Factors Affecting Germination of Teliospores of *Puccinia obtegens*

S. K. TURNER, Former Research Assistant, A. KWIATKOWSKI, Exchange Scientist from Poland, P. K. FAY, Associate Professor of Agronomy, Department of Plant and Soil Science, and D. C. SANDS, Associate Professor, Department of Plant Pathology, Montana State University, Bozeman 59717

ABSTRACT

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Extracts of Canada thistle (*Cirsium arvense*) roots stimulated germination when flooded over teliospores on water agar. Maximum percent germination was obtained in darkness at 21 C. Percent germination was greater after 6 than after 3 mo of storage at 4 C and varied with collection dates (no germination before 15 August) and locations.

Additional key words: biological weed control, *Puccinia punctiformis*

Puccinia obtegens (Link) Tul. (2), an autoecious, host-specific rust on *Cirsium arvense* (L.) Scop. (Canada thistle), is a potential biological control agent for this weed (13). *C. arvense* plants are stressed by systemic infection that may occur after infection by uredospores or basidiospores (5,12). Systemically infected shoots usually die by August in Montana.

Germination of teliospores is poor and erratic; therefore, they have not been used effectively as inoculum for Canada thistle. Germination of teliospores of *P. obtegens* is inconsistent (5,12), resulting in infrequent pycnia. The importance of teliospores, the overwintering spore stage in the rust's life cycle, is unclear because the fungus also overwinters as mycelium in the host's root system.

Teliospore dormancy has been reported for *P. graminis* var. *tritici* and *P. carthami* (7,9). Buller (5) observed germination of *P. obtegens* teliospores after refrigerated storage. Menzies (12) reported 1% germination of *P. obtegens* teliospores that were stored over the winter at 5 C, then kept moist for 6 wk at room temperature. Cotter (7) found that environmental conditions such as freezing and alternate wetting and drying stimulated germination of dormant teliospores of *P. graminis* var. *tritici*.

Certain volatile compounds stimulated (3,8) or inhibited (1) urediospore and teliospore germination. Nonhost and host plant exudates have stimulated teliospore germination of *P. carthami* (9). Klisiewicz (9–11) showed that germination of *P. carthami* teliospores was stimulated by host plant exudates flooded on water agar.

The objective of this research was to determine if *P. obtegens* teliospores could be stimulated to germinate so they could be used to inoculate Canada thistle.

MATERIALS AND METHODS

Canada thistle tissue containing teliospores of *P. obtegens* was collected during the summer and fall of 1980 from 19 locations in Montana. Plant material containing teliospores was also collected from one site at Bozeman over a period of 3 mo. All plant material was air-dried and stored at room temperature until October 1981, then refrigerated at 4 C.

Teliospores were obtained by comminuting dried Canada thistle leaves (0.5–2 g) in 100 ml of sterile distilled water (SDW) in a Waring Blendor for 1 min. The suspension was strained through four layers of cheesecloth and centrifuged at 10,000 *g* for 5 min. The supernatant suspension was removed immediately or after 12 hr, and teliospores were used for germination tests at both times. Germination tests were performed in 9-cm plastic petri plates containing 10 ml of 2% Bacto water agar on which teliospores were spread with a camel's-hair brush.

Canada thistle extract was prepared by comminuting 10–20 g of fresh roots, stems, or leaves in 100 ml of SDW for 1 min. The suspension was strained through four layers of cheesecloth. In germination tests, the extract was atomized uniformly on the agar medium bearing teliospores until the surface was wet. Controls were atomized with distilled water.

In germination tests, teliospores were incubated at 21 C in a controlled-temperature chamber for 96 hr unless otherwise stated. Three replicate plates were used for each germination treatment. Percent germination was recorded by observing 250 teliospores (50 in each of five microscope fields at ×100) per plate. Germination from one or both teliospore cells was recorded as one germinated spore.

Spores collected at Bozeman on 18 and 30 September 1980 were tested at 12, 15, 18, and 21 C. Separate extracts from root, stem, and leaf tissue were prepared and tested as described. The experiment was conducted twice. Germination was assessed after 72 and 96 hr of incubation.

Teliospore collections from different locations were tested for germination. Each spore lot was treated with host root extract and tested two to five times depending on the number of teliospores available. To determine the effect of light on germination, teliospores were exposed daily to white fluorescent light for 12 hr versus complete darkness. All plates contained teliospores brushed onto the medium saturated either with host root extract or water (for controls). Plates were incubated at 21 C and observed for germination after 48, 72, and 96 hr.

