

## Attempts at Germination of Teliospores of *Puccinia coronata* var. *avenae*

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The failure of teliospores of the cereal rust fungi to germinate readily is a major obstacle in the genetic study of pathogenicity. To determine the inheritance and relationships of pathogenicity characteristics it is necessary to obtain the sexual stage of pure cultures grown free from contamination. To establish the sexual stage an efficient method of inducing germination of teliospores produced in isolation is needed. We attempted to stimulate germination of greenhouse-produced and field-collected teliospores of the oat crown rust fungus, *Puccinia coronata* Cda. var. *avenae* Fras. & Led. A preliminary report has been made (17).

Previous studies on inducing germination of dormant teliospores have been inconclusive or contradictory. Dormancy of teliospores of several of the cereal rust fungi was shortened by alternate periods of freezing and thawing or of wetting and drying (1,5,6,8,9,10,14). On the contrary, Hoerner (3,4), and Vakili (16) did not obtain germination of teliospores of *P. coronata* and *P. recondita*, respectively, except when naturally overwintered. Theil and Weiss (15) were unable to shorten dormancy of *P. graminis* teliospores by alternate freezing and thawing. Lambert (7) obtained but limited germination by thorough wetting followed by freezing. Theil and Weiss (15) shortened dormancy by treatment with dilute citric acid solution, but Lambert (7) was unable to repeat this. Lambert (7) also tested dilute solutions of benzaldehyde, salicylaldehyde, citraldehyde, several alcohols, the 16 essential oils used by Noble (13) in his attempts to induce germination of *Urocystis tritici*, ethylene gas and chlorohydrin which Denny (2) found effective in stimulating potato germination, straw extracts, and various concentrations of nitrate and other salts ranging in pH from 5.0 to 8.2. All of these were of little value in shortening the dormancy period.

### Materials and Methods

Numerous teliospore collections of *P. coronata* races 202, 203, 294, 295, and two pathogenically distinct cultures of race 293 designated A and B were studied. Each collection was dried at room temperature for 4 days, placed individually in a small cloth bag, and stored at 34° F.

**PHYSICAL STIMULATION**—One collection each of races 202, 203, 293B, and 295 was subjected to alternate periods of wetting and drying each day for 20 days followed by alternate periods of freezing (—5°F) and thawing (room temperature) at 5 day intervals for 40 days. Upon each transfer from the cold, a sample of teliospores was removed and tested for germination. In a second experiment, telial material of the same races was soaked overnight and frozen in ice at —20° F for periods up to 70 days, a technique similar to that of Johnson and Newton (6). Samples were removed at approximately 10 day intervals and tested for germination. In a third experiment, telia of races 202, 203, 294, and 295 were alternately soaked in running tap water and dried at approximately 12 hour intervals for 30 days. The telia were then alternately frozen and thawed at 5 day

intervals for 60 days. Samples were removed periodically and tested for germination. In a fourth experiment, telia were individually removed from the leaves, placed under a cover slip on a glass slide and gently crushed. When sufficient spores were obtained they were placed on a 2 x 2 glass slide and incubated in a petri dish and filter paper moist chamber for 96 hours. The slides with spores were removed at approximately 12 hour intervals and examined for germination. Field collected telia were not available at the same time as these experiments but were also treated in a similar manner upon collection.

Greenhouse-produced telia of all cultures, as well as telia collected in the field, were placed in small cloth bags and overwintered outdoors on the ground from October 25 to May 26. This material was tested for germination at 10 day intervals from March 20 to May 26. Telia produced outdoors on oat plants and overwintered thereon, both on standing and prostrate culms, were collected and tested for germination at the same times.

CHEMICAL STIMULATION—Telial material of race 295 was treated with 0.5% sodium hypochlorite for 30 seconds, divided into equal parts, and submerged in either a 1, 2, or 3% solution of citric or lactic acid for 1 to 60 minutes, following the approach of Theil and Weiss (15). The telia were then stored in small vials, removed at the end of 6, 10, 17, 23, 30, 34, and 40 days, and tested for germination.

Field-collected telia were treated with a 1% solution of citric acid for 3 minutes, divided into equal lots, and treated with either a  $10^{-3}$  or  $10^{-5}$  solution of the following chemicals: L-arginine monohydrochloride, citric acid, 2-4 dinitrophenol, ferric citrate, furfural, glutathione, indoleacetic acid, maleic hydrazide, L-naphthaline acetic acid, phosphoglyceric acid, and thiourea. Treatment was facilitated by evacuating the atmosphere surrounding the treating solution to 0.05 mm of mercury for 30 minutes following submersion of the telial specimens. They were removed and the teliospores observed for germination at 3 subsequent 12-hour intervals. After 36 hours the telia were placed in the freezing compartment of a standard refrigerator, removed at 5 day intervals, and tested for germination.

GERMINATION TESTS—These were conducted by placing telial material in moist chambers constructed from petri dishes and filter paper. Subsequently, telia were removed individually, placed on a microscope slide, gently crushed to separate the spores, and observed microscopically for germination. After the initial observation the slides were returned to moist chambers and re-examined 12 and 36 hours later. In some instances, to detect germination occurring at a low frequency, telial material was suspended in moist chambers for 16 to 24 hours directly above leaves of *Rhamnus cathartica* seedlings. Ten days later the seedling leaves were examined microscopically for sporidial infection.

### Experimental Results

Attempts to shorten or break the dormancy of teliospores of *P. coronata* var. *avenae* by alternate wetting and drying, freezing and thawing, leaching with water, mechanical separation, overwintering out of doors, and combinations of some of these failed. However, teliospores produced

in the field during the autumn of 1958 and naturally overwintered on the plants, germinated readily the following spring.

Treatment of greenhouse and field-collected teliospores with 2 or more concentrations of 13 chemicals chosen for special characteristics failed to break dormancy.

#### Discussion

In nature, teliospores of *P. coronata* germinate after overwintering. The physiological or morphological modifications which render the spores germinable are not known. The climatic conditions during natural overwintering may activate enzymatic systems in the spore, thus breaking dormancy. The mechanical action of freezing and thawing may modify the cell wall of the spore and thus increase permeability. Wetting of telia-bearing plant material may release degradation products which physiologically or morphologically modify the spores, thus rendering them germinable.

The inability to induce germination of greenhouse and field-collected teliospores, regardless of the stimulation, in comparison with success obtained in germination of teliospores naturally produced and overwintered suggests that natural conditions were not simulated closely enough in the experiments to influence key processes involved in germinability.

#### Summary

Attempts to break the dormancy of *P. coronata* var. *avenae* teliospores by several physical and chemical means and make them germinable failed. Teliospores produced on oats outdoors and overwintered thereon prior to collection germinated readily, indicating that the treatments did not influence key processes to the same degree as do natural conditions which break the dormancy period of teliospores.

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