

Completing the Life Cycle of *Uromyces phaseoli* var. *typica* on Bean Plants

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We wish to thank D. J. Hagedorn, R. W. Saettler, and J. R. Venette for bean rust fungus cultures and Isaac Wahl for advice.

Scientific Journal Series Paper 10155, Minnesota Agricultural Experiment Station, St. Paul, MN 55108.

Accepted for publication 19 May 1978.

ABSTRACT

GROTH, J. V., and B. D. MOGEN. 1978. Completing the life cycle of *Uromyces phaseoli* var. *typica* on bean plants. *Phytopathology* 68: 1674-1677.

A technique was developed for germinating teliospores of the bean rust fungus, (*Uromyces phaseoli* var. *typica*), which allowed all five spore stages to develop on bean plants. Teliospores germinated on water-agar plates, beginning about 3-4 wk after a brief washing. Pycnia were obtained by inverting those plates over young bean plants in a

greenhouse. Pycniospores were transferred between pycnia with sterile toothpicks. Aeciospores were collected, and host leaves were inoculated and incubated using the same methods as were used for urediospores. The technique has worked with many collections of the bean rust fungus that produced telia readily.

The bean (*Phaseolus vulgaris* L.)-bean rust fungus [*Uromyces phaseoli* (Pers.) Wint. var. *typica* Arth.] host-parasite system is being widely used in a variety of fundamental studies of rust fungi. The system is attractive for quantitative studies of rust reactions because large, uniform leaf surfaces are readily obtained and maintained. In other respects, the pathogen is as easy to work with as other rust fungi. A major limitation of the bean rust fungus for some types of studies has been the lack of a technique for completing the life cycle. Genetic studies of the pathogen, in particular, have been precluded. Earlier workers have reported pycnia and aecia of this macrocyclic, autoecious rust in the field and in the greenhouse (7, 8, 9, 11). The first report was by Andrus (1) who obtained all spore stages in a greenhouse, but who did not present information on how the teliospores can be induced to germinate, or how the rest of the life cycle can be completed. Harter et al. (7) germinated teliospores in water on glass slides, but they did not infect plants with basidiospores. Freshly harvested teliospores of a similar species, *Uromyces fabae*, will germinate after 6-7 days of incubation at 16 C (10). This paper describes a simple and effective series of procedures that can be used in crossing rust races or for obtaining the various stages of those rust genotypes that produce telia.

MATERIALS AND METHODS

The bean cultivars used were the fully susceptible Pinto 114 and Pinto 166. Numerous rust collections from dry edible beans grown in Minnesota, North Dakota, and Michigan were tested. Most of these gave virulence levels on 44 bean cultivars similar to those previously reported (5), and all produced telia readily. In contrast, culture

W73 of the pathogen (6) grown on cultivars Bountiful or Topcrop never was observed to produce telia, and could not be used. Such cultures are not unusual (7). Inoculation was with urediospores as reported earlier (5). Uredia, and all other spore stages, developed on plants kept under high-output fluorescent lamps. In the greenhouse, telia formed around uredia at all experimental temperatures except those that were high enough (35 C and above) to kill the uredia. Telia became noticeable from 2-3 wk after inoculation. Telia formed earlier at 21 C than at 24 C, but total amounts of teliospores did not seem to be affected by those temperatures. This effect of temperature on timing of appearance of teliospores was shown earlier (12). Longevity of primary bean leaves could be increased considerably by removing the apical meristem of the plant. Leaves were harvested just prior to senescence, usually about 4 wk after inoculation. Then they were washed in running cold water for 3 days to remove possible inhibitors and to reduce populations of contaminant organisms. Teliospores were removed from the leaves either by scraping them with a spatula or by gently rubbing them while they were under water. Spores were concentrated by allowing them to settle after which the water was decanted. Concentrations of up to 2×10^6 spores/ml were thus obtained. We did not attempt to store teliospores after finding that freezing spore suspensions greatly reduced their germinability on water agar. Approximately 10 drops of such spore suspensions were placed on the surfaces of double glass-distilled water agar (2%) in petri plates, and the suspension was spread evenly over the area of the plate. These plates were then stored at room temperature (24 C) for several weeks. Urediospores, which also were present, quickly germinated and decomposed after about 2 wk.

