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Low Potential of Teff (*Eragrostis tef*) as an Inoculum Source for *Verticillium dahliae*

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Teff (*Eragrostis tef*) is a fine stemmed, tufted annual grass and gluten free small grain (Stallknecht et al. 1993). The use of teff in ancient civilization has been traced back to 3359 BC. The common name hails from a thio-Semitic root word "tff", which means "lost" – possibly referring to the small seed size. Ethiopia is the center of origin for teff and it is still grown there today (Mengesha et al. 1965). Teff is generally not cultivated outside of Ethiopia (Stallknecht et al. 1993). Teff accounts for a large part of the grain grown in Ethiopia, which is approximately half of the cereal hectacerage in 1996 (Brown 1999) and 35% of cereal hectacerage in 2014 (USDA Foreign Agriculture Service). Teff is planted due to its traditional role as a foodstuff for generations for certain ethnic groups, but also due to its nutritional properties such as high calcium and iron and versatility in growing sites from waterlogged to drought sites (Stallknecht et al. 1993).

Planting teff requires a firm, moist seed bed because of small seed (<1 mm diameter). Seeding rates of teff vary from 2.3 to 9 kg/ha, with 5 to 8 kg/ha generally recommended (Stallknecht et al. 1993). Teff seeded at higher densities can smother weeds (Miller 2010). Teff germinates rapidly when planted an average depth of 1.2 cm, although emergence may be delayed until the root system is sufficiently developed (Stallknecht et al. 1993). Forage yields for teff grown in South Dakota ranged from 4 to 11 t/ha depending on the frequency of mowing (Boe et al. 1986). Frequent mowings have been suggested because of the tendency of the seed to shatter (Stallknecht et al. 1993). Mowing generally starts 45 days after sprouting and on 30 day intervals thereafter (Miller 2010). Teff has not been subject to a "summer slump", as some cool season perennial grasses can be, and can have consistent yields in hot weather (Miller 2010).

Little is known about the susceptibility of teff to many diseases, despite what is known about how to grow teff. This is especially true in the United States. In Ethiopia, teff rust (*Uromyces eragrostidis*) and head smudge (*Helminthosporium miyakei*) are important pathogens which can cause losses of 10-15% in yield (Ketema 1997). Early sown teff in Ethiopia can have problems with damping off caused by *Drechslera poae* and *Helminthosporium poae* (Ketema 1987). Other than damping off, teff has not been found to be susceptible to other important soilborne diseases, such as Verticillium wilt.

Teff could be grown in rotation with potato in the Columbia Basin to help reduce soil populations of *Verticillium dahliae*. Confirmation that teff cannot serve as a host for *V. dahliae* must be complete before teff can be employed as part of a crop rotation in a potato production system to reduce *V. dahliae*. Barley, a monocot, has been reported to be susceptible to Verticillium wilt, although *V. dahliae* was also reported as unlikely to become a major pathogen of barley or other cereal crops (Mathre 1989).

Verticillium dahliae infects a wide range of plants, making it one of the most important pathogens of dicotyledonous crop plants in North America (Bhat et al. 2003; Bhat and Subbarao 1999; Fordyce and Green 1960). Despite the wide host range of *V. dahliae*, individual isolations vary in aggressiveness when introduced to different plant hosts under controlled conditions. Aggressiveness in this case refers to increased severity of disease symptoms or increased production of overwintering inoculum, called microsclerotia. Aggressiveness of individual *V. dahliae* isolations has also been noted in the field (Bhat and Subbarao 1999). *V. dahliae* aggressiveness may occur over a relatively short period of time as *V. dahliae* reproduces within both annual and perennial crop hosts such as pepper and mint, respectively (Bhat et al. 2003; Fordyce and Green 1960). These aggressive *V. dahliae* isolates are called host-adapted pathotypes (Bhat and Subbarao 1999), which can be shortened to pathotype for ease of explanation.

