A novel red clover virus associated with root colonization by *Olpidium* sp.

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Diseased red clover (*Trifolium pratense*) was observed in 2-year-old plants in a variety trial seeded at Prairie du Sac, WI. Similar symptoms were observed in space-plant nurseries at multiple disparate locations (Arlington, WI; Lancaster, WI; Marshfield, WI; Prairie du Sac, WI). Often healthy plants removed from the nurseries to the greenhouse developed disease symptoms. In the field, symptoms have appeared on 1- to 4-year-old plants, usually in late summer. Initial symptoms included yellowing of leaves (or sometimes reddening of leaves). Once discoloration began, younger leaves showed some stunting, malformation, and chlorosis. Symptoms were most severe in regrowth after forage harvests with complete plant death usually occurring within one to two forage harvests after symptom onset. This disease appears to be the primary agent of plant death in the U.S. Dairy Forage Research Center red clover breeding program.

Diseased plants were assayed for viral, fungal, and bacterial pathogens to identify the cause of the disease symptoms. Two types of virus-like particles were observed by transmission electron microscopy in partially-purified extracts from symptomatic leaves. The first were 30 nm spherical particles resembling those of cryptic viruses. The second were rod-shaped particles 18 nm in diameter with a prominent central canal. These particles were of varying length and had a strong tendency to aggregate end-to-end. In appearance the resembled virions of varicosaviruses. There was no evidence for the presence of phytoplasmas using a PCR-based assay. To investigate the role of the putative varicosavirus in disease development, soil and roots from diseased plants was collected, half autoclaved and half left untreated. These soils were mixed with a commercial potting mix, and planted with red clover. Plants from the untreated soil showed leaf yellowing and presence of rod-shaped viral particles. Roots of diseased plants were infected with a chytrid fungus, *Olpidium* sp., and *Thielaviopsis basicola*, a fungus with a broad host range causing root rot. Both fungi are known to infect red clover roots (1, 2). *Olpidium* is known to be a vector for varicosaviruses. It is unclear whether *Olpidium* infection causes significant direct damage to roots, but may predispose roots to infection by other pathogens and pests. Attempts to mechanically transmit the virus have not been successful to date. Cloning and characterization of the rod-shaped virus is in progress.

1. Skipp, R. A., and Deverall, B.J. 1990. Selection for persistence in red clover: influence of root disease and stem nematode. New Zealand Journal of Agricultural Research 33:319-333.
2. Tewari, J.P., and Bains, P. 1983. Fungi associated with the roots of clover in Alberta. I. *Olpidium brassicae* and *Ligniera* sp. Canadian Plant Disease Survey 63:2.