Effect of Host Plant Materials and Temperature on Germination of Teliospores of Puccinia carthami

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ABSTRACT

Teliospores of *Puccinia carthami* germinated poorly or not at all in water, on water agar, or on various agar media. Germination was stimulated on water agar supplemented with materials derived from leaves, stems, and roots of safflower. Germination of teliospores of collections C1 and C2 was greatly stimulated on safflower extract agar. C2 teliospores received some stimulation from medium containing extracts from nonhost plants, but C1 spores were not stimulated. Teliospores of the two collections germinated best at 18-24 C with optimum for germination at about 24 C, but differed in amount and rate of germination. The different germination traits recurred among teliospores from re-established infections on safflower.

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Teliospores of the autoecious, macrocyclic rust *Puccinia carthami* Cda. are seed-borne and soil-borne, on, or separated from, debris of infected safflower (*Carthamus tinctorius* L.). Teliospores produce basidiospores at germination which infect emerging safflower seedlings. Seedling infection has been demonstrated by the placing of rusted safflower debris bearing teliospores in the soil (4, 6), or by the use of infested seed (1) or other methods (3, 9).

Teliospore germination in laboratory studies suggests that germination is inconsistent and may be influenced by viability, dormancy, and temperature (4). Teliospores which germinated poorly in water appeared to germinate better in soil, as indicated by subsequent rust development on seedlings at 15, 20, and 25 C (6). It was suggested that soil constituents or the host might affect germination. Teliospores from rusted leaves soaked in water overnight germinated best at 12-18 C, but did not germinate at 7-8 C and 26 C or above (4). However, teliospores germinated at lower temperatures, as seedlings became infected in soil at 5 C (1).

Laboratory techniques used for germinating teliospores involve mostly incubation of the spores in water or on water agar medium with various modifications in incubation treatment (2, 4, 6, 11). The objective of this study was to develop a method to induce germination of dormant teliospores on agar medium, and to determine the effects of host plant materials, and temperature and time on germination of teliospores.

MATERIALS AND METHODS.–Dry, rusted

leaves were collected from safflower plants in two separate areas of the Sacramento Valley during August 1970. Teliospore suspensions were obtained by homogenizing the leaves in a blender in sterile tap water for 30-50 sec and straining the homogenate with cheesecloth. After each of two centrifugations of the spore suspension in a clinical centrifuge, the spore mass was resuspended in sterile tap water. Following the third centrifugation, the water was decanted and the spores were spread on paper to dry for 30 min at 42 C, 30 min at 36 C, and 12 hr at 30 C. Dried spores were stored in glass petri dishes at 23-24 C. The two collections of teliospores were stored separately, and labeled C1 and C2 for identification in experiments. By methods previously described (8, 10), C1 and C2 teliospores were each found to represent a distinct race.

Teliospores were germinated on 15 ml agar media in plastic petri dishes. Spores were dispersed singly with few spore clumps, by use of an atomizer. Dishes of seeded media were incubated at 24 C, and received a 10-hr photoperiod daily with 25 ft-c white fluorescent light. Effect of temperature was studied in controlled temperature incubators. One hundred single spores were scored in a single sweep across the diameter of a dish. Each treatment was repeated 2 or more times. Germination data are recorded as percentage germination, and represent total spores germinated from one or both cells by production of a four-celled promycelium. Germination tests were run during a 9-month period after spore harvest.

The effect of plant materials on germination was studied on water agar. Water agar in dishes was flooded with water solutions of root exudates from safflower or nonhost seedlings cultured in sterile distilled water for 7 days, and with water solutions of materials leached from autoclaved leaves and stems of dead safflower, steeped in sterile distilled water for 1 hr. Water was decanted after 1 hr, and spores were seeded on the agar. Spores were dispersed on agar in which fragments of autoclaved safflower stems and leaves were embedded, and on agar from which roots of live seedlings were removed.

Safflower and nonhost plant extracts for agar media were prepared by boiling 200 g of stems of 6-week-old plants in 1 liter of tap water for 20 min. Sugars or other known nutrients were not added to the media. The pH of the media was 6.8-7.0.

RESULTS.—*Germinability of teliospores in soil.*—Safflower seeds were planted in autoclaved soils infested with rusted safflower leaves bearing C1 or C2 teliospores. Abundant rust pustules developed on cotyledons and hypocotyls of safflower seedlings at 12, 18, and 24 C, but not at 30 C.

Germination in liquid or solid media.—Twenty-five to 30% of C2 teliospores germinated within 72 hr in sterile tap water, in 0.1 M sucrose solution, or sterile soil extract. Germination was inconsistent between submerged spores and spores floating on the surface. Submerged spores germinated very poorly. Germination tests did not yield satisfactory data, because clumping of floating spores occurred, and sucrose and soil solutions became contaminated.

