Germination of Teliospores of Karnal, Dwarf, and Common Bunt Fungi After Ingestion by Animals

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ABSTRACT

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Teliospores of *Tilletia indica, T. controversa*, and *T. caries* were fed to leghorn chickens or grasshoppers (*Melanoplus sanguinipes*) or placed in the rumen of a rumen-fistulated Holstein cow. Viable teliospores were present in all feces collected. After ingestion by grasshoppers, germination of *T. indica*, *T. controversa*, and *T. caries* teliospores was reduced slightly (70.0, 88.5, and 89.2% of controls, respectively). Only *T. indica* teliospore germination was reduced significantly. Ingestion by leghorn chickens or passage through the intestinal tract of a Holstein cow significantly reduced teliospore germination of these fungi but did not prevent it. Quarantines established to prevent the movement of these pathogens should consider feces-derived products as potential sources of introduction.

The passage of teliospores through the digestive tracts of various animals has been reported to have either deleterious (3,4) or inconsequential (1,5,9,11) effects on teliospore germination. Appel and Riehm (3) reported that *Tilletia caries* (DC.) Tul. teliospores did not germinate after passage through the alimentary tract of calves, goats, and sheep. Aebi (1) reported that passage of *T. controversa* Kühn teliospores through an unidentified fly larva did not reduce germinability. Likewise, Hoffmann and Purdy (9) observed no significant reduction in the germination of *T. controversa* teliospores recovered from earthworm castings.

Knowledge of teliospore viability after ingestion by animals is important when bunt-infested grain is fed to animals whose spore-laden feces are in turn used as fertilizer or where the dissemination of bunt teliospores may be enhanced by birds or insects. Quarantines established to prevent the movement of bunt-contaminated grain may miss an important avenue of potential introduction by animal and animal-product movements. Both *T. indica* Mitra and *T. controversa*, incitants of Karnal and dwarf bunt, respectively, are objects of stringent international quarantines (2,10,13). The purpose of this study was to assess the survival of *T. indica*, *T. caries*, and *T. controversa* after ingestion by grasshoppers (*Melanoplus sanguinipes* Fabricius), leghorn chickens, or a Holstein cow.

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MATERIALS AND METHODS

Teliospores. Teliospores of *T. caries* and *T. controversa* originating from diverse geographic locations throughout the northwestern United States were collected from wheat cultivars grown in artificially inoculated nurseries near Logan, UT. The teliospore mixtures thus represented a broad range of pathogenic, physiological, and morphological types of these fungi. *T. indica* teliospores were obtained from the Instituto Nacional de Investigaciones Agrícolas de México as infected kernels from a mixture of cultivars grown in commercial wheat fields in the Yaqui and Mayo valleys of Sonora. The *T. caries* and *T. controversa* teliospores were 1–4 mo old, and those of *T. indica* were 18–20 mo old.

Ingestion and recovery of teliospores.

About 40 *T. caries-* or *T. controversa*-bunted wheat heads were placed in the rumen of a rumen-fistulated Holstein cow that had been maintained on an alfalfa diet. Samples of feces were collected after 2 days and continued up to 7 days. Feces containing the greatest number of teliospores were obtained after 2–3 days. A rumen-fistulated Holstein cow was used because the cow would not voluntarily eat enough bunted spikes to ensure that teliospores would be recoverable. The teliospores were recovered by pouring a suspension of 50 g of feces in 500 ml of water through a column of seven nesting sieves with pore diameters of 2,000, 355, 250, 75, 45, 38, and 25 μm. The teliospore-containing filtrate was vacuum-filtered through a 15-μm nylon mesh. Most of the teliospores of *T. caries* and *T. controversa* have a diameter greater than 15 μm and were retained on the surface of the mesh. A suspension for plating was prepared by collecting the teliospores in a 10-ml volume of water. *T. indica* was not used in the cow-ingestion experiment because of quarantine regulations.

Mature grasshoppers (*M. sanguinipes*) collected from Green Canyon, Cache County, UT, were fed bunt sori (*T. caries* or *T. controversa*) or bunt-infected kernels (*T. indica*) for 4 days. The grasshoppers ate sori and bunt-infected kernels preferentially over healthy kernels. After 4 days, 50–100 mg of feces was collected, crushed gently, and suspended in a 25 ml of 0.01% Triton X-100 (w/v) in water. The suspension was filtered through nylon mesh and then through a vacuum filter apparatus containing a 15- or 25-μm mesh, depending on whether the smaller teliospores of *T. caries* and *T. controversa* (18–25 μm) or the larger teliospores of *T. indica* (38–42 μm) were the objects of collection (6). The suspension for plating was prepared by collecting teliospores retained on the mesh in 10 ml of water.