The potato-aggressive pathotype of *V. dahliae* could still infect and persist within teff and maintain its aggressiveness until it can infect a potato plant again. This hypothesis was recently confirmed by Linde et al. (2016) in a separate fungal pathosystem involving *Rhynchosporium commune*, a pathogen of weedy barley grass and barley, where it was determined that "barley grass acts as an important ancillary host to *R. commune*, harbouring highly virulent pathogen types capable of transmission to barley." This observation underlies the need to verify the maintenance of pathogen aggressiveness as inoculum transfers between weeds and crops. It is therefore important to verify whether aggressive pathotypes of *V. dahliae* can or cannot infect teff and produce microsclerotia. If *V. dahliae* can infect teff and produce microsclerotia, then it is necessary to confirm that aggressiveness is sustained upon infecting potato. An understanding of any possible quantitative increase of *V. dahliae* on teff is important if teff is to be employed as a short-season forage in rotation with potato to avoid increasing *V. dahliae* microsclerotia in soil that could infect future potato crops.

Quantification of *V. dahliae* in teff and eggplant.

Comparisons were made for the aggressiveness of *V. dahliae* isolates to teff and an eggplant cv. Night shadow in two greenhouse trials in 2014 and 2016. The eggplant cultivar was selected as a host that is susceptible to all *V. dahliae* isolates. A total of eight *V. dahliae* isolates were used (Table 1) and one noninoculated control. Experimental units were arranged in a randomized complete block design with three replications in a greenhouse each year.

Table 1: The *Verticllium dahliae* isolates used in in two trials in 2014 and 2016 to evaluate if teff (*Eragrostis tef*) is susceptible to *V. dahliae* infection, and if teff to is susceptible to potato or mint aggressive pathotypes of *V. dahliae*.

Vd Isolate Name	VCG [b]	Pathotype	Original Host [c]
111	2B	Mint	Mint
155	2B	Mint	Mint
381	2 A/B	-	Watermelon
461	2	-	Tomato
625	2B	-	Sugar Beet
653	4A	Potato	Potato
Sunflower	2A	-	Sunflower
Vmd4	2 A/B	-	Tomato

[b] VCG: vegetative compatibility group

[c] Host original isolate had infected. Strains originating from potato or mint are the potato or mint strains, respectively.

Seedlings no greater than 10 cm in length were inoculated by submerging the hypocotyl and primary root in an agitated conidial suspension of one *V. dahliae* isolate at a concentration of approximately 1.0 X 10^6 colony forming units (CFU)/ mL. Plants were allowed to grow for four months before being dried for four weeks to facilitate the formation of *V. dahliae* microsclerotia. The number of *V. dahliae* microsclerotia from collected stems and roots was evaluated in a total of 1 g per dry stems or roots by placing ground plant matter (particle size <4 mm^2) on a semiselective medium for *V. dahliae.* The number of microsclerotia was enumerated after incubating for 10 days.

Differences in the number of observed *V. dahliae* microsclerotia for each *V. dahliae* isolate on a weed or crop host was determined by PERMANOVA (Primer-E Ltd, v7, Devon, UK). Familywise type I error for many pairwise comparisons was controlled using a false discovery rate (FDR) to adjust PERMANOVA p-values to smaller values than 0.05 to ascertain truly significant differences. The *V. dahliae* isolate with the greatest microsclerotia counts within one weed or crop was determined if this one pairing of weed and isolate was greater than comparisons between the seven other *V. dahliae* isolates within that same host with a minimum of 100 permutations on the PERMANOVA.

Results of *V. dahliae* Quantification in teff and eggplant.

Both teff and eggplant were infected by at least one isolate of *V. dahliae*, as indicated the presence of microsclerotia from within the plants (Figs. 1-2). However, teff developed no disease symptoms (data not shown).

V. dahliae infections of teff often yielded small numbers of microsclerotia (< 3 microsclerotia per gram of stem, < 5 microsclerotia per gram of stem, Figs 1-2.). No single *V. dahliae* isolate produced more microsclerotia than the other seven isolates in teff or eggplant stems (false discovery rate corrected $P < 0.006$) and roots (false discovery rate corrected $P < 0.007$) in 2014 and 2016 (Figs. 1-2). Infected eggplant produced significantly greater mean numbers of *V. dahliae* microsclerotia than teff in both stems ($P < 0.02$) and roots ($P < 0.02$) in 2014 and 2016.

Fig. 1: The mean number of *Verticllium dahliae* microsclerotia observed in the stems and roots of teff and eggplant for each *V. dahliae* isolate in 2014. No one *V. dahliae* pathotype produced significantly greater numbers of microsclerotia than all 7 other isolates in stems (false discovery rate corrected $P < 0.006$) and roots (false discovery rate corrected $P < 0.007$). The controls for both teff and eggplant were noninoculated.