TABLE 1. Effect of safflower plant materials on germination of *Puccinia carthami* teliospores from two collections (C1, C2) at 24 C

Media	Germination within 72 hr	
	C1[a]	C2
	%	%
Water agar	0	13
Water agar + root exudates in water	15	52
Water agar + leaf and stem fragments	29	70
Water agar + root exudates in agar	34	56
Water agar + substances leached from dead leaves in water		37
Water agar + substances leached from dead stems in water		48

[a]Effect of substances leached from leaves and stems on C1 teliospores was not determined.

Less than 1% of C2 teliospores germinated on potato-dextrose agar, cornmeal agar, or lima bean agar. Germination on Bacto peptone and proteose peptone agar was comparable to that obtained on water agar (15% in 72 hr and 20% in 7 days). The C1 teliospores did not germinate on any of the liquid or solid media.

Effect of safflower plant materials on germination.—Teliospore germination was highly stimulated by materials derived from safflower roots, stems, and leaves (Table 1). Germination of C1 teliospores, which heretofore had not germinated on different media, was induced. Germination was not stimulated by exudates from roots of nonhost plants.

Germination tests on safflower extract agar consistently yielded high percentages (70-90% in 4-7 days) of germinated spores. Extracts from different safflower cultivars were equally effective in stimulating germination.

Germination of C2 spores on most nonhost extract media was comparable to or better than that on water agar within 96 hr (Table 2). Whereas C2

TABLE 2. Germination of *Puccinia carthami* teliospores on water agar containing host and nonhost extracts

Extract source plant	% Germination of C2 teliospores[a] at 24 C	
	72 hr	96 hr
Carthamus tinctorius L.	50	74
Zea mays L.	26	29
Helianthus annuus L.	24	30
Gossypium hirsutum L.	18	18
Sesamum indicum L.	13	17
Pisum sativum L.	11	14
Vigna sinensis (Torner) Savi	3	5
Water agar	13	16

[a]C1 and C2 are different teliospore collections. Thirty per cent of C1 teliospores germinated within 72 hr on safflower extract agar, but none germinated on other plant extract agars within 96 hr.

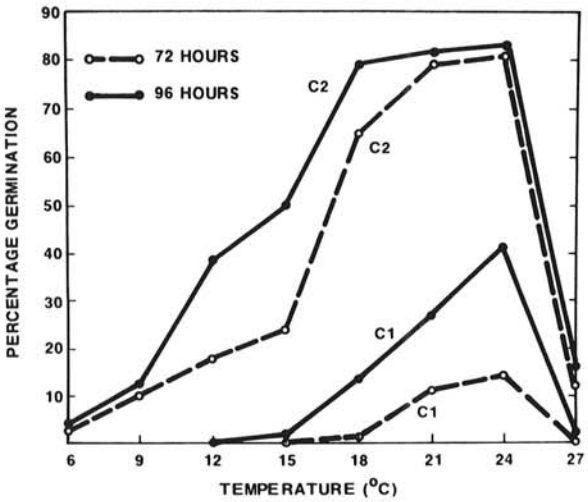

Fig. 1. Effect of time and temperature on germination of teliospores of two races of *Puccinia carthami* on safflower extract agar.

spores continued to germinate on safflower extract agar, little or no change occurred in total germination on nonhost agars after 96 hr. C1 spores were not stimulated by nonhost extracts.

Unwashed C1 and C2 spores, from field collections and re-established infections in the greenhouse, germinated as well as did washed spores on safflower and water agars.

Effect of temperature and time on germination.—Teliospores were incubated at 3-C intervals from 6-30 C on safflower extract medium. Maximum germination of C1 and C2 teliospores occurred at 24 C (Fig. 1). The rate of germination was greatest at 18-24 C; however, with time, germination at lower temperatures approximated total germination at 18-24 C. The C2 teliospores began to germinate within 24 hr at 9-27 C and within 48 hr at 6 C. The C1 teliospores did not germinate during the first 48 hr. Some C1 teliospores germinated at 6-12 C within 7 days. Teliospores of neither collection germinated at 30 C. The same differences in germination of C1 and C2 teliospores (Fig. 1) were obtained with successive generations of spores from re-established infections.

DISCUSSION.—Data presented show that germination of teliospores of *P. carthami* is stimulated by materials from the host plant. Spores of other plant pathogenic fungi are stimulated by plant extracts and diffusates (7) and root exudates (5). Teliospores may require stimulatory substances for germination in nature. It appears that safflower debris may be a factor affecting teliospore germination in nature when moisture and temperature are favorable, notwithstanding the fact that other stimulatory or inhibitory materials exist in the soil which may also affect germination. Stimulation of spore germination in the absence of live safflower plants would effect a reduction in inoculum potential.

Teliospores formed in nature remain in a dormant state, at least until moisture is supplied by seasonal rains. Some required a resting period of 5 or 6 months (4). That germination of C1 and C2 spores was induced soon after harvest indicates that a long period of dormancy may not be required if environmental conditions are favorable for germination.

Data on germination of C1 and C2 teliospores suggest that races may have different germination requirements. Thus, utilization of teliospores of different races may account for discrepancies in germination data at different temperatures (1, 4). Similar differences which recur among successive generations of C1 and C2 teliospores suggest that germinability may be affected by inheritance.

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