Female leghorn chickens about 10 wk old were fed a diet consisting (by weight) of 70% wheat kernels, 5% crushed bunt sori or infected wheat kernels, 15% soybean meal, 5% meat and bone meal, 4.25% limestone, 0.25% sodium chloride, 0.25% dicalcium phosphate, and 0.25% of a vitamin and trace mineral mix. Four chickens were fed the above diet containing either *T. indica, T. caries*, or *T. controversa* for 2 hr. Feces were collected over the following 4-hr period on paper sheets. A suspension of 10 g of feces and 100 ml of water was filtered through two layers of cheesecloth and 60-μm nylon mesh. The teliospore-containing filtrate was vacuum-filtered through 15- or 25-μm mesh nylon and collected in 10 ml of water as before.

Teliospore viability. The teliospore suspensions were centrifuged for 2 min (1,000 *g*) and the supernatant discarded. The pellet was immersed for 1 min in a 5% (v/v) commercial laundry bleach solution (0.26% $NaHClO_3$, pH 10) to surface-sterilize the teliospores and recentrifuged (1,000 *g*). The pellet was rinsed twice and resuspended in sterile deionized water to a concentration of 2,000–3,000 teliospores per milliliter. Teliospores were applied to water agar plates in 0.3-ml aliquots and incubated under continuous illumination (30 $\mu E\ m^{-2}\ s^{-1}$, cool-white fluorescent). *T. caries* teliospores were incubated 1 wk at 15 C, *T. indica* teliospores were incubated 2 wk at 15 C, and *T. controversa* teliospores were incubated 6 wk at 5 C. These incubation periods are optimal for teliospore germination of these fungi (8,12). Teliospores from each treatment were applied to four or five plates, and 200–300 teliospores were counted on each plate at the end of the incubation periods to determine the germination percentage.

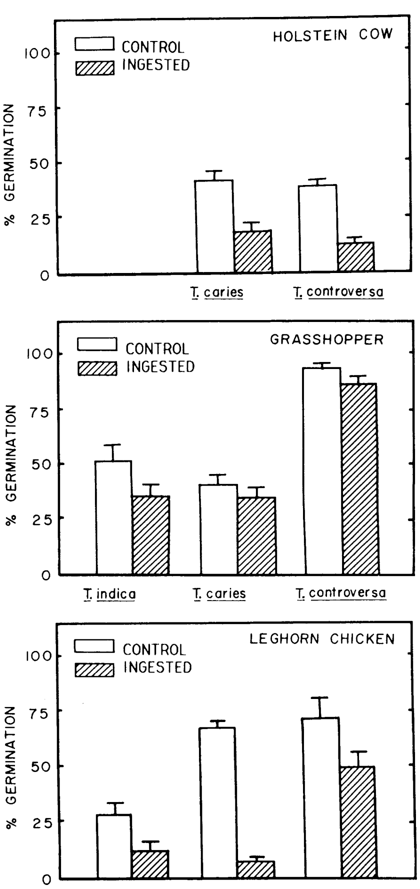

Fig. 1. Percent germination of teliospores of *Tilletia indica, T. caries*, and *T. controversa* after ingestion by a Holstein cow, grasshoppers (*Melanoplus sanguinipes*), or leghorn chickens. Bars represent one standard deviation.

RESULTS

Viable teliospores were present in the feces of all the animals, although germination was always reduced compared with uningested controls (Fig. 1). Ingestion by leghorn chickens significantly (Student's *t* test, $P = 0.05$) reduced teliospore germination of *T. indica, T. caries*, and *T. controversa* to 46.2, 8.3, and 71.7% of the uningested controls, respectively. Grasshopper ingestion reduced *T. indica, T. caries*, and *T. controversa* teliospore germination to 70.0, 89.2, and 88.5% of the controls, respectively. Only *T. indica* teliospore germination was reduced significantly. *T. caries* and *T. controversa* teliospores from cow feces germinated 36.1 and 32.2% of the controls, respectively, both significantly less than the controls.

DISCUSSION

Ingestion by the three animals tested did not completely inhibit teliospore germination of any of the three bunt species. Therefore, the feces of animals that have ingested bunt-contaminated plant products should be considered potential sources of inoculum. Viable bunt teliospores could be widely disseminated by the importation and use of bunt-contaminated, animal-derived fertilizers and also by the natural movement of grain-eating migratory animals. It is a common practice to feed severely bunt-contaminated grain to feedlot animals (7). The mechanical application of manure from feedlots as fertilizer would deposit teliospores on the soil surface in a manner conducive to natural infection of wheat (8,10,13). Sanitation by treatment of fertilizer products by heat or fumigation is unsatisfactory because these fungi are tolerant to such treatments (7,12).

Our results support the suggestion of Aebi (1) that insects may play a role in the dissemination of *T. controversa*. We observed the feeding of grasshoppers on mature heads of wheat infected with common and dwarf bunt in preference to uninfected kernels both in field plots and in colonies maintained in the laboratory. Wide distribution of the insect's feces could enhance the dispersal of these fungi without a marked reduction in their pathogenic potential.

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