after 2 days in 18, 20 and 23 month-old material; for 3, 5, 7 and 8-month-old leaves, only 24 hr, was needed for new sporulation. Densest sporulation was with 3-month-old material.

From green infected leaves kept in a deep freezer (-50°C) within blotting paper in cartons, for 3, 7.5, 8.5, 10, 10.5 and 11 months, new sporulation took place only on 3-month-old material, after 24 hr moist chamber. From green infected material kept in paper bags in a refrigerator (ca 4—6°C) for 5 and 10 months, no sporulation occurred in the 5-month-old material, but sparse sporulation took place on half of the leaves of the 10-month-old material.

4. Germination

Infected leaves from Rehovot were incubated in moist chambers until sporulation; drops of distilled water were then placed on the lesions; the drops, with new conidia, were taken up by Pasteur pipette and deposited on distilled water agar, with or without addition of chloramphenicol in the agar, in petri dishes. These were held in darkness in an incubator at 5, 10, 19, 24, 27 and 32°C. In another experiment, conidia from leaves from Neve Ya'ar and Yasur were tested at 25°C under continuous fluorescent light, and some from Neve Ya'ar were tested on a laboratory bench at 26°C during daylight. Aside from that, Neve Ya'ar conidia were tested at 25°C in darkness.

Germination was ascertained by conidial count under the microscope usually every 1 or 2 hours.

TIME 1 - 9 DAYS

TIME 1 - 9 DAYS

TIME 2 - 21 DAYS

Results

For the Rehovot spores, results, as shown in Graph show:

1. No germination occurred at any temperature until after 3 hr.
2. At most temperatures tried, germination could be considered rather early, compared to that of many ~~plant~~ pathogenic fungi, starting at ca 4 ~~hr~~ hr (9, 24, 27°C).
3. Once begun, germination, percentage-wise, was very rapid, including those temperatures e.g. 32°C, 10°C in which the start of germination was delayed.
4. Since germination reached ca 100%, it shows that all conidia were viable to start with, and that no dormancy existed.
5. Germination at 5°C (not in graph) took place but was greatly delayed: 0% at 40 hr, but 97% at 52 hr.

For Neve Ya'ar conidia at 25°C in darkness, results were very close to those of Rehovot at 24°C.

For Neve Ya'ar conidia at 25°C in fluorescent light, 0% at 2.5 hr but 100% at 4.5 hr, and for Yasur conidia, germination had just begun already at 2.5 hr (20%), and at 5 hr reached 98%.

For Neve Ya'ar conidia at 26°C on lab-bench, 0% at 3 hr and 98% at 5 hr.

Results (cont.)

1. Chloramphenicol in the water agar completely inhibited germination. This is surprising, considering that this antibiotic had no effect on growth of the fungus, and even was present in harvested mycelium with agar medium used to successfully inoculate nutsedge.
2. There did not appear to be any influence of light vs. darkness on ability of conidia to germinate. In the 'darkness' treatment, however, conidia were exposed to light each time a microscope count was made.

5. Inoculation of Purple Nutsedge by *C. nr. caricis*

a. From Conidia from Infected plants

Conidia were obtained by inducing sporulation from blotched leaves from different localities. Five or six leaves were placed in distilled water in a large test-tube, adding 'Tween 20' adjuvant at 4 drops/l. Spores were shaken into suspension in a Vortex apparatus for 2 min., after which conidia were measured by haemocytometer.

The suspension was sprayed onto potted plants grown from tubers obtained from various localities. The inoculated plants were held for varying periods under conditions allowing foliage to remain wet at different temperatures, e.g. dew chamber or transparent plastic covers, after which they were held in a greenhouse bench or in growth chamber at known temperatures.

Example: Conidia from Palmahim were inoculated on plants from various sources at 18–20°C, 12 h light: 12 hr darkness in a chamber for 36 hr., then held in a growth chamber at 25°C.

Results: 18 days passed until typical symptoms appeared, particularly near leaf tips, which dried up. Young and old leaves were infected. Sporulation was noticed. Plants from all sources proved susceptible. As conidia of *Pyricularia higginsii* had been present in the suspension, the spot disease incited by this fungus also appeared on all leaves, starting 7 days after inoculation.

An experiment using inoculum from Neve Ya'ar and from Yakum was undertaken in January 1989, with 2.6×10^5 and 1.9×10^5 conidia/ml, respectively, applied till run-off; leaf wetness period was only 24 h. Control plants were sprayed with water. A small enclosed room in a greenhouse, with no temperature control and with greatly fluctuating daily temperatures was used, as a fine misting apparatus there allowed excellent leaf wetness. The potted plants were then set on the greenhouse bench and

watering was from below, keeping leaves dry for the duration of the experiment. There was no air movement within the greenhouse room.

Results: (1) No lesions appeared on control plants.

(2) Plants survived, and the disease progressed in spite of the great daily temperature changes, the outer limits being 4°C and 44°C .

(3) The first lesions to appear were at 14 days.

(4) The number of lesions were counted at 20, 28, 40 and 47 days after inoculation. There were 3 categories of blotches:

'A' = till 5 mm diam.

'B' - 6-10 mm diam.

'C' = >10 mm diam.

At 21 and 28 days, almost all lesions were of category 'A' and 'B', but by 40 days almost no small 'A' lesions could be seen, and at 47 days, none at all, meaning that new lesions no longer appeared. As no lesions had appeared on the many control plants, it is probable that the few 'A' lesions seen at 40 days at least, took that amount of time to make their appearance (latency).

(5) As almost no large 'C' lesions were found at 21 days (obviously not at 14 days), and few at 28 days, the appearance of many 'C' lesions at 40 days means that the length of time for growth of lesions to full size could often be well over 3 weeks. We found, however, that sporulation could occur on small as well as large blotches.

(6) At 40 days, a new situation came about — the drying-up of some entire leaves, particularly the older ones, when a number of large lesions were present on them. It was sometimes difficult to discern any longer the lesions within these dead leaves, but sporulation took place on them when leaves were placed in a moist chamber. As drying up of older leaves, as a result of natural conditions, occurred in adjacent control plants to a much lesser degree than did drying-up in the treated plants, we ascribe

most drying-up in the latter to the effect of presence of lesions. If our fungus produces the toxin cercosporin as does *C. caricis* in North Carolina (Blaney et al. 1987), it is conceivable that it might be responsible in part for this reaction on older leaves.

b. From Infected Straw

In four different experiments, nutsedge straw (dead infected foliage) from different localities was employed to infect healthy plants. The straw was kept dry for periods ranging from 20 to 300 days prior to their use. The potted plants were inoculated by wetting the straw placed on the soil surface, by spraying it with tap-water with Tween 20 (4 drops/l); leaves were kept wet for 3 or for 4 days, by covering plants with plastic bags; plants were then held in a greenhouse bench in 3 experiments (10°C–33°C) and in a growth chamber in the remaining one (16–20°C). Watering of plants was from below the pots. Plants were examined daily for presence of lesions.

