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Host specificity of Puccinia canaliculata, a potential biocontrol agent for Cyperus esculentus

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Abstract

Aim was to clarify the rust fungus *Puccinia canaliculata* for release in a new environment where it may be utilized as a microbial herbicide. A strain of the rust was introduced from the USA into the Netherlands and kept in quarantaine. The susceptibility of *Cyperus esculentus* and several other species of the Cyperaceae was tested.

C. esculentus 'leptostachyus' from five locations was susceptible to *P. canaliculata*. *C. esculentus* of a yet unidentified biotype from two locations was moderately susceptible (fewer and smaller pustules than on 'leptostachyus'), whereas plants of the same biotype from a third location were resistant to the rust. *C. esculentus* 'esculentus' (from one location) and *C. esculentus* 'sativus' (a crop) were also resistant to the rust.

C. albostriatus, *C. alternifolius*, *C. flavescens*, *C. rotundus*, *Carex hirta*, *Eleocharis palustris*, and *Scirpus maritimus* were resistant to *P. canaliculata*. On *Cyperus fuscus*, *P. canaliculata* produced very small urediniosori (less then 1 mm in diameter); the sori were surrounded by a zone of necrotic plant tissue.

From the observations on *C. fuscus* we concluded that the (potential) host range of *P. canaliculata* is wider than we originally expected. For safety reasons, it was decided not to release it in the Netherlands.

Additional keywords: biological weed control.

Introduction

Cyperus esculentus was only recently introduced into the Netherlands. Due to its large vegetative growth capacity it may cause considerable losses of field crops. In addition, it is difficult and expensive to manage by farmers because of its insensitivity to many herbicides. For the purpose of eradication of the weed, a series of legal measures including a prohibition to grow root crops on infested land, were imposed on farmers (Naber and Rotteveel, 1986). A multidisciplinary research group was established in order to develop suitable eradication strategies for infested fields. From the beginning, biological control was taken into view as a possible part of integrated control systems.

In the United States, at the University of Georgia, *Puccinia canaliculata* (Schw.) Lagerh. is being evaluated as a microbial herbicide to control *C. esculentus*. Natural epidemics start only in late summer and have hardly any effect on the host plant (Phatak et al., 1983). Inoculations early in the growing season result in reduction of shoot growth and tuber formation by 50-75 %. Rust-infected plants also become much

more sensitive to herbicides. Integration of biological and chemical control results in a rapid decline of a *C. esculentus* population (Callaway et al., 1987).

P. canaliculata is a macro-cyclic, heteroecious rust which has been reported from North- and South-America, Africa, and East-Asia (Arthur, 1934; Gäumann, 1959). It has not been reported from Europe, unless it is identical with *P. romagnoliana*, which has been found in the Mediterranean area (Gäumann, 1959). In 1905, Arthur (cited by Arthur, 1934) succesfully inoculated *Cyperus esculentus* with aeciospores of *P. canaliculata* from *Xanthium* sp. Callaway et al. (1985) succeeded to infect *Helianthus annuus* and *X. strumarium* with teliospores in a glasshouse. *Ambrosia trifida* (Arthur, 1934) and Heliopsis parvifolia (Cummins, 1959) have also been reported as alternate hosts. The urodiniostage seems to be confined to *Cyperus* spp. The urediniostage of the rust strain from Georgia seems to be confined to clones of *C. esculentus* (S.C. Phatak, personal communication, 1984). Plants of *C. esculentus* can be infected at temperatures between 4 °C and 34 °C, the optimum range being 20-26 °C. In the optimum range, a dew period of at least 8 hours is required (Sutker, 1983).

Elaborate safety testing schemes have been developed to estimate the host range of biological weed control agents (Commonwealth Agricultural Bureaux, 1978). Those schemes meet the demands of the plant quarantaine authorities of Australia, Canada, New Zealand and the United States. They include testing of the target weed, other recorded host plants, species that are closely related to the target weed, and desirable species that never have been exposed to the agent organism.

Release of an agent organism into a new environment, i.e. from one continent to another, is only considered if all test plants are resistant. Before *P. canaliculata* could be released into the Netherlands, testing the susceptibility of several non-target plants was found to be necessary. The highest priority was given to testing the susceptibility of some native species belonging to the Cyperaceae, and some *Cyperus* spp. grown as ornamentals. To do the testing, a climate room was arranged as a quarantaine facility.

