Completion of Life Cycles of Puccinia hordei and Uromyces scillarum on Detached Leaves of Their Hosts

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ABSTRACT

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Puccinia hordei was cultivated through its pycnial and aecial stages on detached green leaves or segments of fleshy bulb scales of *Ornithogalum narbonense* or *O. brachystachys*. The uredial and telial stages were cultured on detached leaves of *Hordeum spontaneum* or *H. vulgare*. The detached green leaves were maintained on cotton pads soaked in 40 μg/ml benzimidazole solution and the bulb scales were maintained on water-saturated pads. The duration of a cycle averaged about 40 days. Germination of dormant teliospores was induced by floating them on water for 48-72 hr. Longevity of the gametophytic stage, ordinarily limited to 14-21 days on detached green leaves, was extended for over 2 mo on fleshy bulb scales maintained at 18-20 C, and for 5 mo on those kept at 5 C. Prolonged culture of pycnia facilitated crossing and hybridizing of rust fungus isolates virulent or avirulent on leaves of the main and alternate hosts. The microcyclic *Uromyces scillarum* was cultured from telia to telia on fleshy bulb scales of its host, *Leopoldia maritima*, and formed telial clusters similar to those that appear in nature on the host's green leaves. The same results were obtained by inoculation of bulb scale of *Urginea maritima* with germinating teliospores of *Uromyces scillarum* isolated from the green leaves of that host.

Puccinia hordei Otth, the pathogen of brown leaf rust of barley, completes its life cycle in Israel on native *Ornithogalum* spp. This alternate host plays a decisive role in the perpetuation of the fungus (Z. K. Gerechter-Amitai, *unpublished*, and 2, 3). A similar situation exists in other Mediterranean countries (4). Investigations in Israel (3) corroborate d'Oliverira's findings (10) that the genus *Ornithogalum* is important in the origination of new physiologic races of *P. hordei*. Significantly, several genera of indigenous Liliaceae carry the pycnial and aecial stages of several *Uromyces* spp.that attack wild barleys in Israel. They also harbor *Uromyces* microcyclic rusts that are correlated with the heteroecious *Uromyces* spp. that attack barley (1). Studies on the biology, genetics, and evolution of the organisms associated with the barley leaf rust diseases require genetically uniform plant material protected from contamination, and cultured in controlled-environment cabinets. The culture of rusts on detached leaves offers these advantages (5, 7, 8). Infection has been more easily attained on detached leaves than on intact plants in some rusts (12). Completion of life cycles on detached leaves by autoecious and heteroecious rusts has been reported (7, 8).

In this paper, we report the culturing of *P. hordei* and the short-cycled *Uromyces scillarum* (Grev.) Wint. on detached leaves. We also demonstrate the usefulness of fleshy bulb scales as a growth substrate.

MATERIALS AND METHODS

Hosts.—The main hosts of *P. hordei* were selections of *Hordeum spontaneum* C. Koch, which responded differentially at the seedling stage to the rust cultures. Seedlings of barley cultivar Nigrate (C.I. 2444) and a local barley cultivar Amidon were used as standard susceptible hosts for multiplication of inoculum. The alternate hosts of *P. hordei* were accessions of *Ornithogalum narbonense* L. and *O. brachystachys* C. Koch. The pycnial and aecial stages of the fungus occur in plants of both species in natural populations. Some clones of *O. narbonense*, selected after several years of testing (numbered 4624, 4634, 7203, and 7205), had leaves that were resistant to isolates avirulent to specific selections of *H. spontaneum*, and susceptible to other isolates virulent to the same selections of *H. spontaneum*. Resistance of leaves of *O. narbonense* was recognized by the smaller pycnial and aecial clusters with fewer cups surrounded by necrotic lesions, and also by reduced excretion of nectar (9). Bubls were harvested in late spring when the aboveground plant parts were desiccating and disappearing. The bulbs were stored at 32 C in dry soil or in paper bags under dry conditions to prevent sprouting. Germination of bulbs was induced by maintaining them at 20 C under humid conditions.

Uromyces scillarum inhabits in Israel natural populations of geophytic Liliaceae species, such as *Leopoldia maritima* (Desf.) Parl., that grows on sandy soil of the Central Coastal Plain, and *Urginea maritima* (L.) Bak., which is common in many parts of the country.