Results

1. No infection resulted from use of 300 -day-old straw, and very few lesions appeared when 180-day-old straw was used. Straw kept in the laboratory for 90 days incited *Cercospora* lesions after 12 days; other straw dried outside for 20 days gave lesions after 11 days.

2. Only the lower leaves that came in contact with the infective straw developed symptoms of the disease, under the conditions obtaining in the greenhouse, e.g. no dew, no splash. Two weeks after first symptoms appeared, new leaves emerged, but remained free of the disease.

Conclusions

Infected nutsedge straw, dried naturally in the field or in shade or dried in a plant press, should constitute an efficient means for inoculating nutsedge. Outside, under the influence of dew or rain to wet foliage, and wind or rain to transport spores to healthy leaves, this method is likely to succeed in infecting all leaves, rather than only the lower ones.

c. Inoculation in the Field from Newly Infected Leaves

Pieces of fresh leaves with lesions were affixed with thread to healthy leaves of plants growing in a field at Rehovot during winter (January). The first lesions appeared 10–13 days from beginning of the treatment, at those loci, the inoculum falling off eventually.

Lesions reached a diameter of 4–10 mm by 20 days, with sporulation. The number of lesions had increased greatly by 27 and 34 days, some of them being small, and these are considered to be largely a second generation of infections, from conidia produced on the primary lesions. Control plants growing 10 m from the inoculated ones showed no lesions throughout the experiment.

Conclusions

Previous observations in a weed plot at Rehovot had shown that the disease proceeded during winter, as long as the leaves had not withered from particularly low temperature. The results of this experiment confirm these observations. It was surprising that symptoms could already appear in only 10–13 days, considering that the very earliest times ever obtained in any of our experiments was 7 days and usually took 2 weeks or more. The paradox was that the optimal temperatures found for growth, formulation and germination of our fungus were considerably higher than those occurring in the field in question in January, and it would be expected that no infection should take place. This anomalous situation

(winter infection) might possibly be explained either by predisposition toward plant susceptibility brought about by cold weather, or by particular susceptibility of the plants resulting from plant reaction during inoculation in the cold weather. Either might compensate, in infection, for poor performance at low temperature of growth, sporulation and germination. Another possibility could be that cercosporin toxin production might be enhanced at low temperature by conidia on the host's leaf or that it does not break down so easily at low temperature. All these hypotheses have yet to be tested.

d. Inoculation Experiments using Mycelium Produced on Solid Media

The inoculum consisted of mycelium alone, comminuted in an Ultra-Turrax, with mycelial particles separating in a Vortex apparatus for 2 min with addition of 4 drops of Tween 20/1 water. The suspension was observed under the microscope and no conidia were found. The mycelial fragments, C.F.U.s (colony-forming-units) were counted with a haemocytometer.

Plants were sprayed till run-off and kept in moist chambers for periods ranging from 24 hr to 8 days. Among the 19 trials, other conditions also differed: source of inoculum, light regime, temperature. Inoculum was derived from 6 geographically disparate localities: Zikim, Rehovot, Palmachim, Neve Yaar, Yasur and Moledet. Plants originated at 7 localities: N. Negev, Zikim, Rehovot, Palmachim, Neve Yaar, Yasur and Huleh Valley.

In one experiment, comminuted mycelium in sterile distilled water was held in a covered beaker in a refrigerator for 5 weeks, before spraying on plants. In another experiment, inoculum was placed on only the upper or lower side of leaves, effort being made to prevent transfer of any inoculum to the other side during the leaf-wetting period or afterwards.

Results

1. Mycelium lacking conidia was capable of inciting formation of typical *Cercospora* lesions.
2. All sources of inoculum were capable of inducing lesions, and subject plants from all sources were shown to be infectible to either some or all isolates.
3. Plants from one locality might become infected by an isolate, whereas plants from another locality could remain uninfected by that isolate when tested at the same time.
4. Although there did not appear to be good correlation between particular isolates and particular plants as regards infection, in a number of experiments, isolates from one locality could not infect plants from the same locality, e.g. Zikim, Palmachim, Yasur, at the same time succeeding in infecting plants from other locations (in no instance, however, did such an isolate never attack plants from the same locality in other experiments).
5. The earliest appearance of lesions (Moledet isolate grown on Difco PDA on N. Negev plants) was in 7 days and on Huleh and Yasur plants in 12 days. Seven days is the shortest incubation period ever found here. Nevertheless, this isolate and the conditions of inoculation etc. (48 hr leaf wetness period, constant 25°C, 12 hr light: 12 hr darkness, old isolate-90 days in culture on agar medium) failed to cause lesions on Rehovot and Neve Ya'ar plants inoculated at the same time as N. Negev plants.
6. There did not appear to be clear correlations among the parameters of isolate, growth medium, age of inoculum from agar medium, host, temperature, wetness period, and light regime on the one hand and disease development on the other.

7. Thick-walled mycelial particles were seen to have sprouted hyphal extensions on leaves during the leaf wetness period, and no sporulation had occurred on the mycelium. Penetration of leaves by hyphae could not be clearly seen under the light microscope or SEM.
8. The comminuted mycelium stored in a refrigerator for 5 weeks in sterile distilled water proved capable of inducing *Cercospora* lesions or nutsedge 16 days after inoculation (temperature 16–20°C, leaf wetness 3.5 days, 12 hr light:12 hr darkness).
9. Inoculation on either only upper or lower sides of leaf yielded typical *Cercospora* lesions.

e. From Mycelium Produced in Liquid Media

The liquid medium used for preparing mycelium for a series of inoculations on nutsedge was V-8 broth. The mycelium was grown for 14, 21 and 60 days at a constant 25°C in shake culture, at 100 rpm; some of the mycelium was washed free of substrate, some not. Mycelium was comminuted with addition of Tween 20 adjuvant, and sprayed on plants. Leaf wetness lasted 4 days in a greenhouse with daily fluctuating temperature 13–28°C, and held in the greenhouse throughout the rest of the experiment 12–32°C.

Results

1. Mycelium from liquid culture is capable of inciting the disease.
2. Symptoms first appeared for some treatments in 8–10 days, 10 days for others, and 14 days for yet others.
3. There was no clear correlation between age of plant at time of inoculation (3–4 leaves, 3–5 leaves and 6–7 leaves) and timing of first lesions. All leaves were susceptible, including all those on 6–7 leaf plants.

4. Comminuted mycelium washed free of the liquid V-8 broth and that still carrying broth with it were equally capable of infecting plants.

6. Host Range

Test plants in pots, along with nutsedge as a control, were sprayed with comminuted mycelium obtained in culture in PDA; leaves were kept wet for 40 hr at 25°C, 12 hr light; 12 hr darkness, and then kept at 25°C in a growth chamber.

Results

Purple nutsedge yielded typical lesions.

No symptoms appeared on the following:

Dicots — tomato, peanut, sunflower, cotton.

