Durability of Secondary Sporidia of Floret Infecting *Tilletia* Species under Laboratory and Field Conditions: Implications for Epidemiology

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Abstract: Secondary sporidia resulting from germinating teliospores of some *Tilletia* species initiate local infection of wheat, rice and rye grass florets and cause the diseases rice smut, Karnal bunt, and rye grass bunt, respectively. Secondary sporidia are considered to be fragile and to require very high humidity to survive longer than a few hours. To examine this, secondary sporidia from potato dextrose agar or water agar cultures of *T. horrida*, *T. indica*, *T. walkeri*, and the seedling infecting species *T. tritici* were deposited via natural liberation onto lids of Petri dishes and were air dried and maintained at 10–20% RH at 20–22°C, and at 40–50% RH at 18°C. After various time periods lids were inverted over fresh PDA to determine viability. Dried sporidia regenerated after 30 days at 10–20% RH and 60 days at 40–50% RH. Commonly, 18 hours after lids containing dried sporidia were inverted over PDA, newly produced secondary sporidia were present on the agar that had produced hyphae capable of infecting plants. Sporidia initially dried rapidly at 10% RH or dried over 10 hours had no difference in viability. In experiments using *T. horrida* and *T. indica* in wheat and barley fields, secondary sporidia on Petri dish lids held up to 46–49 days 20–25 cm from the soil surface in the canopy rapidly regenerated as mentioned above, even after several diurnal periods that included temperatures above 38°C and relative humidity below 10%. It appears sporidia can survive for extensive dry periods in common field conditions and then rapidly regenerate and infect plants under humid rainy conditions associated with the diseases.

Keywords: *Tilletia horrida*; *T. indica; T. walkeri*; sporidia

Tilletia horrida, *T. indica*, and *T. walkeri* are smut fungi that infect florets during heading and cause the diseases rice smut, Karnal bunt of wheat, and rye grass bunt, respectively. Secondary sporidia of these fungi are forcibly discharged and germinate to produce hyphae which initiate infection. Secondary sporidia are thin-walled propagules that are considered to require very high relative humidity to survive longer than a few hours.

It is thought that teliospores germinate at heading time and produce sporidia that infect plants. It has been suggested that there must be some sort of "safety mechanism" or "timing mechanism" to stimulate teliospore germination or sporidial germination during heading to insure ample inoculum is present to produce infection. Otherwise, teliospores would undergo a hypothesized "suicidal germination" prior to the host being in a susceptible growth stage. However, teliospores likely germinate whenever conditions of moisture and temperature are proper, regardless of the growth stage of the host. Teliospores germinate well under moist conditions over a broad temperature range of 5 to 25°C. Teliospores require 4–5 days to germinate under optimal conditions and then more time is necessary to increase secondary sporidia to concentrations sufficient to cause disease. The concept of teliospores germinating at heading time when plants are susceptible is inconsistent with observations that only a day or two of conducive weather during the plant susceptibility period is required to induce disease. This study was conducted to determine the longevity of secondary sporidia under field and laboratory conditions to evaluate their potential for within season survival of the pathogen.

MATERIAL AND METHODS

Cultures of hyphae and secondary sporidia of *T. horrida, T. indica*, *T. walkeri*, and the seedling infecting species *T. tritici*, which causes common bunt of wheat, were grown in 9 cm plastic Petri dishes on 2% potato dextrose agar (PDA) and 2% water agar (WA). During growth, naturally liberated secondary sporidia were deposited onto the lids of the dishes due to static electrical forces. After 1 to 5 days of growth, lids were removed from the Petri dishes and were air dried at 10–20% RH for 1 day.

For laboratory experiments, sporidia on Petri dish lids were maintained over an empty Petri dish bottom either on a lab bench under ambient laboratory conditions of 10–20% RH at 20–22°C, or in an incubator at 40–50% RH at 18°C. After various time periods the lids were placed over fresh PDA plates and sealed with Parafilm to determine if secondary sporidia could regenerate. The experiments were repeated a minimum of four times for each species at the low RH, and three times at the higher RH.

