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## Introduction

Buckwheat (*Fagopyrum esculentum* Moench) is a low-acreage specialty crop grown in North Dakota, and few yield limiting diseases have been identified in the state. It is an ideal crop for short growing seasons, because it matures relatively quickly. In 2008 variety trials in Minot, North Dakota in 2008, stem lesions, defoliation and premature senescence was observed for the first time. Samples were sent to the NDSU Plant Diagnostic Lab, but symptoms observed in the field and laboratory (discolored pith) were not consistent with any known disease that had been described on buckwheat.

## Methods

Prematurely senescing buckwheat plants were submitted to the Plant Diagnostic Laboratory at North Dakota State University in September 2008. Symptoms included lesions on stems with underlying light brown to grey discolored pith or no pith. Symptomatic tissue was surface sterilized and plated on 1/2-strength acidified potato dextrose agar, and two different fungi were isolated from affected stem tissue – one from discolored pith tissue (Isolate 1), and one from necrotic stem tissue (Isolate 2). Only Isolate 1 was further characterized by obtaining the sequence of the internal transcribed spacer rDNA region and comparing it to sequences from GenBank and type cultures.

A randomized complete block design with five varieties inoculated with either Isolate 1 or Isolate 2 and replicated four times was implemented in a controlled environment (growth chamber) with temperature maintained at 25 C and light:dark cycles of 16h:8h. Varieties of cultivated buckwheat used included Mancan, Manor, Kona, and two unnamed genotypes 26 and 29.

For each variety, Cone-tainer® pots were sown with three seeds and emerged seedlings were thinned to one plant per pot after emergence. Seventeen-day old seedlings were clipped at zero or one node above the cotyledon leaves and inoculated with either Isolate 1 or Isolate 2 by inverting 200-µl pipette tips containing a plug of the fungus (grown on potato dextrose agar) over the cut end of the stem (Figure 1). The cultivated buckwheat variety Mancan was used as a non-inoculated control by inverting a 200-µl pipette tip that had been seeded only with a plug of sterile potato dextrose agar.

One week after inoculation, plants were evaluated using three assessment techniques:

- Incidence: Presence (1) or absence (0) of symptoms,
- Severity: Where 0 = no symptoms; 1 = tip of inoculation site slightly discolored; 2 = darkened lesion only at tip of inoculation site with little expansion; 3 = stem darkened and shriveled just to next node; 4 = lesion expanded below next node, and
- Lesion length.

Re-isolation attempts were performed on selected symptomatic plants from the trial.

## Results and Discussion

No symptoms developed on any plants that were inoculated with Isolate 2; thus, Isolate 2 was not further characterized. For Isolate 1, disease developed on 18 plants of 20 that were inoculated, and the pathogen was successfully re-isolated from selected symptomatic plants. Symptoms included a tan lesion that extended below the point of inoculation (Figure 2), with pycnidia of the pathogen forming within the lesion after about 2 weeks. No significant differences in incidence or severity among varieties were observed (Table 1). However, significantly shorter lesions developed on genotype 29, compared to Mancan and Manor, suggesting that a varietal response to infection may exist.

Isolate 1 (designated AR 4603) first appeared cream to tan when grown on potato dextrose agar (Figure 3). By approximately four weeks of growth under ambient conditions and 12h:12h light:dark cycle, the colony had gradually darkened and pycnidia were generated. Culture characteristics of the pathogen were similar to that of *Phomopsis* or *Diaporthe* species. The sequence of the internal transcribed spacer rDNA region of AR 4603 (Figure 4) shared 99% homology with that of *Diaporthe stewartii* A.L. Harrison when compared with the type culture (CBS193.36). *D. stewartii* has been associated with *Cosmos* species and has been reported on wild buckwheat (*Eriogonum* species) (Dr. Amy Rossman, USDA, personal communication). *Eriogonum* species and other wild buckwheat (*Fallopia convolvulus*, also known as black bindweed) are in the same family as but are not closely related to cultivated buckwheat (*F. esculentum*).

In 2009, the disease was not observed in Minot, ND variety trials (same farm as initially detected in 2008, different field), nor were symptoms found on volunteer buckwheat growing in the same location as the initial 2008 detections. Scouting for the disease in the variety trials at NDSU North Central Research Extension Center will continue in 2010. Further study of this pathogen may be warranted, particularly since a varietal response to lesion length appears to exist. In a preliminary trial, plants appear to be more susceptible to infection as they approach maturity (data not shown). To the best of our knowledge, the host range of this pathogen has not been described and could influence future management strategies if this disease becomes a recurring problem in North Dakota, and it has not been reported on cultivated buckwheat (*F. esculentum*).

**Figure 4.** The sequence for the ITS1 region of AR 4603 is 99% homologous (544/547) with that of *D. stewartii*. Nucleotides in blue are identical to and those in red differ from the sequence of the same region for *D. stewartii* type culture (CBS193.36):

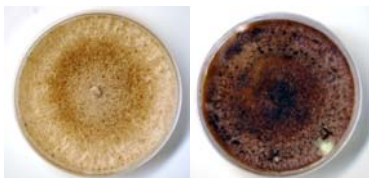
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CCGTTGGTGAACAGCGGAGGGATCATCTGCTGGAACCGCCCTGGCGCACCCA
GAAACCCCTTTGTGAACCTTATACCCATACCTGTTGCTCGGCGCAGCCGGCCCTTT
TCGACAAAGGCCCCCTGGAGACAGGGAGCAGCCGCGCGCGCCCAACCAAAAC
TCTGTTTCTATAGTGAATCTCTGAGTAAAAAACATAAATGAATCAAAACCTTCAAC
AACGGATCTCTGGTCTCTGGCATCGATGAAGAAGCAGCGGAATGCGGATAAGTAA
TGTGAATTGCGAGAATTCAGTGAATCATCGAATCTTTGAACGCGACATTCGCGCCCTC
GGTATTCCGGAGGGCATGCCTGTTCCAGCGCTCATTCAACCCCTCAAGCTGGCTT
GGTATGGGCGACTGCTGTGAAGAAGCGAGCCCTGAATCTAGTGGCGAGCTC
GCCAGAACCCGAGCGTAGTATTACATCTGCTGGAAGGCCCTGGCGGTGC
CCTGCCGTTAAACCCCAACTCTGAAATTTGACCTCGGATCAGGTAGGAATAC
CC
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**Table 1.** Pathogenicity of *D. stewartii* on varieties of cultivated buckwheat

Variety	Disease Incidence (0=no symptoms; 1=symptoms)	Disease Severity (0-4 scale)	Lesion Length (cm)
Mancan	1.00 a	3.62 a	4.06 a
Manor	0.88 a	3.13 a	4.44 a
Koma	0.88 a	3.13 a	3.33 ab
26	1.00 a	3.88 a	3.84 ab
29	1.00 a	3.63 a	2.89 b
Control	0.00 b	0.00 b	0.00 c
LSD(P=0.05)	0.20	0.75	1.17
CV	24.39	25.75	37.35



**Figure 1.** To inoculate a plant, a stem is snipped above the first or second node. A pipette tip (200-µl capacity) is then inverted over a colony of the pathogen growing on potato dextrose agar to collect a pathogen-and-agar plug, which remains in the wider portion of the pipette tip. The tip is then placed snugly over the cut end of the stem.



**Figure 3.** *D. stewartii* first appears cream to tan on potato dextrose agar (left). Pycnidia form within a few weeks. Over time, the colony darkens (right).



**Figure 2.** A diseased stem (left) shows a typical lesion with black pycnidia forming within (non-inoculated control at right).

## Acknowledgements

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