Monocots: Gramineae - sorghum

Cyperaceae: *Scirpus maritimus*, *Carex* sp.

Cyperus (?) *cyperoides* L. Kuntze.

Distribution map of *Cercospora* nr. *caricis*
on Purple nutsedge (*Cyperus rotundus*) in Israel.

SUPPRESSION OF SOILBORNE PLANT DISEASES IN COMPOST-AMENDED MEDIA

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Suppression of several plant diseases was observed in compost-amended media in greenhouse experiments. In order to evaluate the role of the compost microflora in the suppression of the pathogenic fungi, we examined the propagules after incubation in the medium using light and scanning electron microscopes. Sclerotia of *Sclerotium rolfsii* were incubated in composted grape marc (CGM), raw grape marc (GM), and peat media. The sclerotia rapidly lost viability in the CGM medium but not in the peat or in the GM. In the CGM it was observed that the sclerotia were shrunken and invaded by antagonists.

Pythium aphanidermatum was grown on nylon monofilament fabric in petri dishes containing liquid growth medium. After 24 h of incubation the nylon fabric containing the fungal mycelium was transferred to composted separated cow manure (CSM), and peat media. The media had been previously adjusted to a water tension of 10 mbar and this was maintained during the incubation period. In the CSM medium the *Pythium* mycelium lysed within 24-48 h but not in the peat medium, in which the mycelium was viable even after 14 days. With neither a light nor a scanning electron microscope could we observe direct parasitism of *Pythium* by other fungi or bacteria. Addition of glucose in the compost or peat medium resulted in an increase of microbial respiration: 24 h after the glucose amendment, the respiration rate in the CSM medium was five times higher than in the peat medium, indicating that the compost microflora react more vigorously to exogenous amendment than the peat microflora, thus probably keeping available nutrients from the plant pathogens. These results emphasize the importance of the medium's microflora in the suppression of plant pathogens in compost-amended media.

FUNGAL DISEASES OF THE NOXIOUS WEED *CYPERUS ROTUNDUS* IN ISRAEL

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The perennial weed purple nutsedge (*Cyperus rotundus*) is very problematic in Israel, mainly on summer crops and in orchards, and few herbicides are capable of even partly controlling it. Indigenous pathogenic fungi are therefore being sought for its biocontrol. At least three have been found here that are widespread, and have now been characterized: (i) *Pyricularia ligulalis* Luttrell (= *Dactyaria ligulalis*), first found in Alabama, is known in South America, Sudan and Australia. This is the first record from the Mediterranean Basin and Middle East; we identified it also on *Cyperus longus* from Anatolia. Grown in pure culture here, it incites small lesions on *C. rotundus* leaves, mainly in summer under damp conditions. (ii) A rust, comprising uredial and telial stages, corresponds morphologically to *Puccinia romagnoliana* Maire & Sacc., which differs from *P. conclusa* Thum. by the presence of many narrow paraphyses in the telia. Telia are hypophyllous, long and narrow, opening tardily, then pulverulent. (iii) *Cercospora* sp., which does not fully match any of the many named species on Cyperaceae in morphology and/or symptoms induced on hosts. Symptoms: Large, long-elliptical chestnut-brown lesions, sometimes with narrow yellow halo, on leaves, stems, and inflorescence peduncles and leaves, often coalescing; girdled inflorescence stems collapse. Morphology: Sporulation hypophyllous, in rows within lesions, with sparse stromal tissue

in substomatal chamber. Conidiophores dark, often closely geniculate near apex, 0-3 septate, as many as 20 in each fascicle, 37-50 x 2.5-5 µm. Conidia hyaline, obclavate, subtruncate at base with dark scar, 60-200 x 2.5-4.5 µm, 4-25 distinct septa under lactophenol. Grown in pure culture, it sporulates poorly, but best on V-8 agar of *Urtica*-decoction agar under continuous or intermittent near-UV illumination. Vegetative growth: minimum ca 10°C, optimum ca 25°C; sporulation on leaf: minimum ca 10°C, maximum < 34°C; germination: minimum 5°C, maximum > 32°C.

Inoculation by comminuted mycelium without any conidia induced typical symptoms, with sporulation in the lesions. Young and mature leaves were equally susceptible, with symptoms appearing from 10 to 22 d after inoculation, depending on temperature and on length of leaf wetness.

SOME FACTORS AFFECTING TELIOSPORE AND BASIDIOSPORE GERMINATION OF *Puccinia Xanthii* - A POTENTIAL MYCOHERBICIDE OF *XANTHIUM* SP. - AND CONDITIONS NECESSARY FOR SUCCESSFUL INFECTION OF THE WEED

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Xanthium sp. is an annual weed which presents a problem in cotton fields in Israel in summer. *Puccinia xanthii* (Schw.) Schr., a microcyclic, monoeccious fungus, was found to occur naturally on *Xanthium* sp. growing in wet areas. When alternating hot/cold treatments were given, they appeared to stimulate both teliospore and basidiospore germination. Liquid additives derived from uninfected freshly dried green leaves of *Xanthium* sp. plants, added to cultures of *P. xanthii*, generally appeared to inhibit teliospore and basidiospore germination; some increase, however, was found when additives produced from uninfected, naturally dried brown leaves, were used. Temperatures within the range of 15-30°C had little effect on teliospore germination; however, basidiospore germination decreased as the temperature increased. Light had little effect on teliospore germination but completely inhibited basidiospore germination at higher temperatures. The conditions necessary for successful infection of *Xanthium* sp. plants were found to be a pre-soaking period (by floating) of the inoculum material in 0.5% sucrose, and a very high level of humidity to ensure that host leaves remained wet during the infection period. A temperature between 28 and 35°C is needed to encourage good production of teliospore-bearing sori after the infection period.

B: HEAT TREATMENT AND SOLARIZATION, COVERING AGENTS (ANTI-TRANSPIRANTS) AND OTHER PHYSICAL METHODS

WEAKENING OF *FUSARIUM* STRUCTURES BY SUBLETHAL HEATING

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Sublethal heating at 38-42°C of conidia and chlamydospores of *Fusarium oxysporum* f.sp. *niveum* resulted in a weakening effect beyond the immediate heating effect. This was expressed in

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I. RESEARCH ON *ALTERNARIA HELIANTHI* ON *XANTHIUM STRUMARIUM*

A Partial Host Range and Evaluation of Potential as a Biological Control Agent

Various species of *Alternaria* have been considered and studied as potential biocontrol agents against weeds (Walker and Sciumbato 1979 and Walker 1982). *Xanthium* species (Allen et al. 1983 and Quimby 1983) are affected by *Alternaria helianthi* (Hansf.) (Tubaki and Nishihara 1969). Some response of *Xanthium strumarium* (common cocklebur) to this fungus might be expected because the weed is of the Tribe Heliantheae in Compositae as is its major host, cultivated sunflower (Herr and Lipps 1982, Sackston 1983, and Shane et al. 1981).

