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Teleutospores as origin of systemic infection of Cirsium arvense by Puccinia punctiformis

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Abstract

Teleutospores of *Puccinia punctiformis* were used for germination and inoculation experiments. The percentage of germination increased with increasing root extract concentration separately from the spore-suspension present in the same Petri dish.

Volatile substances in root extracts of the thistle plant markedly promoted the germination of the teleutospores.

Inoculation experiments showed that underground parts of plants of *C. arvense* can be infected. The percentage of diseased shoots emerging from inoculated root segments was higher than of those emerging from seeds. Three types of systemically infected shoots could be distinguished.

The development of spermogonia on the diseased shoots indicates that at first the hyphae are haploid, but later on become dikaryotic as shown by the formation of uredosori. Teleutospores are thus responsible for the systemic infection of the plants.

Additional keywords: thistle plant, rust fungus, root extracts, germination, inoculation, local infection.

Introduction

Cirsium arvense (L.) Scop. is a weed spread worldwide (Haggar, 1986; Moore, 1975; Müller, 1976). This thistle can be controlled by systemic herbicides (Müller, 1976). The costs of herbicides and the side effects, especially in natural environments, have promoted interest in considering integrated control techniques (Haggar, 1986; Anonymus, 1978).

Puccinia punctiformis (Str.) Röhl is an autoecious brachy-type rust. Especially spring shoots of *C. arvense* show a systemic infection by hyphae of the rust. The systemically infected shoots are pale and slender and die before flowering. The leaves bear spermogonia and uredosori, which can be observed predominantly in April and May. Later in the season other plants of *C. arvense* are infected by uredospores which leads to formation of localized uredosori on the leaves (local infection). Teleutosori are predominantly formed in autumn.

The role of teleutospores in the life-cycle is discussed by several authors. Menzies (1953) reported that in her experiments only a few teleutospores germinated which hardly lead to infections of thistle plants. She concluded that it is unlikely that the

greater part of the systemically infected shoots will be the result of infection by basidiospores, produced by germinating teleutospores. Buller (1950) proved that infection of seedlings by basidiospores is possible and results in a systemic infection with scattered hyphae, which produce spermogonia. He obtained better results in germination experiments with teleutospores than Menzies did.

Menzies (1953) stated that infection by the uredospores leads to isolated mycelium in the leaves. This mycelium later grows out into the roots and invades, before or after hibernation, the root-buds as they arise.

Turner et al. (1982) obtained a high percentage of germination when teleutospores were kept in the dark for 96 hours at 21 °C by adding root extracts. French and Turner (1984) reported that the stimulus in the root extracts is volatile, fat soluble and physically similar to other rust spore stimulators as nonanal, beta-ionone and 5-methyl-2-hexanone.

Our investigations intended to elucidate the role and function of the teleutospores in the life-cycle of the rust. First we have looked at the germination of the spores and secondly infection experiments were carried out on the underground parts of thistle plants.

Materials and methods

Teleutospores were collected from shoots of *C. arvense* in the grasslands near the river Waal at Nijmegen. The spores were stored in a refrigerator (circa 4 °C) for two months and then kept at room temperature for 4, 6 or 11 weeks. A slide with teleutospores, suspended in distilled water, was placed in a Petri dish together with two other slides, on which 0.5 ml root extract or water as control. On the bottom of the dish some water was added to assure a high humidity. The Petri dishes were kept in the dark for 96 hours at 21 °C (Turner et al., 1982). The percentages of germination were determined after incubation, by examining 250 spores of each slide under a light microscope (Leitz GmbH Wetzlar). A spore was defined as germinated when the length of the germination tube reached half of the smallest diameter of the spore.

The extracts of the roots were prepared by squashing roots in a morter with quartz sand in water. The suspension (0.044 g/l) was centrifuged at 1500 RPM during ten minutes (using a Hetlich AHT 5200 centrifuge). The supernatant was passed through a Büchnerfilter and sterilized by a miliporefilter. The used roots for the three trials of the experiment were obtained from plants respectively 10, 11 and 16 weeks after sowing. These plants were cultivated in pots in a greenhouse.

Teleutospores, stored on dried leaves during two months in the refrigerator (circa 4 °C) and, thereafter, 3-5 months at room temperature (ca 20 °C), were used for inoculating pieces of roots and just germinated seeds of *C. arvense*. It was observed that the teleutospore fraction always contain some uredospores as well. Therefore, we tested the germination capacity of these uredospores before the inoculations were done. However, no germination was observed in all samples tested. The uredospores do not survive the long storage at room temperature.

Roots were taken from thistle plants grown in the greenhouse from seed sown 3 months before the first inoculations. The roots were cut in pieces, 3 cm long, according to Özer (1969). The spores were applied to the root segments with a hairbrush. The root segments were placed in pots (diameter 8 cm) filled with garden soil, 0.5 cm under

the soil surface and kept in the greenhouse.

Three years after collection, seed of *C. arvense* have been laid on wet filter paper to germinate. Just after germination they were inoculated with teleutospores and kept in pots like the root segments.