When teliospores had begun to germinate, the plates containing teliospores were inverted over recently emerged bean seedlings in the greenhouse. Glass or

transparent plastic cylinders 15 or 30 cm high and 10 cm in diameter were used both to hold the plates in place over the plants and to ensure high relative humidity on the leaves. Plates remained over the plants for 2-14 days. Plants were removed from the cylinders and placed over moist sand in the shade at 22-26 C for 1-2 days to prevent leaves from being damaged or killed by desiccation. Pycnia began to appear as chlorotic flecks only on upper leaf surfaces about 10 days after teliospore plates were first suspended. Numbers of pycnia increased afterward, probably as a result of basidiospores produced later. Pycnia were formed at temperatures of from 22 C to about 26 C, and when pots were placed in both shady and sunny locations. Higher temperatures seemed to be detrimental to pycnium formation, so that shade, where temperatures within the cylinders remain closer to ambient, was preferable. Otherwise, rigorous control of

environmental conditions was not essential to basidiospore and pycnia formation. About 1 wk after a pycnium had first appeared, cloudy white nectar containing pycniospores became noticeable in its center. Pycniospores were transferred from one pycnium to another with sterile wooden toothpicks. Assuming that the two mating types occurred in equal frequency, we transferred pycniospores from seven donor pycnia to each receptor pycnium, ensuring that the opposite mating-type was obtained with over 99% confidence. To prevent insects from fertilizing pycnia, plants were moved into insect-excluding cages after pycnia first appeared. Aecia formed directly beneath the fertilized pycnia about 9-12 days after fertilization at temperatures of 22-26 C and low- or intermediate relative humidities. The aecial peridia ruptured at approximately 1 wk, or later, subsequent to appearance of the aecia. Aeciospores were