Six teliospore collections from Bozeman from 29 July to 21 October 1980 were tested for germination in February 1981. Leaves with teliospores from each collection were homogenized separately and left in the homogenate for 12 hr before they were placed on water agar, saturated with host root extract, and incubated for 96 hr at 21 C with 12 hr light daily.

Teliospore collections from Hilger, Lewistown, Winifred, and Bozeman, MT, were tested for germination after 3–6 mo of storage at 4 C. Teliospores soaked in homogenate for 12 hr were placed on water agar, saturated with root extract, and incubated at 21 C for 96 hr in darkness.

RESULTS

Root and stem extracts of Canada thistle stimulated teliospore germination. Teliospore germination increased over that of the control when spores were treated with root or stem extract at incubation temperatures of 15, 18, and 21 C. The highest germination percentage in all treatments occurred at 21 C. Germination percentages were significantly different between the control and root extract-treated teliospores. Germination percentages increased from 3.7 (untreated spores) to 32% at 18 C and from 7.6 to 44% at 21 C. Water extracts of stem tissue were less stimulatory than root extracts. Germination at 18 and 21 C

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increased from 3.7 and 7.6% for the control to 11.9 and 14.1% for teliospores treated with stem tissue extracts, respectively. Leaf extracts did not stimulate teliospore germination.

In a separate experiment, teliospore germination was influenced by temperature. Teliospores treated with root extracts were incubated for 96 hr in darkness at temperatures of 12–24 C, which increased at three-degree intervals. Germination occurred at all temperatures tested; however, the highest germination percentages, 21.2 and 28.9, occurred at 18 and 21 C, respectively. Germination increased from 0.9% at 12 C to 12.4% at 15 C. Germination declined from 28.9 at 21 C to 0.8% at 24 C.

Teliospores under a 12-hr photoperiod showed significantly lower germination than those incubated in complete darkness. Germination increased from 19.7% in light to 32.4% in complete darkness.

Teliospore germination percentages varied among spore lots collected at different sites and dates. Plant tissue containing telia was collected from nine widely separated locations from 5 June to 18 September 1980. Percent germination ranged from 0 to 38, with germination occurring only in samples collected on 15 August or later. A second study was initiated to determine the effect of collection date. Germination of teliospores from systemically infected Canada thistle leaves collected at the same location six times from 29 July to 21 October 1980 ranged from 0 to 36%. The highest germination percentages occurred when plant tissue was collected in September and October.

Percent germination of teliospores from four locations was greater after 6 than after 3 mo of storage at 4 C (Table 1). Germination also increased in samples from the other three locations after 6 mo of cold storage.

Table 1. Percent germination of *Puccinia obtegens* teliospores collected at four locations in Montana in 1980 and stored at 4 C for 3 or 6 mo

Months of cold storage	Percent germination[a,b] per collection location			
	Hilger	Lewistown	Winifred	Bozeman
3	3.50	0.10	2.10	2.30
6	14.10	8.80	16.40	63.30
LSD 0.05	4.12	2.12	3.41	6.62

[a] Mean value of three replicates.
[b] Teliospores were allowed to germinate on water agar containing root extracts of Canada thistle for 96 hr at 21 C without light.

DISCUSSION

Germination of teliospores of *P. obtegens*, as with *P. carthami* (9), was stimulated by host plant extracts. Klisiewicz (10) demonstrated that a stimulatory substance in safflower extract was volatile.

Temperature influenced teliospore germination. The optimal temperature range for germination (18 to 21 C) occurs in soil during late spring and summer within the geographical distribution of Canada thistle and *P. obtegens* in North America (6). The optimal temperature for germination of wheat stem rust races differs (8). Further testing of *P. obtegens* teliospores might disclose races that would germinate at lower soil temperatures. Germination at lower soil temperatures presumably would initiate infection earlier in the growing season, which would be more conducive to widespread infection.

Percent germination was higher when spores were incubated in complete darkness than when subjected to light. Teliospores of *P. obtegens* are normally borne on plant debris that becomes soilborne, providing conditions more conducive for germination of the spores. Incorporation of teliospore inoculum into soil would enhance germination.

Because most of the teliospore samples collected from separate locations did not germinate, environmental conditions during development might influence germinability. Collection date may also be an important factor. Teliospore germination percentages increased when spores were collected later in the growing season. Waters (14) found that low light intensity and/or nutrient stress induced formation of *P. obtegens* teliospores. Spore immaturity may explain lower germination percentages of teliospores from samples collected early in the growing season.

P. obtegens teliospores can be used as inoculum for biological control of *C. arvense* if the following factors are considered: 1) Collection should be done in September and October after a period without adverse weather conditions such as rain or snow. 2) Root extracts of the host plant ensure maximum germination.

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