Field experiments were conducted in four different fields including sprinkler irrigated winter wheat and spring barely fields at Aberdeen, Idaho using *T. horrida*, and in flood irrigated durum wheat and spring wheat fields near Phoenix, Arizona using *T. indica*. Petri dish lids from PDA cultures containing dried sporidia described above were taped to 31 cm wooden stakes. The staked dish lids were placed within the crop canopy 20 to 25 cm from the soil surface with the sporidial side of the dish facing slightly toward the soil at an angle of about 70–80°C. Uninoculated control dish lids were placed in the fields in Arizona because Karnal bunt is known to occur naturally in these fields. When the experiment began, the spring barley was at boot stage, the winter wheat was at heading, the durum wheat was at flag leaf stage, and the spring wheat was at flag leaf emerging stage. In the winter wheat field, sporidia were applied to leaves about 25 cm from the soil surface by rubbing leaves directly on cultures. Petri dish lids were collected approximately every seven days in Idaho and about every 14 days in Arizona. Temperature and relative humidity at the level of the dish lids in the field were monitored with WatchDog electronic data loggers placed within small instrument shelters. The data loggers did not record humidity below 20.7% so it was necessary to estimate humidity by comparing RH away from the crop canopy recorded by a nearby National Oceanic and Atmospheric Association instrument array after crops neared maturity and fields were dry.

In separate experiments the effect of initial drying time on survival of all four species was tested by drying lids: (i) by immediate exposure to 10% RH, (ii) over a water mist placed in a Petri dish bottom, (iii) over filter paper soaked with 0.5 ml water placed in a Petri dish, and (iv) over filter paper soaked with 1.0 ml water placed in a Petri dish. Lids dried over the water treatments were canted slightly over the dish bottom to allow air exchange and eventual drying. After drying, the plates were left at 10–20% RH and 20–22°C for four days and then inverted over fresh PDA and sealed with parafilm. This experiment was repeated twice.

RESULTS

Secondary sporidia that adhered to Petri dish lids commonly were arranged in reticulate or linear patterns, likely due to static electrical forces. In the laboratory experiments, secondary sporidia were commonly able to regenerate after drying for 30 days at the lower RH, and after 60 days at the higher RH. In some experiments sporidia regenerated after 45 days at the lower RH, and after 90 days at the higher RH. Most remarkably, 18 hours after dried sporidia on lids were inverted over fresh PDA, it was common to see newly formed secondary sporidia that had germinated to produce long hyphae on the PDA surface. Secondary sporidia originating from WA cultures regenerated similarly to those originating from PDA cultures.

In the field experiments, rapid regeneration of secondary sporidia occurred within 18 hours, like that described above, from dish lids held in the fields for up to 42 to 46 days when the crop was mature or near maturity and the fields were dry. This occurred despite wide fluctuations in temperature and relative humidity in the field environments during the period which included several days where the temperature exceeded 43°C with less than 10% relative humidity. During earlier growth stages when the crop was still green there were several diurnal periods when the relative humidity dropped below 35% for several hours. No sporidia were generated from

the uninoculated control lids placed in the Arizona fields.

In the experiments to determine the effect of initial drying time on survival, any moisture on the lids dried within about one minute after direct exposure to the dry air in the lab. In plates where water was added, the moisture in the bottom dish evaporated after approximately 1 h in the misted plates, 4 h in the 0.5 ml plates, and 10 h in the 1 ml plates. There was no apparent difference in the initial drying treatments on the eventual regeneration of sporidia.

DISCUSSION

The results of these experiments present new information that demonstrate secondary sporidia, the infective agents of the diseases, are highly durable for extensive periods under very dry environments, and under somewhat typical field environments even after the crops are mature. This is contrary to reports that state high humidity is required for survival of sporidia. This information indicates that secondary sporidia have a more substantial role in pidemiology than previously thought. It appears that teliospores that germinate and produce hyphae and secondary sporidia during conducive periods well in advance of the susceptibility period of plants (during and after heading could produce effective sporidial inoculum that can lay dormant in dry field environments for extensive periods nd then rapidly regenerate and infect plants during humi rainy conditions normally asociated with the diseases.