Dry Rot of Rutabaga Caused by *Fusarium avenaceum*

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Abstract. The rutabaga, also known as the swede turnip, is grown on ≈ 2000 ha in Canada. During the spring of 2006, a grower in Prince Edward Island noticed an advanced level of decay in his stored rutabagas (cv. Thompson Laurentian). About 80% of the stored crop was affected. Lesions on the surface of affected roots were circular to ovate and ranged in size from 10 to 50 mm. The lesions were light brown, with dark borders and some concentric zones evident near the perimeter of the affected tissue. Root tissue within the lesions was shrunk and often wrinkled. Sectioning the root through the lesion revealed an internal advancing dry rot, with an irregular border and cavities that contained white mycelium. Isolation from diseased tissues yielded fungal cultures, which were determined to be *Fusarium avenaceum* using morphological and molecular criteria. Successful completion of Koch's postulates determined that *F. avenaceum* was indeed the causal agent of rutabaga dry rot. To the authors' knowledge, this is the first report of *F. avenaceum* causing disease in rutabaga in Prince Edward Island, and likely only the second observation of its occurrence in North America. Dry rot, incited by *F. avenaceum*, may need to be considered as part of the spectrum of postharvest pathogens of rutabaga.

The rutabaga [*Brassica napus* L. (Napobrassica group)], also known as the swede turnip, is a member of Brassicaceae, and in North America is predominantly grown in California, Colorado, Wisconsin, and Minnesota, as well as several Canadian provinces. In Canada, production occurs on ≈ 1500 ha, with the majority in Ontario (41%), Quebec (29%), and the Atlantic provinces (25%) (Statistics Canada, 2007). Prince Edward Island rutabaga production accounts for $\approx 13\%$ of total production (Statistics Canada, 2007). Much of the crop is stored, sometimes for periods of up to 9 months. Rutabagas are commonly stored at temperatures near 0 °C to reduce postharvest decay, and at a relative humidity greater than 95% to reduce root shrinkage. Rutabagas are particularly susceptible to bruising, especially during mechanical harvesting, which can lead to the development of rot in storage. For this

reason, much of the crop in some production areas, including Prince Edward Island, is hand-harvested.

During the spring of 2006, a grower in Prince Edward Island noticed an advanced level of decay in his stored rutabagas (cv. Thompson Laurentian). These rutabagas (9 ha) had been harvested in mid October (typical harvest period in Prince Edward Island). After shipments to early markets, $\approx 122,500$ kg (30% of the harvested crop) was placed into storage. Symptoms of disease were first noticed toward the end of January and, by mid February, 80% of the stored crop was affected and ultimately unmarketable. Lesions on the surface of affected roots were circular to ovate and ranged in size from 10 to 50 mm (Fig. 1A). The lesions were light brown, with dark borders and some concentric zones evident near the perimeter of the affected tissue. Root tissue within the lesions was shrunk and often wrinkled. Sectioning the root through the lesion revealed an internal advancing dry rot, with an irregular border and cavities that contained white mycelium (Fig. 1B).

Sections of root tissue (10 $\times$ 5 $\times$ 3 mm) were excised from the margins of lesions from five diseased rutabagas with a sterile scalpel. The sections were then surface sterilized in 0.6% sodium hypochlorite for 2 min, rinsed twice in sterile distilled water, and blotted dry on sterile filter paper (Whatman

no. 4, Whatman, Florham Park, N.J.). Tissue pieces were then plated onto one-quarter strength potato dextrose agar (PDA) in small Petri dishes (60 $\times$ 15 mm; Fisher Scientific Co., Nepean, Ont.). Petri dishes were incubated in the dark at 22 °C for 7 d. After incubation, fungal growth was noted emanating from all tissue sections. Hyphal tips from the margins of actively growing cultures were removed with a sterile probe and plated onto PDA to generate pure cultures.