Trevathan and Roy (1980) first observed an *A. helianthi*-induced blight of cultivated sunflower in Mississippi in 1978 and 1979. After their paper appeared, a deliberate search was made and the fungus was isolated from common sunflower near Stoneville, MS, in July 1982; this isolate was designated "SV-1," and was deposited in the Agricultural Research Culture Collection, U.S. Department of Agriculture, Peoria, IL (Accession Number NRRL 15814).

Thus, this study was conducted to determine a partial host range for isolate SV-1 of *A. helianthi* and to evaluate whether further research might be justified as to its potential as a biological control agent against common cocklebur.

Culturing techniques and host range evaluations were all conducted according to the methods of Walker (1980, 1982), and Walker and Sciumbato (1979).

Results

The SV-1 isolate of *A. helianthi* was pathogenic to cultivated sunflower and to common cocklebur. Soybean, cotton, rice, grain sorghum, corn, wheat, and 22 other weed and crop species/varieties in nine botanical families showed no response or only minor symptoms.

Conclusions

Thus, further research appeared to be warranted to determine the potential of *A. helianthi* as a biocontrol agent against common cocklebur in geographic areas where sunflower is not a crop. This research was published (Quimby 1989).

Further Evaluation of *Alternaria helianthi* as a Microbial Herbicide for Cocklebur

Cocklebur, *Xanthium strumarium*, is the most troublesome weed in soybeans in Mississippi (Houston et al. 1984) and is one of the most important weeds in the southeastern United States. Herbicides currently in use for cocklebur control need certain environmental conditions and plant growth stages to be effective. The time period available for herbicide application is so narrow that control is often impractical. A broader base of technology is needed for control of cocklebur.

Fungal pathogens have been shown to have great potential as augmented biocontrol agents because of their reproductive capacity, specificity, genetic stability, infectivity, adaptation to mass production, and safety to the environment (Quimby and Walker 1982; TeBeest and Templeton 1985). The use of an endemic fungus rather than an introduced exotic one may be advantageous for creating artificial epidemics for weed control (Daniel et al. 1973).

Alternaria helianthi is an endemic pathogen of wild sunflower (*Helianthus annuus*) in the Southern United States and is one of the many factors limiting commercial sunflower production in the humid south. *Alternaria* blight has only recently been recognized as a problem disease of sunflower in North America (Sackston 1983). *Alternaria helianthi* can be transmitted by sunflower seeds and overwinters in infected plant debris (Herr and Lipps 1982; Jeffrey et al. 1984). Allen et al. (1983) reported that certain *Xanthium* species were susceptible to *A. helianthi*. Simultaneously, Quimby (1983, 1989) reported that *A. helianthi* is also a pathogen of common cocklebur.

Since commercial sunflower production is not feasible in much of the Southern United States and since *A. helianthi* is native to this region, this research was a natural follow-up to a partial host-range study (Quimby 1989) and the suggestion that further evaluation was justified toward augmenting this fungus

as a biocontrol agent. The objectives were to evaluate various spore concentrations and dew periods on *A. helianthi*-induced mortality of cocklebur plants at various stages of growth in a greenhouse study. In addition, a preliminary field test was conducted to rate the performance of *A. helianthi* in the field.

Test cocklebur plants were grown in plant strips (twelve 5.5 cm x 5.5 cm pots/strip) of a commercial potting mix. Cocklebur seeds were rinsed in tap water for 16 h, dried, and stored in the refrigerator at 4 C prior to planting. Greenhouse air temperatures were maintained at 25 to 30 C with approximately 50% relative humidity.

Inoculum of *A. helianthi* was produced in soybean flour/cornmeal liquid culture, blended, poured into pans and incubated according to Walker and Riley (1982). A hemacytometer was used to estimate conidia/ml.

Four cocklebur growth stages were tested using 1-, 2-, 3-, and 4-week old plants. Four spore concentrations (25,000, 50,000, 75,000, and 100,000 conidia/ml) and a control without conidia were sprayed with an atomizer to runoff on plants of each growth stage across four replications. The surfactant nonoxynol (9 to 10 POE)-(p-nonylphenyl)-w-hydroxypoly (oxyethylene) was used in all treatments at 0.05%. One experimental unit consisted of 12 plants. Each treated plant was subjected to 16 h of dew in a dew chamber at 26 C immediately after inoculation and then placed into a greenhouse. The percent mortality was recorded one week after inoculation. A Duncan's multiple range test was used to separate means.

A dew period test was conducted to determine the amount of dew necessary for adequate infection. Dew periods of 0, 4, 8, 12, 16, 20, and 24 h at 26 C were provided to three replications of plants sprayed to runoff with 50,000 conidia/ml. Percent mortality was recorded one week after inoculation and data

were statistically analyzed so that the Least Significant Difference (LSD) could be computed.

A preliminary trial was conducted to evaluate the performance of *A. helianthi* in the field. Cocklebur in the 3- to 4-leaf stage in soybeans was sprayed to runoff with ~~1~~ million conidia/ml of 0.05% nonoxynol surfactant. The weeds were sprayed at dusk. One 5-meter row, 1-meter wide constituted one replication; four replications per treatment were treated. The check plots were sprayed with surfactant solution without conidia. The field test was bordered, down-wind 1-km, with sunflower as a trap crop to determine how far the fungus would move during a growing season and to what extent.

Results

Effective control of cocklebur with *A. helianthi* was obtained in these greenhouse tests. Seedlings in the cotyledon to first-leaf stage were most sensitive to *A. helianthi*. Seven days after inoculation, 100% of the one-week-old plants were killed across all inoculum concentrations. As the plants increased in size, resistance increased. Four-week-old plants were the least susceptible where mortality increased as spore concentration increased; 100,000 conidia/ml produced 85% mortality at the 4-week growth stage.

A spore concentration of at least 50,000 conidia/ml would be necessary for adequate infection of 2- to 4-week old plants in greenhouse and growth chamber studies. A higher mortality rating across all growth stages was recorded with 75,000 and 100,000 conidia/ml as compared to 50,000 conidia/ml (Table 1).

Greater than 90% mortality occurred at 12 h of dew across three replications using 50,000 conidia/ml. This is in agreement with Allen et al. (1983b), who reported that maximum infection of sunflower leaves by *A. helianthi* occurred with a 12 h or greater dew duration.

Conclusions

Results of the preliminary field trial showed that *A. helianthi* can potentially be a useful microbial control agent for cocklebur. A secondary or repeating cycle of the disease did not occur on the sunflower trap crop even though all the inoculated cocklebur leaves were destroyed. Since less than 1% *Alternaria* blight developed on these sunflower plants and since this was equivalent to the natural disease development on sunflower control plants, it is concluded that cocklebur cannot serve as a host for the repeating or secondary cycle of this disease.

Within a few hours after spore germination, apparent phytotoxicity occurred to cocklebur but little to no sporulation of the fungus occurred on cocklebur plants. It appears that cocklebur is a dead-end-host for this fungus. This factor is probably the underlying reason that *A. helianthi* has never been found in natural stands of cocklebur even though the fungus is ubiquitous and endemic to the Southern United States. Allen, et al. (1983a) also reported that *A. helianthi* has not been observed under field conditions on *X. spinosum* nor *X. pungens* in Australia.