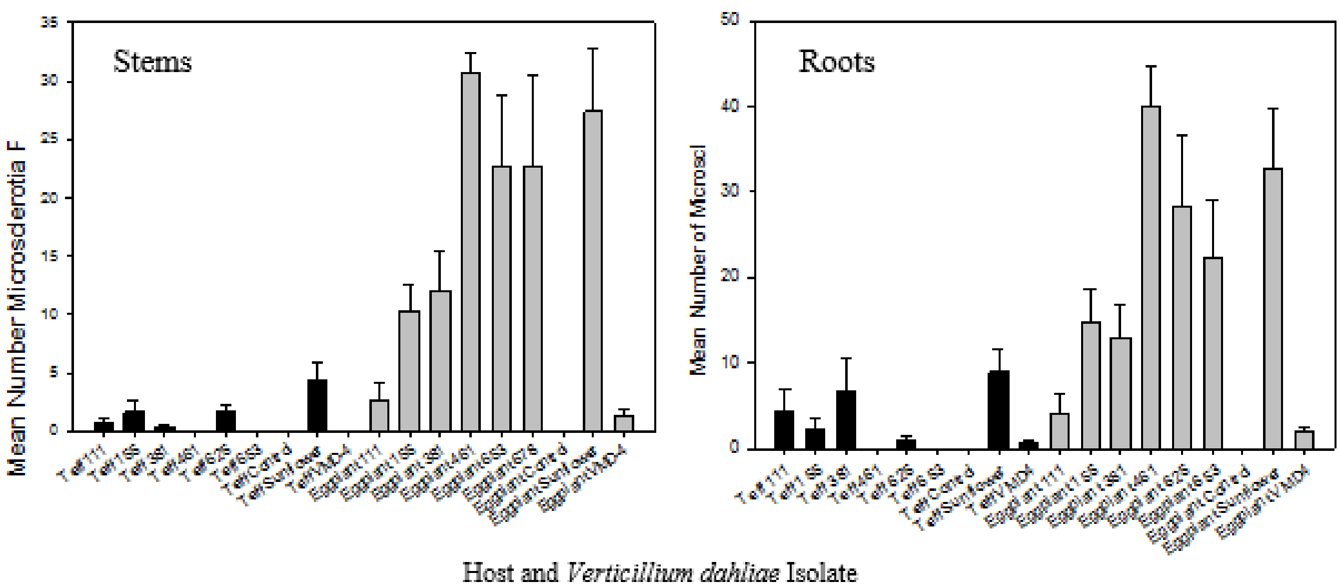
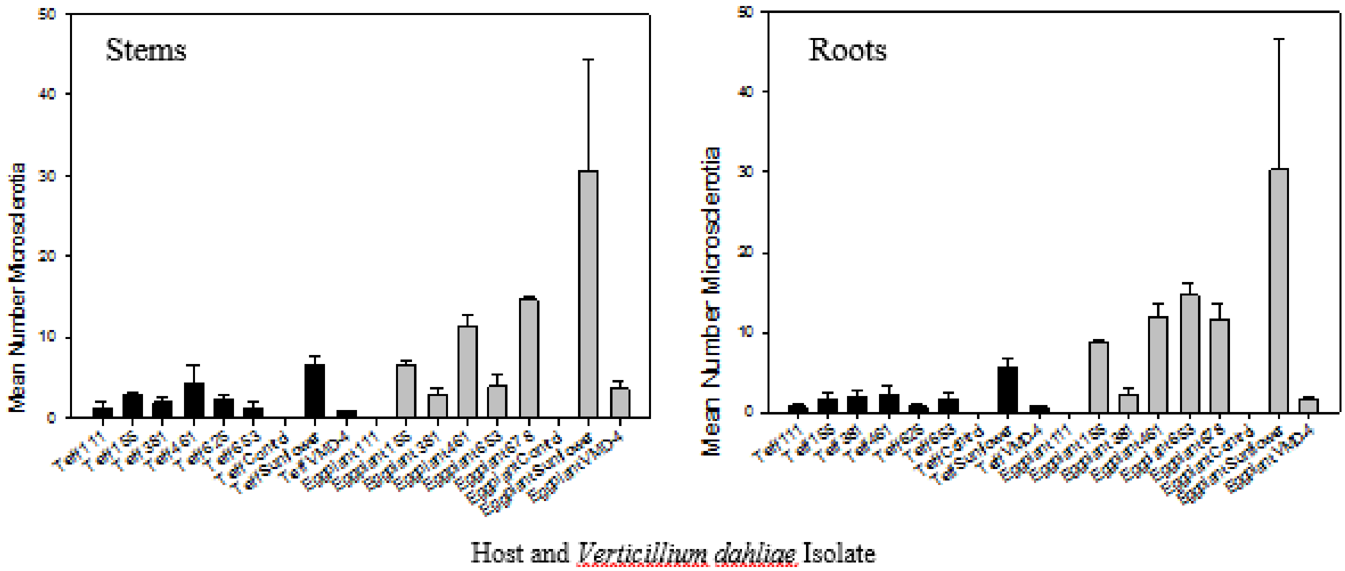