All isolates were morphologically similar, so one was chosen for more detailed identification. To identify the fungal isolate, the following procedures were carried out using DNA sequencing methods and micromorphological observations. Pure cultures were grown in 9-cm polystyrene Petri dishes on PDA for 5 d at 22 °C in the dark. Fungal mycelia were scraped from the surface of the agar using a sterilized scalpel, and total genomic DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN, Valencia, Calif.) according to the manufacturer's instructions. One microliter of aliquot was then analyzed on a 1.5% agarose gel in 0.5 $\times$ Tris–borate–ethylenediamine tetraacetic acid (EDTA) buffer to estimate the concentration and quality of the DNA.

Specific primer sets were used to amplify a 700-bp fragment of the translation elongation factor α gene (EF1- α) region (Barasubiye et al., 2005). Polymerase chain reaction (PCR) amplification was carried out in an Eppendorf cyclor (Brinkmann Instruments, Westbury, N.Y.). Each reaction mixture contained 10 ng (usually 1 μ L) diluted template genomic DNA, 1 $\times$ Titanium Taq buffer (10 $\times$), 3.5 mM MgCl₂ included in 10 $\times$ buffer, 0.1 mM each of four deoxyribonucleotide triphosphates, 0.08 μ M of each primer, and 1 U of Titanium Taq DNA polymerase in a total of 10 μ L. An initial denaturation step of 95 °C for 3 min was followed by 34 amplification cycles of denaturation (the first five cycles at 95 °C for 45 s, annealing at 68 °C for 45 s, and extension at 72 °C for 90 s). Cycles 6 to 10 and 11 to 34 used the same parameters except that the annealing temperature was 66 and 62 °C, respectively. After the 34 cycles were completed, the amplicons were incubated for an additional 8 min at 72 °C for a final extension. A negative control (without DNA template) was used to test for DNA contamination of reagents and reaction mixtures. To estimate the concentration and quality of PCR, 1 μ L amplicon from the PCR reaction was run on a 1.5% agarose gel with 0.5 μ g ethidium bromide/milliliter and 0.5 $\times$ Tris borate EDTA buffer for 45 min. DNA bands were visualized on an ultraviolet transilluminator.

Sequencing of the EF1- α template was performed using the BigDye Terminator V2.0 ready reaction kit from Applied Biosystems (Foster City, Calif.) and 0.5 $\times$ reactions. An estimated 10 to 20 ng of each amplicon was sequenced in both directions using the same primers described earlier for PCR. Sequencing reactions were analyzed with the ABI 3100-Avant automated sequencer

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Fig. 1. (A) External symptoms of dry rot of rutabaga incited by *Fusarium avenaceum*. (B) Internal decay of rutabaga after artificial inoculation of wounded roots with *F. avenaceum*. Note the control inoculation (agar disk only) on the left.

(Applied Biosystems-Hitachi). Contigs were assembled and sequences were edited using SeqMan version 6.1 (DNASTAR, Madison, Wisc.). The sequence was deposited in GenBank (accession no. EF105293) and it was used for searching for similar sequences in various databanks, including *FUSARIUM-ID* v. 1.0 (Geiser et al., 2004) and GenBank using the BLASTn program for nucleotide–nucleotide analysis (version 2.0, National Center for Biotechnology Information, U.S. National Institutes of Health, Bethesda, Md.). Using the BLASTn program, the five most significant sequences of *Fusarium avenaceum* (accession nos. AJ543520, AJ543519, AJ543518, AJ543517, and AJ543516) with 99.8% similarity to our strain were considered to confirm identity. A score higher than 1218 for the top five sequences was obtained, and the expectation E value was 0.0.

The culture was also grown on Spezieller Nährstoffarmer Agar (Nirenberg, 1981) with

filter paper at room temperature ($\approx 22^\circ\text{C}$ under mixed near-ultraviolet light), examined microscopically, and compared with taxonomic descriptions by Nelson et al. (1983) and Gerlach and Nirenberg (1982). The results obtained by DNA sequencing of EF-1 α were supported by micromorphological observations (shape of macroconidia). The culture was identified as *F. avenaceum* (Fr.:Fr.) Sacc., and it was deposited in the Canadian Collection of Fungal Cultures, Ottawa, under the accession no. DAOM 237752.