In addition, Allen et al. (1983c) showed that sunflower is most susceptible to infection by *A. helianthi* during anthesis. Since the fungus would be used for weed control during the early soybean growing season, it is likely that sunflower would not be affected due to simple escape. Sunflower is not a major crop in much of the Southern United States. This factor coupled with the fact that cocklebur appears to be a dead-end-host for *A. helianthi* and the success of this biological control agent in these preliminary tests justifies testing it further for control of this major weed in the Southern United States.

Table 1. The effect of inoculum concentrations of *A. helianthi* on mortality of cocklebur growth stages.

Treatment ^a	% Mortality ^b
GS1/IC2	100.0 a
GS1/IC3	100.0 a
GS1/IC4	100.0 a
GS1/IC5	100.0 a
GS2/IC5	100.0 a
GS3/IC4	100.0 a
GS2/IC4	97.9 a
GS3/IC5	97.9 a
GS3/IC3	95.9 a
GS4/IC5	83.4 b
GS2/IC3	83.3 b
GS3/IC2	68.8 c
GS4/IC4	37.4 d
GS4/IC2	16.7 e
GS4/IC3	16.7 e
GS2/IC2	12.5 e
GS1/IC1	0.0 f
GS2/IC1	0.0 f
GS3/IC1	0.0 f
GS4/IC1	0.0 f

^aGrowth stages x inoculum concentrations, where: GS1 = one-week old plants, GS2 = two-week-old plants, GS3 = three-week-old plants, GS4 = four-week-old plants. IC1 = 0 spores/ml (control), IC2 = 2.5×10^4 spores/ml, IC3 = 5.0×10^4 spores/ml, IC4 = 7.5×10^4 spores/ml, IC5 = 10.0×10^4 spores/ml.

^bMeans followed by the same letter are not significantly different at the 0.05 level by Duncan's multiple range test.

II. RESEARCH ON *ALTERNARIA CRASSA* ON *DATURA STRAMONIUM*

Studies of Biotic and Abiotic Factors Influencing the Potential of This Fungus as a Biological Control Agent

Jimsonweed (*Datura stramonium*) is an annual broadleaf weed that is imperfectly controlled with herbicides in soybeans (Oliver and Howe 1980). ~~An effective~~ An effective biological control agent for jimsonweed could serve as an essential part of an integrated weed management program in soybeans.

In 1984, a leaf-spotting fungus, *Alternaria crassa* (NRRL #18136), was isolated from infected leaves of jimsonweed that were collected in a soybean field near Southaven, Mississippi, USA (Boyette 1986). Boyette (1986) indicated that the host range of this fungus is restricted to jimsonweed and 'Marion' and 'Beefsteak' tomatoes (*Lycopersicon esculentum*).

The objectives of this research were to determine the optimal dew period, inoculum concentrations, plant growth stages, and air temperature regimes required for biocontrol of jimsonweed with *A. crassa*.

General methods were similar for the studies described previously. Inoculum for all tests was produced after Boyette (1986) and Walker and Riley (1982). Jimsonweed seeds were mechanically scarified with sandpaper. Plants were grown in a commercial potting mix that was supplemented with a slow-release fertilizer (N/P/K, 14:14:14) and contained in 11 x 33-cm peat strips. Each strip contained 12 plants. Greenhouse temperatures were 28-32 C with 40 to 60% relative humidity. The day length was about 12 h with 1650 $\mu\text{E}/\text{m}^2/\text{s}$ photosynthetically active radiation (PAR) at midday. Unless otherwise stated, seedlings in the cotyledonary stage of growth were inoculated with a spray mist of *A. crassa* spores in 0.02% (v/v) nonoxynol surfactant at about 900 l/ha (permits complete plant coverage at about 9.35 $\mu\text{l}/\text{cm}^2$ of plant leaf area). Control plants were sprayed with surfactant solution without fungus. Percent weed control was

expressed as percent of weeds killed 2 weeks after inoculation. Dry weights were taken of green tissue at harvest, combined within each replication, and total biomass reduction expressed as a percentage of surfactant controls. The tests were arranged in a randomized complete block design with three replications of each treatment; the experiments were conducted twice.

To determine response to dew period duration, jimsonweed seedlings in the cotyledonary stage were sprayed with 0, 100,000, or 1 million conidia of *A. crassa*/ml in surfactant as above. The treated seedlings were then placed in a dew chamber at 25 C for 0 h, 6-12 h (in 1-h increments), 18 h, or 24 h. Following their exposure to the various dew durations, plants were placed on greenhouse benches and observed for disease development.

To determine the effects of plant growth stage at time of inoculation, and the effect of various inoculum concentrations, seedlings in the cotyledonary, 1- to 2-leaf, 3- to 4-leaf, or 5-leaf to maturity (flowering) stages of growth were inoculated with conidia concentrations of 0, 1,000, 10,000, 100,000, or 1 million conidia/ml. The plants were then exposed to dew in a dew chamber for 12 h at 25 C, after which they were placed on greenhouse benches, and monitored for disease development.

To investigate the effects of various air and dew temperature regimes on the activity of *A. crassa*, seedlings were sprayed with spores at concentrations of 0, 1,000, 10,000, 100,000, or 1 million conidia/ml, which represents 0, 9, 90, 900, and 9,000 conidia/cm² of leaf area, respectively. The seedlings were then placed in dew chambers for 12 h, with dew temperatures of 20, 25, or 30 C. Then the plants were moved to environmental chambers with day/night temperatures of either 24/13 C, 29/18 C, or 35/24 C, respectively, with 14-h photoperiod during the 'day' temperature period and about 840 $\mu\text{E}/\text{m}^2/\text{s}$ PAR and a relative humidity of 50%.

Results

Alternaria crassa killed jimsonweed over a broad range of dew period durations at 25 C. Although some infection occurred at all dew periods tested under both inoculum concentrations (100,000 and 1 million conidia/ml), periods <8 h did not provide satisfactory weed mortality. At least 9 h of dew was required to kill about 80% of fungus-inoculated weeds at an inoculum concentration of 100,000 conidia/ml. By increasing the inoculum concentration 10X to 1 million conidia/ml, about 80% control was achieved after only 8 h of dew. Although 100% mortality was not achieved with all fungal treatments, many plants were severely stunted. The total biomass of plants inoculated with 100,000 conidia/ml was reduced by 86% vs. 94% reduction with 1 million conidia/ml under 8 h dew. Seedlings suffered 100% mortality at the high spore concentration under 9 h dew.