Each pot was wrapped in a plastic bag to prevent infections with spores from neighbouring plants bearing sori or from elsewhere. Water was added to the plant via the bottom of the pots. During a period of 42 days the pots were examined for the emergence of plants and whether or not they were bearing spermogonia and/or uredosori. After this period the plants became too large and began to show damage, due to growing under plastic coverage.

The greenhouse had a minimum temperature of 16 °C and, during sunny days, a maximum of 25 °C. The experiments were done in threefold; each time using 20 root pieces and 20 just germinated seeds per treatment.

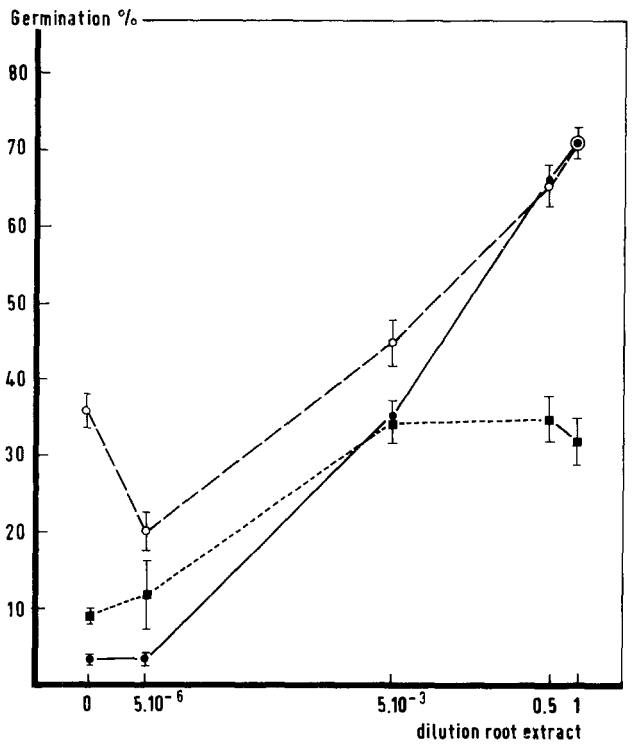

Fig. 1. Germination of teleutospores of *P. punctiformis* in distilled water in presence of different dilutions of root extracts of *C. arvense*; original concentration 44 μg/ml.

Trial	Age (in weeks) of:	
	teleutospores	extracted root segments
1 •———•	12	10
2 o– – – –o	14	11
3 ■--------■	19	16

Results

The results shown in Fig. 1 indicate that spores do not germinate well in water alone; with one exception (trial 2). The percentage of germination becomes higher with increasing concentration of root extract.

Inoculation experiments demonstrated that underground parts of plants of *C. arvense* can be infected (Table 1). The percentage of shoots that were diseased was higher with shoots emerging from root segments than those emerging from seeds. In the first trial with root segments the number of emerging shoots were low, probably caused by inoculation of segments that were too young.

Table 1. Number of diseased shoots after inoculation of root segments and just germinated seeds of *C. arvense* with teleutospores of *P. punctiformis*. In each trial 20 segments of 3 cm or 20 just germinated seeds were inoculated.

Trial number	Age of roots segments at time of inoculation (in months)	Number of pots with shoots		
		total	systemically infected	locally infected
Root segments				
1	3	9	5	1
2	4	20	11	3
3	5	18	14	0
Seeds				
1		20	2	0
2		12	3	3
3		18	4	1

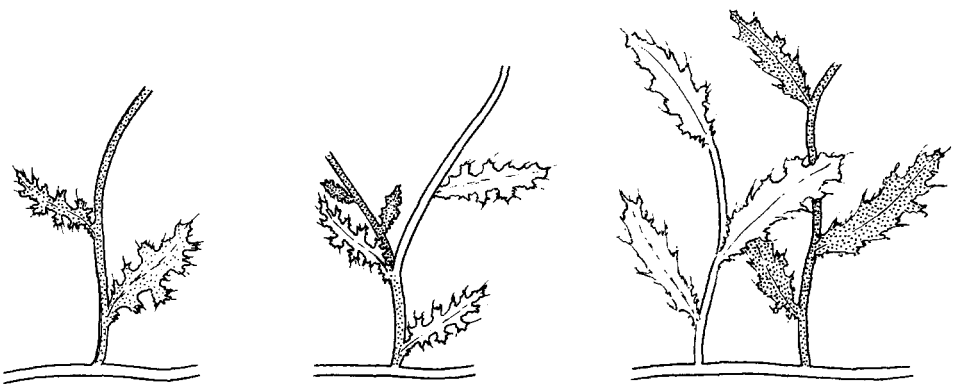

Fig. 2. Three types of systemically infected plants of *C. arvense* as a result of inoculations of root segments by teleutospores of *P. punctiformis*.

Table 2. Three different disease patterns of systemically infected plants (A, B or C according to Fig. 2) after inoculation of root segments and just germinated seed of *C. arvense* by teleutospores of *P. punctiformis*.