Fig. 2: The mean number of *Verticllium dahliae* microsclerotia observed in the stems of teff and eggplant for each *V. dahliae* isolate in 2016. No one *V. dahliae* pathotype produced significantly greater numbers of microsclerotia than all 7 other isolates in stems (false discovery rate corrected $P < 0.006$) and roots (false discovery rate corrected $P < 0.007$). The controls for both teff and eggplant were noninoculated.



Discussion.

Teff was confirmed as a host for *V. dahliae*, as indicated by the presence of microsclerotia in stems and roots of test plants. The microsclerotia production of *V. dahliae* in teff was consistently less than in eggplant cv. Night shadow in both greenhouse experiments. Teff likely produces fewer microsclerotia than other Verticillium-susceptible hosts in the field.

The number of microsclerotia produced in teff did not differ between the mint and potato pathotypes of *V. dahliae* in this study. It is unlikely that teff infected by *V. dahliae* will proliferate microsclerotia of mint or potato-aggressive pathotypes relative to susceptible eggplant and potato cultivars, respectively. Taken all together, this information demonstrates that teff can return *V. dahliae* microsclerotia to the soil, but the effect of widespread planting of teff on *V. dahliae* microsclerotia would be less than the widespread planting of eggplant or potato cultivars susceptible to Verticillium wilt. Evaluation of the maintenance of potato pathotype aggressiveness upon reinfecting potato after completing the lifecycle within teff was unnecessary given the few *V. dahliae* microsclerotia that were recovered from teff for isolate 653 (potato pathotype).

The numbers of microsclerotia returned to soil are important for Verticillium wilt development in susceptible crops planted in the future. Substantially greater or fewer microsclerotia will lead to more or less disease, respectively. An increase of microsclerotia in soil and in the severity of Verticillium wilt was observed in the Red River Valley of Minnesota and North Dakota with the continued use of Verticillium wilt susceptible potato cultivars over several years (Slattery, 1981). Teff as part of crop rotation and Verticillium wilt management strategy is unlikely to return many microsclerotia in the field, especially compared to a Verticillium-susceptible host such as eggplant. Environmental factors, plant stress, and the susceptibility of the particular crop host will also contribute to disease development in the field.

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Upcoming Potato Conferences in Pocatello and Kennewick

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Idaho Potato Conference & Ag Expo
Jan. 18-19, 2017

Pond Student Union Building, Idaho State University, Pocatello, Idaho

The 49th Annual University of Idaho Potato Conference and 38th Ag Expo will be held on the Idaho State University campus in Pocatello, 8 a.m. to 5 p.m., Wednesday, Jan. 18, and 8:30 a.m. to 12:30 p.m., Thursday, Jan. 19.

The conference covers a wide range of industry topics ranging from variety development, management practices and economics, marketing and political issues. There are Idaho and Washington Department of Agriculture Pesticide Handler credits available. There is also a series of nine presentations on industry topics presented in Spanish. The Ag Show is held in Holt Arena and there is an extensive Trade Show in the ballroom of the SUB. There is also a Grower-Speaker Social Wednesday evening, poolside at the Red Lion Inn.

For more information and links to registration, see:

https://www.uidaho.edu/cals/potatoes/conferences/idaho-potato-conference

Washington/Oregon Potato Conference
January 24-26, 2017

Three Rivers Convention Center, Kennewick, Washington

This conference includes a Spanish-language session on Tuesday morning, a Cultivar Performance Workshop on Tuesday afternoon, General Sessions on Wednesday and Thursday, plus numerous side meetings, evening events, and a top-notch tradeshow.

For more information and a link to registration, see:

http://potatoconference.com