Mortality was significantly increased at all growth stages by increasing the inoculum concentration. Greater overall mortality was caused by inoculum concentrations of 100,000 and 1 million conidia/ml than with the lower concentrations tested. A concentration of 10,000 conidia/ml killed 50 to 60% of the seedlings in the cotyledonary growth stage, but was ineffective on larger plants. Seedlings up to the 4-leaf stage were killed with 100,000 conidia/ml but larger plants required an order of magnitude higher concentration for 90% control. Responses in biomass reduction were similar. A concentration of 10,000 conidia/ml reduced biomass of cotyledonary stage seedlings 75%.

Weed control was significantly reduced by day/night air temperatures of 35/24 C at all inoculum concentrations tested. The intermediate temperature regime of 29/18 C appeared to provide the most favorable environment as 100% mortality occurred at 100,000 and 1 million conidia/ml. At the post-inoculative temperature of 24/13C, 100,000 conidia/ml killed only 57% of the treated

seedlings. Nearly as many (52%) of the seedlings were killed by 10,000 conidia/ml at the more favorable day/night temperatures of 29/18 C.

These results indicate that *A. crassa* has potential as a biological herbicide for controlling jimsonweed. The environmental requirements are relatively narrow for this fungus to be effective in causing mortality of jimsonweed. However, these conditions would be expected to occur during a typical growing season in the midwestern and southeastern United States. These results also suggest that death of jimsonweed may not be necessary to suppress the competitiveness of the weed below acceptable economic threshold levels. Additional studies will be conducted in the field to further evaluate the biocontrol potential of *A. crassa* against jimsonweed as results of experiments conducted in controlled environment chambers may not reflect results that might be obtained under actual field conditions.

This research has been published (Boyette and Turfitt 1988).

III. RESEARCH ON *COLLETOTRICHUM TRUNCATUM* ON *SESBANIA EXALTATA*

Studies of host range and virulence as a potential mycoherbicide

Hemp sesbania (*Sesbania exaltata*) is a serious weed pest of soybean and rice in the Southeastern United States (McWhorter and Anderson 1979; Palmer 1980), and is increasing in cotton (Bryson 1986). This weed is very competitive in cotton as three plants/m of row for the full season will reduce seed cotton yields by 53% and boll weights by 9% (Bryson 1987).

Although hemp sesbania can be controlled in soybeans and rice with postemergent applications of acifluorfen and imazaquin, no satisfactory postemergent herbicide exists for its control in cotton (Jordan 1985).

In June 1987, an anthracnose pathogen was isolated from leaf spots of hemp sesbania, and was tentatively identified as *Colletotrichum truncatum*. The disease was widespread among the hemp sesbania seedlings, mainly affecting the cotyledons. Symptoms were not found on older seedlings.

These experiments were conducted to determine the host range and virulence of this pathogen. This information is essential as a first step in evaluating the potential of *C. truncatum* as a mycoherbicide for control of hemp sesbania.

Information about the collection, isolation, and culture of the organism follows: diseased leaf samples were collected from the Southern Weed Science Laboratory weed nursery in Stoneville, MS. Microscopic examinations revealed orange-colored masses of conidia being produced in acervuli in the lesions. A mass of spores was transferred into 10 ml of 10% (v/v) ChloroxTM (0.525% sodium hypochlororite) in sterile distilled water. Then 1-ml portions of the spore suspension were pipetted into petri plates of potato-dextrose agar (PDA-A) amended with the antibiotics chloramphenicol (0.75mg/ml) and streptomycin sulfate (1.25 mg/l). The plates were incubated for 48 h at 25 C. Advancing edges of fungal colonies were transferred to PDA-A, and incubated for five days at 25 C

under alternating 12-h light/12-h dark regimes. The light was provided by fluorescent bulbs. The fungus was subcultured on PDA without antibiotics, and preserved in screw-capped culture tubes of twice-autoclaved sandy loam soil, and stored at 4 C.

Inoculum was increased by flooding ~~petri~~ petri plates of PDA-P (amended with L-proline (1.5 g/l) with 1 ml of spore suspension from subculture plates. The plates were incubated for 3 to 5 days under the same temperature and lighting conditions as previously described. Conidia were washed from the plates with sterile distilled water, rinsed twice by centrifugation (994 x g for 10 min.) and adjusted to 20 million conidia/ml by adding distilled water. Counts of conidia were determined by hemacytometers.

Hemp sesbania plants and host range test plants (Table 1) were grown from seed in a commercial potting mix contained in peat strips. Each strip contained 12 plants. The potting mix was supplemented with a slow-release fertilizer (14/14/14, N/P/K). The plants were placed on sub-irrigated benches in the greenhouse. Greenhouse temperatures ranged from 25-30 C with 40-90% relative humidity. The photoperiod was 12 h with 1650 uE/m²/s photosynthetically active radiation as measured at midday.

Pathogenicity tests were conducted by inoculating hemp sesbania plants from the cotyledonary stage through the 7-true leaf stage; seedlings were sprayed to runoff with 20 million conidia/ml in 0.02% Tween-80 surfactant. Control plants were sprayed with surfactant solution only.

Immediately after inoculation, the plants were placed in a darkened dew chamber for 12 h at 25 C, then transferred to subirrigated greenhouse benches. The plants were monitored for disease development for 1 wk.

For the host-range tests, test plants (Table 1) were grown under the same conditions as previously described for hemp sesbania. All plants were treated

when in the cotyledonary to 3-true leaf stage of growth, with 36 plants exposed to each treatment. The plants were inoculated, incubated, and monitored for disease development for 3 wks.

The discovery of this *Colletotrichum* offered a unique opportunity for cross-inoculation tests and comparison with other ~~species~~ closely related. Cultures of *C. truncatum* and *C. dematium* isolates that have been reported as pathogens of other leguminous weeds or soybeans were obtained from the Northern Regional Research Laboratory (NRRL), Peoria, IL 61604, and the American Type Culture Collection (ATCC), Rockville, MD 20852, for pathogenicity comparisons (Table 2). Isolates NRRL 15933 (pathogenic to Florida beggarweed, *Desmodium tortuosum*), ATCC 22582 (pathogenic to coffee senna, *Cassia occidentalis*), and ATCC 18013 (pathogenic to soybeans) were cultured on petri plates of PDA. The spores were rinsed from the agar surface with distilled water, centrifuged for 10 min. at 956 x g, and adjusted to 20 million conidia/ml by adding distilled water, and the plants were sprayed to runoff with an atomizer (Table 2). The inoculated plants were given dew treatments as described previously and monitored for disease development for 3 wks.

Results

Leaf lesions, 2 to 4 mm in diameter with dark concentric rings, were observed on cotyledons of the hemp sesbania seedlings collected in the field. Microscopic examinations revealed acervuli containing setae and truncate conidia measuring 18 to 28 by 3 to 5 μ m. Based on characteristics observed in infected tissue and in artificial culture, the fungus was tentatively identified as *Colletotrichum truncatum* (Schw.) Andrus and Moore sensu Sutton (1980); this identification was subsequently confirmed by the Commonwealth Agricultural Bureau, International Mycological Institute, Kew, Surrey, United Kingdom. The fungus has been

deposited with the ARS Patent Culture Collection, Northern Regional Research Center, Peoria, IL 61604, and has been assigned the number CT isolate 18434.