Materials and methods

Conditions in the quarantaine facility. The climate room was divided into four separate compartments of each ca. 1 m^2. In the compartments, pots with plants were placed on a turntable. An Hoagland nutrient solution was regularly supplied on dishes that were placed under the pots. Illumination of the compartments was provided by High Power Intensity bulbs (each 400 W, Philips). The light bulbs were separated from the growth chamber by glass plates. Tap water was running over the glass plates to carry off the radiation heat. Light intensity at a height of 20 cm from the table was 70 $W.m^{-2}$. Relative humidity (RH) in the room was kept at 70 %.

Cultivation of plants. Tubers of *C. esculentus* 'leptostachyus' (five collections, A1-A5), an unidentified variety (three collections, B1-B3), and 'esculentus' (one collection, C) were provided by S. ter Borg (Agricultural University, Wageningen). They were originally collected from different fields in the eastern part of the Dutch province Noord-Brabant. Tubers of *C. esculentus* 'sativus' (a crop) are commercially available. Tubers of *C. rotundus* were provided by A.J.W. Rotteveel (Plant Protection Service, Wageningen). The tubers of both species were placed under running tap water for 48 h to break dormancy, and planted in pots with sand. The pots were placed under

fluorescent tubes (Philips TLD 36W/33), illuminating 16 h a day in the climate room described above. Soon after emergence, the plants were transplanted to pots with pot soil (∅ 11 cm, 3 plants per pot); the pots were subsequently placed on the turntables.

Plants of other species were grown in pots (∅ 11 cm) with pot soil in a glasshouse at a temperature varying from 17 to 25 °C. If necessary, additional illumination was given with High Power Intensity bulbs up to 16 h a day. Seed of *C. flavescens* and *C. fuscus* was derived from H. van de Steeg (University of Nijmegen). *C. albostriatus* and *C. alternifolius* were grown from vegetative material. Shortly before use, they were placed in the climate room on a turntable.

Plants of *Carex hirta*, *Eleocharis palustris* and *Scirpus maritimus* were collected from their natural habitat. They were grown from vegetative materials in pots in the glasshouse as described above. Shortly before use, they were placed in the climate room on a turntable.

Production and storage of urediniospores. In April, 1986, we received urediniospores of *P. canaliculata* from S.C. Phatak (University of Georgia, USA). They had been stored at −73 °C. Upon arrival, a proportion of the spores was stored at −196 °C in liquid nitrogen. Another part was incubated for 2 min in a water bath at 40 °C to break dormancy, and applied on plants of different biotypes of *C. esculentus* (see below). Spores were collected from rust pustules on infected plants. They were either used on the same day (fresh spores), or stored for several days in a refrigerator at 4 °C and 20 % RH (Rowell, 1984).

In January, 1987, we received a second lot of spores from S.C. Phatak. These had also been stored at −73 °C. From this starting material, spores were produced on and collected from plants of *C. esculentus* 'leptostachyus' (A1). They were used as fresh spores, or stored in a refrigerator or in liquid nitrogen until use.

Inoculation and conditions shortly after inoculation. Spores from liquid nitrogen, after breaking dormancy, and those from the refrigerator were incubated 4-6 h at 70 % RH to allow them to rehydrate. Then they were mixed with talc powder in a whirl mixer. With fresh spores, the rehydration step was omitted. Plants were inoculated 20-25 days after emergence or transplantation. The mixture of spores and talc powder was either applied with a paint-brush on the abaxial leaf surfaces (with exception of the 3-5 oldest leaves), or with a pulverizer on whole plants.

Inoculated plants were placed on a turntable in one of the climate room compartments. On the outer edge of the table, a cilindrical frame of 60 cm height was placed and covered with plastic. The plants were wetted with glass-destilled water in an atomizer, and the top of the cilinder was covered with a plastic sheet. Then the lights were turned off, and the plants were kept in the dark or in diffuse light from neighbouring compartments for 16 h. After that time, the lights were turned on again, and a small aperture was made in the plastic cover. As a result, the water slowly evaporated, and the RH declined to about 90 %. The sheet was removed 24 h after inoculation.

Results

Susceptibility of C. esculentus to P. canaliculata. The first series of tests were done with several collections of *C. esculentus*. Either fresh spores, or spores from the refri-

Table 1. Susceptibility of *Cyperus esculentus* to *Puccinia canaliculata* in experiments in 1986.

C. Esculentus collection		Number of experiments	Number of plants tested	Number of infected plants
'leptostachyus'	A1	4	16	14
	A2	2	6	6
	A5	4	18	11
'unidentified'	B1	4	16	7
	B2	1	10	5
	B3	4	25	10

gerator were used. They were applied with a brush. The results are summarized in Table 1.