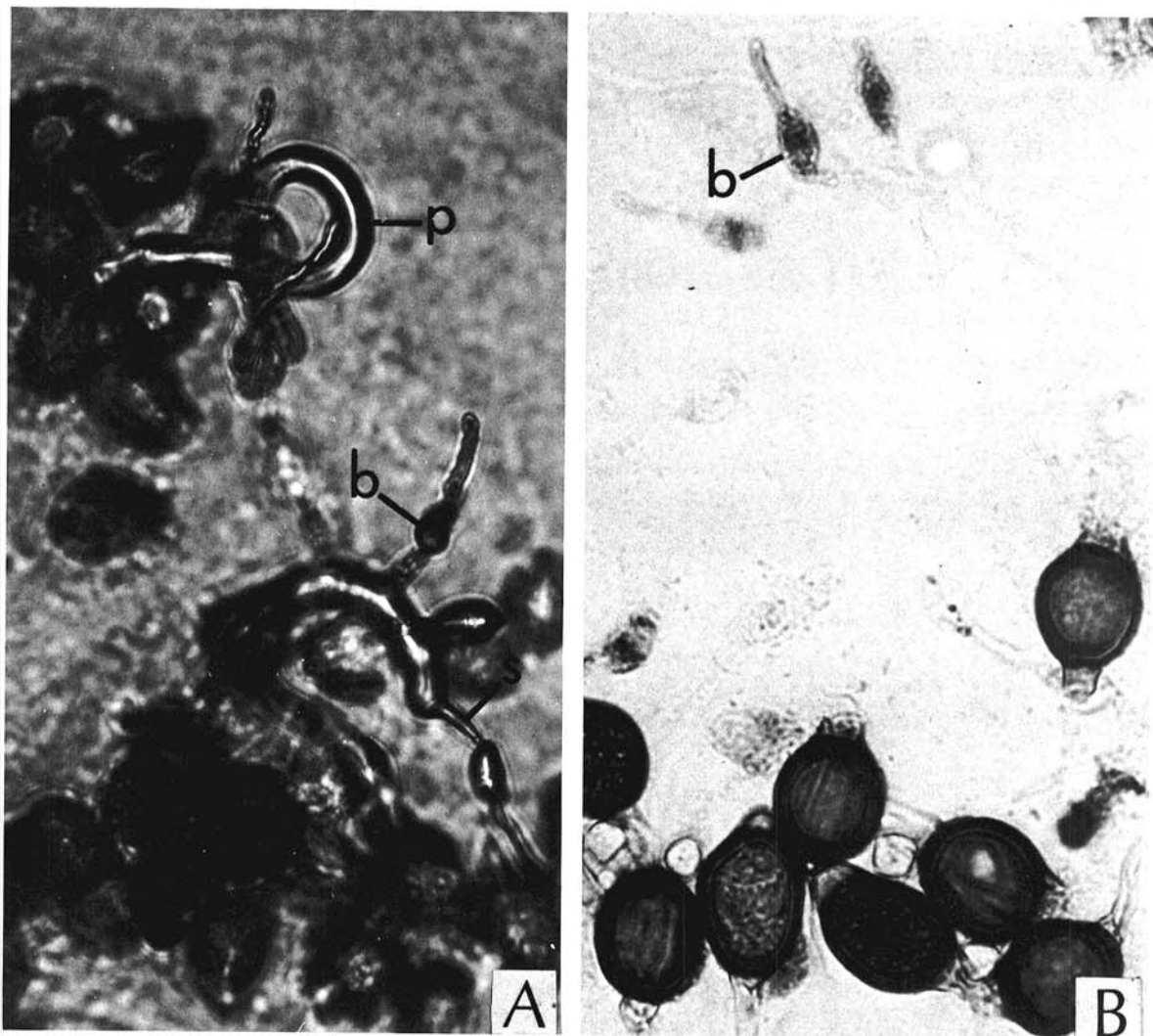


Fig. 1-(A, B). Teliospores of *Uromyces phaseoli* var. *typica* the causal agent of bean rust A) promycelia (p), sterigmata (s) and basidiospores (b) of two germinating teliospores. Note that basidiospores have produced germ tubes. $\times 500$. B) Germinated and non-germinated teliospores on a water agar plate which had been inverted over a bean plant for two wk. Note basidiospores (b), and granular cytoplasm as well as intact apex of nongerminated teliospores. $\times 600$.

collected with a cyclone spore collector into a No. 00 gelatin capsule, and were immediately sprayed as an oil suspension (5) onto healthy bean leaves. No attempt was made to store aeciospores for more than a few days. Inoculated plants were placed in a saturated environment for 18-24 hr at 23 C. Resulting uredia formed 8-10 days later in the greenhouse at 23 C.

RESULTS AND DISCUSSION

As with other rust fungi, the main obstacle to completing the life cycle of the bean rust fungus has been to obtain germination of teliospores. Initial results using established techniques of alternatively washing and drying and freezing and thawing telia on leaves to break teliospore dormancy (4) were unsuccessful. Dinoor (2) found that with *Puccinia coronata* var. *avenae*, such treatment usually was not necessary if telia came from green leaves.

We have obtained teliospore germination in all of many trials (Fig. 1-A). We have used 10 distinct collections representing both single-pustule and mass collections, as well as mixed teliospores that were available from other studies. Teliospores have germinated at all seasons.

A number of variations on the technique were tried, but none had a noticeable effect on amount of germination of teliospores; eg, washing the leaves as little as 3 hr or as much as 8 days, storing newly seeded plates for 1 wk in the refrigerator, adding one drop of a 50 $\mu\text{g}/\text{ml}$ tetracycline suspension to 5 ml of teliospore suspension, and using

telia from field collections and from greenhouse-grown dried bean leaves. Our results do not support an earlier (7) contention that greenhouse-grown teliospores cannot be used to complete the life cycle. In all trials, acceptable, albeit variable, levels of germination were obtained. Usually germination began at about 2-3 wk (rarely at 1 wk) after plating, and continued at low rates (<1% at any single examination for at least 1 mo). At the end of this time, 10-49% of the teliospores had germinated (Fig. 1-B).

We found that when dried water agar plates with teliospores that had been inverted over plants were rehydrated with 5 ml of distilled H_2O , basidiospores were produced abundantly 2-3 days later. Such plates were useful for pycnium production if inverted over plants for only 1-2 days.

The most difficult task in completing the bean rust life cycle was going from basidiospores to pycnia. We had several unaccountable failures in obtaining pycnia when presumably adequate numbers of teliospores were germinating on plates. Numbers of pycnia also varied from time to time, being as high as 300 per leaf to as low as 1-2. For controlled crosses, fewer pycnia are more desirable (Fig. 2-A). Pycnia appeared initially only on the upper leaf surface as chlorotic flecks. After fertilization, aecia developed first on the underside of the leaf and later, occasionally, on the upper leaf surface. Aecia continued to expand as rings or clusters of pustules (Fig. 2-B) as long as the leaf remained healthy. A single collection of aeciospores from a large cluster of aecia was sufficient to provide inoculum for the production of several hundred