CT isolate 18434 was highly virulent on hemp sesbania at all stages of growth that were tested. Cotyledonary plants were all killed within 3 days after inoculations while larger plants required 5 to 7 days to achieve this level of kill (Figure 1).

The reactions of the test plants to CT isolate 18434 are shown in Table 1. All evidence of infection was limited to plants in the Leguminosae. Some infection occurred on 'Bragg', 'Bedford', and 'Hill' cv. soybeans, but it was restricted to small (<3 mm), nonenlarging lesions. Microscopic examinations revealed that the fungus did not sporulate on the necrotic soybean tissue. A related species, *S. drummondii*, was not affected by the fungus.

Of the isolates that were included in the cross-inoculation tests, only NRRL CT isolate 18432 had visibly infected hemp sesbania after 3 weeks (Table 2). Florida beggarweed and coffee senna were not visibly affected by this isolate. 'Bragg' soybeans were slightly infected, but no mortality occurred. ATCC isolate 22583 infected and killed Florida beggarweed seedlings. This isolate also caused slight infection on soybean. ATCC isolate 22852 caused infection and mortality of coffee senna, and moderately infected soybeans. ATCC isolate 18013 was the most virulent on soybeans which were severely infected and died after inoculation. This isolate also caused slight injury to Florida beggarweed, but it caused no visible infection to hemp sesbania or coffee senna.

Conclusions

This is the first report of the occurrence of *C. truncatum* on hemp sesbania. The isolate from hemp sesbania caused slight injury to some soybean cultivars but it did not cause mortality nor did it sporulate on the soybean lesions. Although it is unlikely that this isolate of *C. truncatum* would pose a serious threat to

soybeans or other non-target species, more research is needed to verify these findings.

Other isolates of *C. truncatum* have been evaluated as biological herbicides for controlling the weeds coffee senna (*Cassia occidentalis*) (Gadauskas, et al. 1977), and Florida beggarweed (*Desmodium tortuosum*) (Cardina, et al. 1988). In cross-inoculation tests, none of the isolates was pathogenic to hemp sesbania, nor was the hemp sesbania isolate pathogenic to either of these species.

From these results and from those of others, apparently host specialization may occur in this species, although formae speciales of *C. truncatum* are not recognized by Sutton (1980). Other fungal taxonomists, however, do recognize formae speciales based in part on host range reactions (Von Arx 1970).

These results indicate that *C. truncatum* has potential as a bioherbicide for controlling hemp sesbania. This fungus is highly pathogenic to the weed, and either non-pathogenic or weakly pathogenic to the crop species that were tested. TeBeest (1988) has discovered recently that *C. gloeosporioides* f. sp. *aeschynomene*, the incitant of anthracnose on northern jointvetch (*Aeschynomene virginica*) has a much broader host range than originally reported by Daniel et al. (1973). Thus, more research should be conducted on the host range of CT isolate 18434 of *C. truncatum* to include soybean cultivars and other leguminous crop species of more diverse genetic backgrounds (Wapshire 1974). Additional studies will be conducted to determine the environmental and physical conditions necessary for this fungus to control hemp sesbania, and to test the biocontrol efficacy of the pathogen under field conditions.

This research has been published recently (Boyette 1991).

Table 1. Response of various crop and weed species tested for susceptibility to Colletotrichum truncatum

Family	Disease
Common name, scientific name, cultivar	reaction ^a
COMPOSITAE	
Cocklebur (<u>Xanthium strumarium</u> L.)	I
CONVOLVULACEAE	
Morningglory (<u>Ipomoea</u> spp.)	I
CUCURBITACEAE	
Pumpkin (<u>Cucurbita pepo</u> L.)	
'Jack-O'-Lantern'	I
Squash (<u>Cucurbita pepo</u> var. <u>melopepo</u> (L.) Alef.) 'Golden Summer Crookneck'	I
Watermelon (<u>Citrullus vulgaris</u> Schr.)	I
'Charleston Grey'	
GRAMINAE	
Corn (<u>Zea mays</u> L.) 'Truckers Favorite'	I
Johnsongrass (<u>Sorghum halepense</u> (L.) Pers.)	I
Rice (<u>Oryza sativa</u> L.)	
'LaBelle'	I
'Starbonnet'	I
Grain sorghum (<u>Sorghum bicolor</u> (L.) Moench) 'Texas C-124'	I

Table 1, Cont.

Family/species	Disease
Common name, scientific name, cultivar	Reaction
LEGUMINOSAE	
Alfalfa (<u>Medicago sativa</u> L.) 'Delta'	
Coffee senna (<u>Cassia occidentalis</u> L.)	I
Sicklepod (<u>Cassia obtusifolia</u> L.)	I
Florida beggarweed (<u>Desmodium</u>	
<u>tortuosum</u> L.)	I
Showy croton (<u>Crotalaria</u>	
<u>spectabilis</u> L.)	I
Hemp sesbania (<u>Sesbania exaltata</u> (Raf.)	
Cory.)	S-5
Rattlebox (<u>Sesbania drummondii</u> L.)	I
Northern jointvetch (<u>Aeschynomene</u>	
<u>virginica</u> (L.) B.S.P.)	I
Soybean (<u>Glycine max</u> (L.) Merr.)	
'Bedford'	S-2
'Bragg'	S-2
'Dare'	I
'Davis'	I
'Forrest'	I
'Hill'	S-1
'Hood'	I

Table 1, Cont.

Family/species	Disease
Common name, scientific name, cultivar	Reaction
LEGUMINOSAE (Cont.)	
'Centennial'	I
'Tracy'	I
Peanut (<u>Arachis hypogaea</u> L.)	
'Tennessee Reds'	I
Garden bean (<u>Phaseolus vulgaris</u> L.)	
'Kentucky Wonder'	I
'Romano Pole'	I
'Ohio Pole'	I
'Jackson Wonder'	I
'Henderson Bush Lima'	I
'Lady Cowpea'	I
'White Crowder Pea'	I
MALVACEAE	
Cotton (<u>Gossypium hirsutum</u> L.)	
'Stoneville 213'	I
'Deltapine 61'	I
Prickly sida (<u>Sida spinosa</u> L.)	I
Velvetleaf (<u>Abutilon theophrasti</u> Medic.)	I

Table 1, Cont.

Family/species	Disease
Common name, scientific name, cultivar	Reaction
<hr/>	
SOLANACEAE	
Tomato (<u>Lycopersicon</u> <u>esculentum</u> Mill)	
'Beefsteak'	I
'Marion'	I
Jimsonweed (<u>Datura</u> <u>stramonium</u> L.)	I
<hr/>	

^aReaction: S = susceptible, where 1 equals small (1 mm),
nonenlarging lesions to 5 equals plant death; I = immune.