Inoculation procedure.—Cultures of single-urediospore or single-aeciospore origin were obtained by a method similar to that reported by Hooker and Yarwood (8). The cultures were multiplied on green leaf sections of Nigrate or Amidon in petri dishes, the bottoms of which were lined with cotton pads, saturated with a 40 μg/ml benzimidazole solution. The closed petri dishes were kept at 20 C and illuminated daily with fluorescent and incandescent light of approximately 16,140 lux (1,500 ft-c) intensity.

Teliospores of *P. hordei* (used for inoculation of the alternate host), were produced on detached barley leaves in petri dishes. Various methods were used for breaking their dormancy (1, 7, 8). The best results were obtained by the method of Anikster (1): Teliospores were scraped from green leaves or straw and floated for 48-72 hr on distilled or tap water at 18-20 C. Drops of the teliospore suspension then were distributed on water agar, where they started to germinate after 5-10 days of incubation at 18-20 C. Similar results were obtained by soaking plant-segments harboring telia for 48-72 hr and then distributing the spores on water agar.

The procedure devised by Lumbroso (9) was used to inoculate the alternate hosts. Small portions of leaves with telia were soaked for 48-72 hr in running water and then gently ground with a rubber pestle in a mortar. The detached teliospores were freed from coarse plant particles by washing and screening them through a nylon net into glass vials, and then centrifuging them in 0.05% water solution of Tween-20 (polyoxyethylene sorbitan monolaurate). The supernatant liquid was decanted, and the treatment was repeated several times. Subsequently, the sedimented teliospores were suspended uniformly in water by a magnetic stirrer. Aliquots of 2-3 ml of the spore suspension were pipetted to Büchner funnels lined with filter paper disks. The disks then were removed and dried. The teliospore inoculum on different disks was assumed to be of similar composition and concentration. The filter paper disks then were mounted on the inside of petri dish lids. Green leaf-segments (4-5 cm long, cut preferably from the base of the leaf) and sections of fleshy bulb scales were inoculated. The green leaf-segments were mounted slantwise on glass supports, so that their basal portions were submerged in 40 μg/ml benzimidazole solution or in water. The fleshy scale-segments were placed on a water-soaked cotton pad covering the perforated bottom of a plastic container 5 cm in diameter and 6 cm high. Several scale segments from the same bulb were maintained in the container with their convex surface up to prevent accumulation of condensation water on the surface toward the basidiospores (9). The containers were transferred into wider containers. Water supplied to the wider container reached the inner container through the perforation in the bottom and provided sufficient moisture to the cotton pad throughout the test. The inner containers were tightly capped with petri dish lids lined with filter paper disks bearing teliospores. The filter paper disks bearing teliospores in the inner container were kept moist by water-absorbing strips which connected them with wet cotton pads.

The data reported herein pertain to only two single-urediospore cultures, Z and W, although many rust cultures were investigated. Culture Z, a progeny of three successive generations of selfing, was homogenous for avirulence on green leaves of *O. narbonense* clones numbered 4624, 4634, 7203, and 7205, and produced type 2 infections on seedlings of selections of *H. spontaneum* numbered 562, 563, and 564, but was virulent on green leaves on other clones of *O. narbonense*. Culture W was virulent on green leaves of all clones of *O. narbonense* tested, and formed type 3 infection on seedlings of selections of *H. spontaneum*, nos. 562, 563, and 564.

RESULTS

Completion of the life cycle of Puccinia hordei on detached leaves.—Aeciospores of cultures Z and W, originally developed on intact plants of *O. narbonense*, were inoculated to leaf segments of Nigrate that had been maintained in separate containers on cotton pads. Uredia became visible about 9-10 days after inoculation. Some cultures did not produce telia, whereas other cultures produced uredia and telia simultaneously. In many cultures, including Z and W, telia appeared 5-6 days later than uredia.