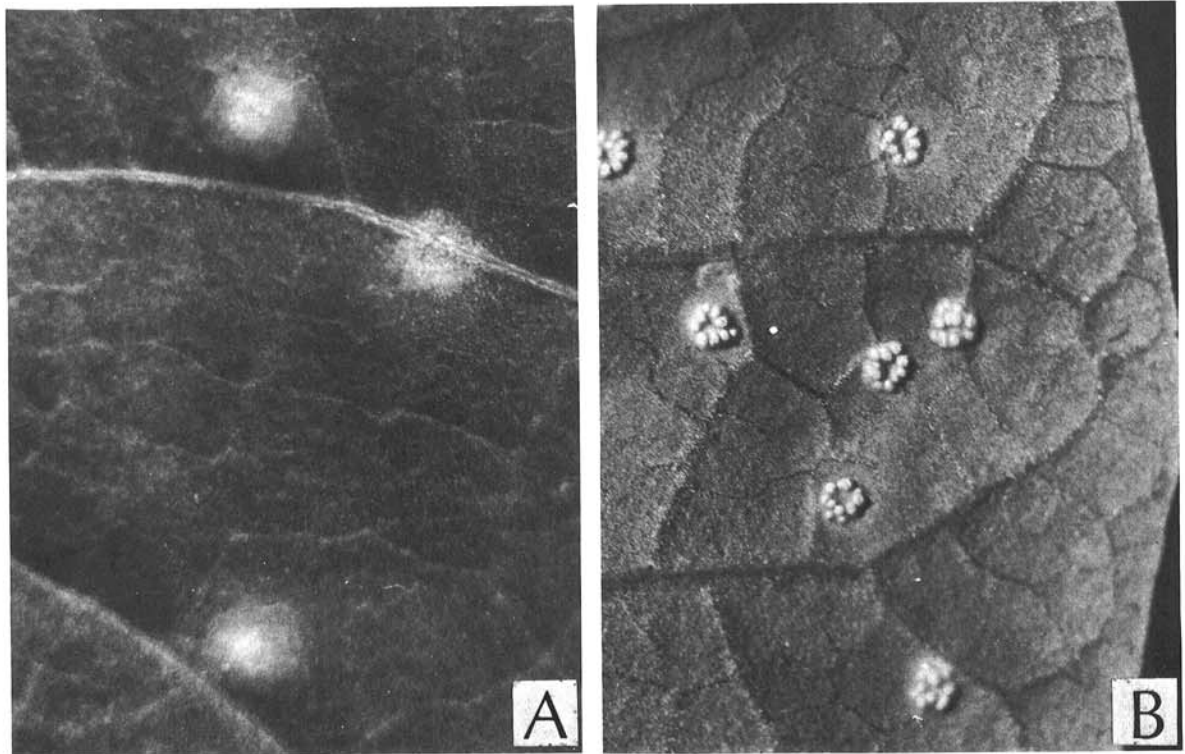


Fig. 2-(A, B). Signs of *Uromyces phaseoli* var. *typica* on Pinto 166 bean leaves. A) White pycnia on upper surface of a bean leaf. $\times 5$. B) White aecial clusters on lower surface of a bean leaf, each the result of the fertilization of a single pycnium. $\times 3$.

uredia on two or three plants.

Crosses were made of two single-pustuled races, one from Michigan and one from North Dakota, that differed interactively in their virulence on two bean cultivars. Each race served as the female parent for half of the crosses. Of 35 isolated pycnia to which pycniospores were (putatively) transferred, 20 produced aecia (the female being of one race nine times and of the other 11 times). The discrete nature of F_1 virulence levels on the cultivars was evidence that individual, major virulence genes were segregating. Three aecia contained two distinct genotypes each. Such mixtures were expected using our multiple crossing technique if one or two heterozygous loci were segregating in the F_1 and if, as with *Puccinia coronata* var. *avenae*, an aecium could result from more than one fertilization event (3). Of the 23 total progeny, 12 were best explained genetically as unequivocal crosses. Five of the remaining eleven were equivocal; they could have resulted either from crossing or selfing. The remaining six were probably the result of inadvertent selfing. Thus, the technique was shown to be usually successful in crossing bean rust races.

From our limited experiment, all five spore stages were readily obtained in the greenhouse and laboratory for cultures of *U. phaseoli* var. *typica* that were capable of producing telia. Since nearly all of our regional rust collections produced telia profusely, we did not explore ways of inducing telia to form in those cultures that did not normally do so. Though this technique was adequate for our purposes, minor changes may lead to increased efficiency.

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