To complete Koch's postulates, healthy rutabagas were inoculated with the isolate of *F. avenaceum*. Five rutabagas (cv. Thompson Laurentian) were washed and air-dried before inoculation. Three wounds (15 mm in depth, 5 mm in diameter) were made with a cork borer on the sides of the root equidistant from each other. One wound in each root was inoculated with a small disk (5-mm diameter)

of PDA (control). The other two wounds on each root were each inoculated with a disk of PDA containing active mycelium of *F. avenaceum* taken from the margin of a 7-d-old culture growing on PDA. After inoculation, the excised root tissue plugs were placed back into the cavities created by their extraction to seal the wounds. Each rutabaga was then placed in a paper bag (Harvest Gold, 4.54 kg; PEI Bag Co. Ltd., Summerside, PEI) and stored in the dark at 10°C and 95% relative humidity in a vegetable storage facility for 5 weeks.

After 5 weeks in storage, the inoculated rutabagas were examined for external and internal symptoms of disease. Symptoms of disease were like those seen in the original diseased samples, including the development of typical dry rot in internal tissues (Fig. 1B). Control wounds inoculated with PDA disks only developed no disease symptoms (Fig. 1B). Isolations from diseased lesions using the same protocols described previously always yielded isolates of *F. avenaceum*, as determined by micromorphological observations (shape of macroconidia).

Fusarium avenaceum is a ubiquitous soil-borne fungus and occurs on a wide range of plant hosts (Booth, 1971). It is widely known as one of the causal agents of *Fusarium* dry rot of potato (Satyaprasad et al., 1997) and, in the Maritime region of Canada, is the cause of close to 15% of the dry rot encountered by growers (R.D. Peters, unpublished data). References to *F. avenaceum* as a pathogen of cruciferous crops are somewhat limited, but *F. avenaceum* has been found to be a pathogen of canola (*Brassica napus* L.) (Lange et al., 2000; Tahvonen et al., 1984), stored broccoli (*B. oleracea* var. *italica* Plenck.) (Mercier et al., 1991), and cabbage (*B. oleracea* var. *capitata* L.) in storage (Geeson, 1983), as well as the cause of head rot of cabbage in the field (Dillard and Cobb, 2006; Dillard et al., 2004). To our knowledge, this is the first report of *F. avenaceum* causing disease in rutabaga in Prince Edward Island, and likely only the second observation of its occurrence in North America, after a brief reference to surface rot, presumably incited by *F. avenaceum*, of swede turnips in Nova Scotia in 1961 (Creelman, 1962). Storage rot of rutabaga incited by *F. avenaceum* is not described in any of the recent guides to diseases of cruciferous crops (Howard et al., 1994; Rimmer et al., 2006). The incidence of this disease in North America is uncertain, and although likely low, the severity of disease development in the storage of concern in this study would indicate that it may need to be considered as part of the spectrum of post-harvest pathogens of rutabaga.

Fusarium species are ubiquitous in soil and often able to colonize plant wounds, sometimes leading to disease development. As mentioned, rutabagas are susceptible to bruising during harvest operations, which in turn also makes them susceptible to opportunistic wound pathogens. Although the diseased rutabagas described here were hand-harvested, root damage can still occur when harvested

roots are gathered and tossed into crates or handled before entering storage. In addition, tractors used to apply pest control products can sometimes damage roots before harvest. Minimizing root injury would be a key cultural practice to reduce storage rot, in addition to maintaining low storage temperatures. Thiabendazole has excellent efficacy for control of dry rot of potato incited by *F. avenaceum* (Hide et al., 1992; Satyaprasad et al., 1997) and is available as a postharvest application; however, this product is currently not registered for use on rutabaga. Chemical control may become an option in situations when *Fusarium* dry rot of rutabaga becomes entrenched.

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