Table 2. Pathogenicity of C. truncatum and C. dematium isolates in cross-inoculation experiments

Fungus	Isolate code	Disease reaction on indicated plant ^a			
		Hemp	Florida	Coffee 'Bragg'	
		sesbania	beggarweed	senna	Soybean
<u>C. truncatum</u>	NRRL 18434	S-5	I	I	S-2
<u>C. truncatum</u>	NRRL 15933	I	S-5	I	S-2
<u>C. dematium</u>	ATCC 22852	I	I	S-5	S-3
<u>C. truncatum</u>	ATCC 18013	I	S-1	I	S-5

^aReaction: S = susceptible, where 1 = small (1 mm) nonenlarging lesions to 5 equals plant death; I = immune.

Figure Legends

Figure 1. Effect of *C. truncatum* on hemp sesbania at various stages of growth. The plants were inoculated with *C. truncatum* spores at a rate of 2×10^7 spores/ml.

IV. RESEARCH ON PHYTOTOXINS: *ALTERNARIA HELIANTHI* AND *A. CRASSA*

Bioassays of Crude Phytotoxins

Duke (1986) has published a review of phytotoxins and their potential to provide new chemistries for weed control in the future. Since this project involved basic research on *Alternaria* species, a natural outgrowth was a preliminary investigation into their potential to produce phytotoxins. *Alternaria helianthi*, a pathogen of sunflower is known to produce several phytotoxins when cultured in liquid media (Tal et al. 1985); however, the biological and chemical characterization of these toxins is incomplete.

Crude toxin was obtained from fungal mycelium and extracted with chloroform or ethyl acetate.

Results

Leaf disc bioassays indicated that sunflower and cocklebur were highly sensitive to 0.2 g dried crude toxin from *A. helianthi* in 10 ml buffer. Electrolyte leakage from the leaf discs was about four fold that of the untreated controls after 72 h. The toxin acted as a plant growth regulator by causing >50% growth inhibition of internodes of sunflower seedlings by 3 weeks after a foliar spray treatment. HPLC analyses using a cyclodextrin column and standards for comparison indicate that a major portion of the toxin is radicinin, which is a known phytotoxin (Robeson et al. 1982) produced by *A. helianthi* and other fungi. A portion of the crude toxin also comigrated with a radicinin standard on TLC plates. Wavelength scans from 190 nm to 450 nm on HPLC peak apices suggest that two previously unreported enantiomeric forms of radicinin probably exist, but further characterization is needed to verify the existence of both forms. Radicinin, purified by HPLC, produced results similar to those of the crude toxin in the leaf disc bioassay.

Alternaria crassa (host = jimsonweed) was discovered to produce a toxin with the same HPLC retention time as radicinin from *A. helianthi* (host = sunflower). Yield (ca. 1.5 g/L) of crude toxin from mycelium of *A. crassa* was 3-fold that from *A. helianthi*. In laboratory bioassays, the crude toxin from *A. crassa* delayed germination of hemp sesbania seeds after 24 h of imbibition; in combination with imazaquin the crude toxin synergistically reduced growth of tomato seedlings by about 50% after 72 h. The crude toxin caused severe wilting of hemp sesbania seedlings when it was applied through the roots.

Conclusions

Further research would be justified on purifying the crude toxin(s) and on developing ways to stabilize the labile compounds. Evidence was obtained that some of the fractions from HPLC were phytotoxic, but they were so labile that identification could not be obtained (through NMR, Infrared Spectroscopy, etc.).

This research was published in abstract form (Quimby et al. 1987).

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f. DESCRIPTION OF COOPERATION

Scientists involved in this project have exchanged correspondence, research reports, and research ideas during the period since initiation. Dr. Reuven Reuveni visited the Stoneville, U.S.A. laboratory in August of 1987 (this trip was not paid for by BARD). He consulted with Dr. Boyette during that visit (Quimby was absent) on his research with Puccinia xanthii and discussed other ongoing research in Israel and the U.S.

Material for fungal growth media unavailable in Israel was shipped to Dr. Kenneth at the Faculty of Agriculture, Hebrew University of Jerusalem at Rehovat.

Dr. Quimby visited Israel in September of 1988. He consulted at the Newe Ya'ar Experiment Station and at the Faculty of Agriculture, Rehovat. A seminar was presented at the Faculty of Agriculture. Quimby spent several days in the field with Kleifeld and Kenneth examining their finds of pathogens and the effects of the pathogens on weeds throughout Israel from the North to the South.

g. EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE AIMS OF THE ORIGINAL PROPOSAL AND THE BENEFIT TO AGRICULTURE

The Israeli team was "on target" with respect to accomplishing the objectives of the original proposal. The research on the life history of Puccinia xanthii at the Newe-Ya'ar Experiment Station led to successful induced infection of cocklebur plants under controlled environmental conditions. Unfortunately, they were unable to achieve infection and control of the weed under more natural, rigorous conditions.

However, their discovery of a 'new' pathogen (Cercospora nr. caricis on purple nutsedge (Cyperus rotundus) led to an important series of studies which was documented in a M. Sc. Thesis by Elinoar Nitzani (Bonne'). The fact that infection can be induced with mycelia (minus conidia) of this fungus could really be important toward commercialization. Since Nitzani achieved infection of healthy plants with straw from diseased plants, this suggests that farmers might be able to manipulate this fungus on their weeds without expensive inputs. Much more research is justified on these points.

The American effort was mostly successful in meeting the objectives of the project. Concentrations of Alternaria helianthi and growth stages of cocklebur were determined for optimal control.

The survey for new pathogens was productive. Dr. Boyette discovered a specialized form of Colletotrichum truncatum which is specific for control of a major weed, hemp sesbania. This weed is a problem in cotton, rice, and soybeans. No good chemical control exists for it in cotton, so a biological alternative is very timely. This fungus was also characterized as to its biology and ecological requirements.

The fungus, Alternaria crassa, which attacks jimsonweed, was further characterized as to its ecological requirements.

The direct benefits to agriculture accruing from this project may not be realized for several years and after more research on the development of the fungi for practical weed control. However, pressure will continue for the development of pest control measures that will reduce the amount of pesticides needed. This research

will provide a basis for further applied research that will actually move the technology to the farm. Of particular benefit is the establishment of a base of biological control research in Israel.

h. LIST OF PUBLICATIONS FROM PROJECT

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i. **ACKNOWLEDGEMENTS**

Many individuals contributed to the success of the USA portion of this project. Special gratitude is expressed to: K.P. Bachman, A. Bassi, B. Bennett, M. Dantzler, A. McWright, L. Merchant, V. Robertshaw, J. Schmidt, L. Turfitt, B. Reinhard, and R. Wilson. ~~The~~ Directors of the Southern Weed Science Laboratory during this period, Dr. C.G. McWhorter and Dr. S.O. Duke, were very supportive through administration and encouragement. The BARD offices in Israel and Beltsville were always helpful. Also, special thanks should go to those who made the trip of Quimby to Israel so interesting and productive: Y. Kleifeld, R. Kenneth, and J. Gressel.