Trial number	Number of systemically infected shoots			
	total	type A	type B	type C
Root segments				
1	4	3	0	1
2	13	0	8	5
3	23	0	5	18
Seeds				
1	3	1	2	0
2	2	0	2	0
3	4	0	4	0

Emerged shoots were predominantly systemically and some locally infected. Most shoots which had been systemically infected, first developed spermogonia, later mostly followed by uredosori. Three different types of systemically infected plants can be distinguished (Fig. 2). The frequency of plants with these types is indicated in Table 2.

Discussion

Our experiments have shown that teleutospores can germinate under appropriate conditions. The influence of volatile substances present in root extracts of *C. arvense* is evident. With increasing concentration of the root extracts the percentage of germinated teleutospores is enhanced. In Trial 3 the highest percentage of germination is already reached at a dilution of 0.005 of the original root extract. The reason therefore could be the age of the teleutospores and/or the age of the used roots for preparing the extracts.

Our experiments show that teleutospores, brought on root segments and just germinated seeds, can infect the underground parts of the thistle plant. This leads to three types of systemically infected plants and it has also been shown that there are more infected shoots upon inoculation of root segments compared with inoculation of just germinated seeds.

Because both infected and non-infected shoots emerge from the same inoculated root segment, it is reasonable that the penetration places on the root are restricted to the shoot primordia, which are directly formed on the roots of the thistle plant. From this point of view, it is also clear that more systemically infected shoots are formed from root segments than from seeds, because the number of sites for penetration will be much higher on the former.

The different types of systemically infected plants (Fig. 2) can be explained as follows: type A is a shoot from an infected primordium, type C are two shoots developed at the same time, emerging from an infected and a noninfected primordium and type B is a shoot that has been infected on a later moment in the primordium develop-

ment, so that the hyphae could not follow the growing tip of the young shoot, but reached the side bud primordium on the shoot just in time.

We found spermogonia on the systemically infected shoots, often, but not always, followed by uredosori. The appearance of spermogonia indicates that the hyphae are haploid as could be expected from infection by basidiospores. Dikaryotic hyphae, which form the following stage, the uredosori, could be the result of hyphae receiving spermatia from a different mating type, or of migration of nuclei from hyphae of one mating type to hyphae of the other type (Buller, 1950). Assuming that insects, which were sometimes observed within the plastic bags, wrapped around the developing plants, were responsible for the spread of some spermatia from one mating type to the other, an explanation of our results can be based on both possibilities.

In our opinion, Menzies (1953) based her hypothesis on the fact that she obtained a very low percentage of germination of teleutospores and a very low percentage of infection by inoculating thistle plants with teleutospores. Therefore she assumed that teleutospores probably did not play an important role in the life-cycle of the rust. We proved that teleutospores germinate well, up to 75%, and that the inoculation of the underground parts resulted in systemic infection of about 50% or more of the emerged shoots.

Menzies (1953) obtained both uredosori and spermogonia on plants after inoculation with uredospores. Our experience is that in uredosori always some teleutospores are formed. It is possible that Menzies has used inoculum, which contained some teleutospores. So the haploid hyphae, bearing the obseved spermogonia, could be a result of teleutospores and then the segregation of nuclei, assumed by Menzies (1953), is not necessary. It is also questionable whether hyphae normally restricted to local growth in the leaves, will pass into the roots.

From our results it seems more appropriate that haploid systemic mycelium is formed after infection with basidiospores, formed by germinating teleutospores, and not after infection with uredospores followed by segregation of nuclei, as stated by Menzies (1953).

We have shown that a high rate of systemically infected plants can be obtained in greenhouse experiments. In the field, however, this rate is low, as we observed, because the spores have to reach the underground parts of the plants. If it is possible to increase the amount of teleutospores/basidiospores which reach the underground parts, the use of these spores will by a useful tool for biological control of *C. arvense*. Investigations on an appropriate application are needed.

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Samenvatting

Teleutosporen van Puccinia punctiformis: bron van systemische infectie van Cirsium arvense

Teleutosporen van *Puccinia punctiformis* zijn gebruikt voor kiemings- en incubatieproeven.

Vluchtige substanties, aanwezig in wortelextracten van de akkerdistel, hebben een grote invloed op de kieming van teleutosporen. Het percentage kiemende teleutosporen nam toe bij toenemende concentraties van het wortelextract, aanwezig in dezelfde petrischaal als de sporensuspensie.

Inoculatieproeven toonden aan dat ondergrondse plantedelen van *C. arvense* kunnen worden geïnfecteerd. Het percentage aangetaste scheuten opkomend uit wortelsegmenten was groter dan dat van scheuten verkregen uit zaden. Behalve systemisch geïnfecteerde scheuten werden ook enkele lokaal geïnfecteerde scheuten aangetroffen. De systemisch geïnfecteerde planten konden worden onderscheiden in drie verschillende types.

De ontwikkeling van spermogonia op de systemisch aangetaste scheuten wijst er op dat het aanwezige mycelium haploid is, zoals ook te verwachten is bij infecties met basidiosporen. Pas later worden de hyphen dikaryotisch, zoals blijkt uit de vorming van uredosori.

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