Exposing the infected barley leaf sections to 25 C

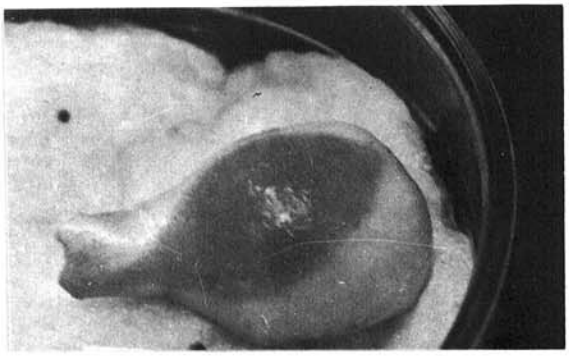

Fig. 1. Cluster of pycnia of *Puccinia hordei* developing on a section of bulb-scale of *Ornithogalum narbonense* in one orange-pigmented spot. Rust infection obtained by artificial inoculation (×3).

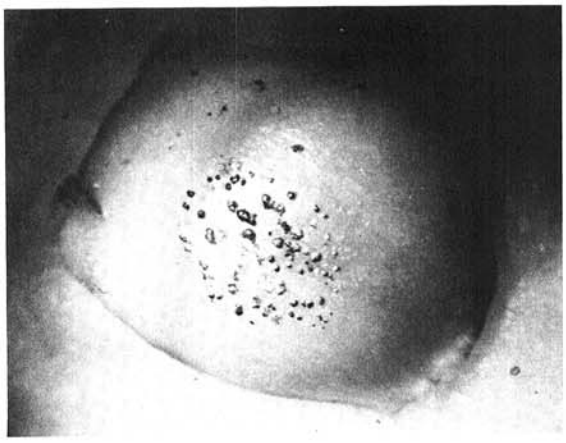

Fig. 2. Group of telia of *Uromyces scillarum* on a bulb-scale segment of *Leopoldia maritima* (×4).

accelerated the development of telia. Inoculum of activated teliospores was distributed on filter paper disks at different concentrations. For example, there were about 40,000 teliospores on some disks. The moistened disks were fastened to the inside of petri plate lids and placed over containers with green leaf-segments of individual clones of *O. narbonense*. Green leaf-segments were substituted by fleshy bulb scales of corresponding clones in parallel tests. Pycnia appeared 10-15 days after exposure, and were more abundant on fleshy bulb scale-sections taken from storage than on sections of bulbs activated in wet soil at 20 C.

Teliospores wetted and air-dried once or twice before inoculation formed a higher percentage of pycnia than teliospores not subjected to that treatment. When scales from stored bulbs were inoculated, seven to ten pycnial clusters developed per 10 cm^2 of scale surface, which was about 10 times the pycnial production on scales of activated bulbs (9). In crowded pycnia, aecial development commenced after 6-8 days, whereas solitary pycnia developed aecia only when their nectar was intermixed with nectar collected by micropipette from other pycnia. Less nectar was produced in pycnia on scale-segments than on attached foliage. Aeciospores transferred from the bulb scale and inoculated to green leaf-sections of barley resulted in development of uredia and telia. Several complete cycles from uredia to uredia were produced on detached leaves of the main and alternate hosts. The duration of a cycle averaged about 40 days. The specific pathogenic characteristics, as expressed by the ability of the fungus to elicit resistant or susceptible reactions on the infected *Ornithogalum* or *H. spontaneum* plants, were preserved throughout the tests.

The longevity of the gametophytic stage of *P. hordei* on green leaf-segments of *Ornithogalum* plants averaged 14-21 days; it was limited mainly by the deterioration and premature senescence of the host tissue. The rust was cultured on fleshy bulb-scale sections for over 2 mo at 18-20 C, and for over 5 mo at 5 C. Segments not infected or moderately infected survived longer than heavily infected ones. Few concentric rings of pycnia were formed during the early period of growth. The pycnial and aecial clusters expanded rapidly (Fig. 1), and eventually embraced the entire scale surface, indicating the systemic nature of the mycelium in the gametophytic stage (6). Colonization of the translucent scale tissue by mycelium was recognized under the microscope at low-power magnification by the aggregation of yellow pigment at the infected site.

Cross sections through the fungus-bearing scale tissue stained in cotton blue solution reveal development of intercellular hyphae invading the host cells by filamentous proliferations. Such haustoria, designated by Rijkenberg and Truter (11) as "intracellular hyphae," differ distinctly in shape from haustoria formed by the uredial stage of the fungus. Similar results were obtained in histologic studies of the pycnial stage of *Puccinia sorghi* (11).