Urediniosori of *P. canaliculata* became visible 9-10 days after inoculation, and the epidermis was ruptured 1-2 days later. Plants of type A were more susceptible than those of type B. This was reflected by the higher percentage of infected plants (78 % compared to 43 %), and also by the pustule size. Those of type A measured 2-3 by 5-8 mm, and those of type B only 1-2 by 2-3 mm. Plants of both types never reacted with chlorosis or necrosis around the urediniosori.

During the next several months, we tried to improve the conditions for infection. An increase of the incubation period at high humidity to 36 or even 48 h had only a positive effect in some occasions with spores that had been stored in the refrigerator. With fresh spores, no infection occurred at all under these conditions. The availability of fresh spores for further experimentation became a limiting factor. Spores that had been stored in liquid nitrogen germinated on leaves, but could not infect susceptible plants.

For further experiments with the second lot of spores from the USA, we used only spores that had been stored in liquid nitrogen, or fresh spores. Plants of a susceptible variety of *C. esculentus* (A1) were included as a reference in each test. 100 % of the inoculated plants of this variety were infected. The number of sori per leaf (avaraged per plant) varied from 1 to 15 between experiments. Results of additional testing of the susceptibility of *C. esculentus* are shown in Table 2. Two additional A-types (A3 and A4) were susceptible. Two B-types that were susceptible in previous tests (B1 and B2) were now resistant. Plants of type C were also resistant.

Table 2. Susceptibility of *Cyperus esculentus* to *Puccinia canaliculata* in experiments in 1987.

C. esculentus collection		Number of experiments	Number of treated leaves	Percentage of infected leaves
'leptostachyus'	A1	7	241	43
	A3	1	69	38
	A4	1	78	59
'unidentified'	B1	1	49	0
	B2	1	49	0
'esculentus'	C	1	58	0

Table 3. Susceptibility of different plant species to *Puccinia canaliculata.*

Plant species	Number of plants	Necrosis	Sporulation
Cyperus esculentus 'sativus'	8	±	–
C. albostriatus	8	–	–
C. alternifolius	8	–	–
C. flavescens	11	–	–
C. fuscus	8	+	+
C. rotundus	8	–	–
Carex hirta	7	–	–
Eleocharis palustris	8	–	–
Scirpus maritimus	8	–	–

Susceptibility of non-target plants to P. canaliculata. During the second half of 1987, non-target plants of 9 different species were tested for their susceptibility to *P. canaliculata* (one or two experiments per species). In all the tests, control plants of *C. esculentus* (A1) always showed a susceptible reaction. The results are shown in Table 3. On leaves of *C. esculentus* 'sativus', tiny necrotic spots were produced. These were interpreted as a hypersensitivity reaction. The other species, with the exception of *C. fuscus*, were resistant. On *C. fuscus*, *P. canaliculata* produced very small urediniosori (less then 1 mm in diameter); the sori were surrounded by a zone of necrotic plant tissue.

Discussion

The required quarantaine conditions for *P. canaliculata* restricted the possibilities for the setup of experiments, especially in the phase of optimizing conditions for infection. Due to the size of the climate room, only a very limited number of factors could be varied in one experiment. We recommend that future investigations with exotic plant pathogens are done in their country of origin. Under the experimental conditions that were eventually chosen to test the susceptibility of non-target plants, 100 % of susceptible plants of *C. esculentus* were infected. However, the germination rate of spores, and the number of pustules per plant were quite low (cf. Sutker, 1983). Nevertheless, the experimental results under the given conditions seemed sufficiently reliable to justify conclusions on susceptibility of *C. esculentus* and non-target species to the rust.

It seems likely, that only plants of *C. esculentus* 'leptostachyus' may be controlled by the tested strain of *P. canaliculata*. This fact by itself is no handicap to further study the potential of the rust as a biological control agent, because 'leptostachyus' is one of the most common varieties of *C. esculentus* in the Netherlands. Eventually, other strains of the rust would be needed to control other biotypes of the weed.

From the results on *C. fuscus*, we concluded that the tested strain of *P. canaliculata* has a wider host range than we expected from known data. Until proven otherwise, other related plant species that are native to Europe, are suspected as (potential) host plants.

According to the international recommendations in determining the host range of

exotic plant pathogens (Commonwealth Agricultural Bureaux, 1978), we decided to terminate research on *P. canaliculata* from North-America, and not to ask permission to release it in the Netherlands. This view could change if *P. canaliculata* is identical with *P. romagnoliana* from Europe.

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