The cultures investigated have been selfed or hybridized and the progenies have been grown through a complete life cycle.

Life cycle of Uromyces scillarum.—This microcyclic rust develops telia only. Telia are grouped in clusters on leaves of *Leopoldia maritima* and *Urginea maritima*. Teliospores first were subjected to the treatment for breaking dormancy, and then inoculated to the fleshy bulb-scale segments cultures, as in tests with *P. hordei*. The resultant telial clusters are shown in Fig. 2. They are similar to those that develop on green leaves in nature.

DISCUSSION

Culturing of *P. hordei* on detached leaves of the main (barley) and alternate (*Ornithogalum* spp.) hosts throughout the complete life cycle offers an easily manageable and economic method for studying genetically homogenous plant material, for obtaining adequate replication, and for eliminating variability in seasonal conditions. Additional advantages of the method are rapid succession of generations that retain the specific pathogenicity of each culture, extending longevity of the gametophytic stage, and controlling contamination.

The prolonged duration of pycnial development on bulb scales was favorable for crossing of cultures of *P. hordei*. Well-distributed pycnia could be fertilized by bringing them in contact with exudate from the pycnia of another specific culture. Pycnia on detached green leaf-segments of *Ornithogalum* spp. are not suitable for crossing because they are relatively short-lived due to premature senescence of the host tissue.

Successful culturing of microcyclic rusts and the pycnial and aecial stage of heteroecious rusts on fleshy bulb scales is likely to advance research on correlated rust species and their co-evolution.

LITERATURE CITED

1. ANIKSTER, Y. 1975. Studies on the taxonomy, biology and evolution of the genus Uromyces on barley in Israel (Hebrew, with English summary). Ph.D. Thesis, Tel-Aviv University, Tel-Aviv, Israel. 142 p.
2. ANIKSTER, Y., C. ABRAHAM, Y. GREENBERGER, and I. WAHL. 1971. A contribution to the taxonomy of Puccinia brown leaf rust of barley in Israel. Israel J. Bot. 20:1-12.
3. ANIKSTER, Y., J. G. MOSEMAN, and I. WAHL. 1976. Parasite specialization of Puccinia hordei Otth and sources of resistance in Hordeum spontaneum C. Koch. Pages 468-469 *in* Barley Genetics III. Proc. 3rd Int. Barley Genetics Symp., Garching 1975, 849 p.
4. CRITOPOULOS, P. 1956. Perpetuation of the brown rust of barley in Attica. Mycologia 48:596-600.
5. DAY, P. R. 1966. Recent developments in the genetics of the host-parasite system. Annu. Rev. Phytopathol. 4:245-268.
6. DIETEL, P. 1918. Die wirtswechselnden Rostpilze. Zentralbl. Bakteriol., Parasitenkd. Infektionskr. Hyg., Abt. 2, Naturwiss. 48:470-500.
7. HENNESY, C. M. R., and W. E. SACKSTON. 1970. Studies on sunflower rust. V. Culture of Puccinia helianthi throughout its complete life cycle on detached leaves of sunflower (Helianthus annuus). Can. J. Bot. 48:1811-1813.
8. HOOKER, A. L., and C. E. YARWOOD. 1966. Culture of Puccinia sorghi on detached leaves of corn and Oxalis corniculata. Phytopathology 56:536-539.
9. LUMBROSO, E. 1973. Studies on the gametophytic stage of barley leaf rust Puccinia hordei Otth. M. Sc. Thesis, Tel-Aviv University, Tel-Aviv, Israel. 83 p. (Hebrew, with

English summary).
10. OLIVEIRA, B. D'. 1939. Studies on Puccinia anomala Rost. I. Physiologic races on cultivated barleys. Ann. Appl. Biol. 26:56-82.
11. RIJKENBERG, F. H. J., and S. J. TRUTER. 1973. Haustoria and intracellular hyphae in the rusts. Phytopathology 63:281-286.
12. WATERS, C. W. 1928. The control of teliospore and urediniospore formation by experimental methods. Phytopathology 18:157-213.