CONTROL AND ERADICATION OF CORKY RINGSPOT DISEASE [1,2]

by
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[2] *Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.*

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Disease Description and Situation in the Columbia Basin. Corky ringspot disease of potato has emerged as a major problem in the Columbia Basin of Washington and Oregon over the past few years. It causes dark rings, arcs, and flecks of dead tissue in tubers and renders them unmarketable. The disease is caused by tobacco rattle virus (TRV). This virus has an extensive host range (Robinson and Harrison, 1989; Cooper and Harrison, 1973) that includes many common weeds and many of the crops commonly grown in rotation with potato. TRV may be spread in the soil by several species of stubby root nematodes *(Paratrichodorus and Trichodorus* spp.) (Brown et. al. 1989; Van Hoof, 1968) but spread of the virus in the Columbia Basin appears to depend on a single, indigenous species, *Trichodorus allius* (Santo, et. al. 1997), that propagates well on many weeds and crops grown in the Columbia Basin. The nematode vectors acquire and transmit the virus while feeding (Taylor and Robertson, 1970) on root cells of their host plants. They can survive in the soil without host plants up to 3 years and still retain an ability to transmit the virus (Van Hoof, 1971 Some chemical treatments (Temik and Telone II) reduce, but do not eliminate (Ingham, 1993), nematodes in soil and may provide single season control of the disease. However, long term control has not been possible in the past since neither the causal virus or the nematodes that spread it could be totally eliminated from contaminated soils.

TRV may be spread to new soils that already contain the nematode vector in true seed of some plants, in infected seed potatoes, and in other infected, vegetatively propagated plants, such as tulip, narcissus, daffodil, and other flower bulbs and roots (Harrison and Robinson, 1978). It is also spread with its nematode vectors in soil clinging to machinery, boots, or the hooves of animals, transplants, and in water. The nematode is often present in Columbia Basin soils when the virus is not present.

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Although the nematode that transmits TRV in the Columbia Basin is native to the soils there, the virus apparently was not present until recent years.We reported the first case of corky ringspot in Washington state in 1975 (Thomas, 1977). Growers were advised then and again in 1990 (Thomas and Santo, 1990) on methods to prevent or reduce spread of the virus to new soils, and the disease initially appeared to remain localized to a few locations. From the first report in 1975 until 1989, only two new cases were diagnosed in our lab. However, after use of Temik was eliminated in 1989, the disease began to appear in new locations that had no previous history of corky ringspot (Thomas, et.al., 1993). By repressing numbers of nematode vectors, the wide-spread use of Temik may have masked dissemination of the virus during these years. At first the spread of the disease was slow, but numerous new cases of corky began to emerge in 1995. Growers reported that entire circles were lost, and major portions of other circles were excluded from the market.

This rapid expansion of the disease is troubling because the disease has not previously been eliminated from contaminated soils and the chemical control method that provides adequate control (fumigation with Telone II) is expensive and is subject to being removed from the market by regulation. In Oregon, R. E. Ingham (Ingham, 1993) tested a wide variety of chemicals. Only fumigation with Telone II or treatment with Temik alone and in various combinations provided good control. More recently, Santo et.al.(Santo et. al. 1998) found that Temik treatments applied under new regulations reduces but does not provide adequate control of corky ringspot disease on contaminated soils. Thus, only Telone II is available to provide good chemical control of the disease.

Two aspects of the epidemic in the Columbia Basin are unusual. First, the tuber symptoms are not always typical, and often appear unusually severe and expansive in the tuber. Infected tubers in the Columbia Basin often do not have the typical rings and arcs of dead tissue but, rather, have internal flecks and spots similar to those diagnosed as internal brown spot. Furthermore, while the rings and arcs of dead tissue typically break through the skin, symptoms of infected tubers in the Columbia Basin are often completely internal. These atypical symptoms could be due to the different environment of the Columbia Basin or they may be attributable to a different strain of the virus.

Another unusual aspect of the epidemic in the Columbia Basin is that the disease is often present throughout entire circles in the first year. Tobacco rattle virus normally enters a field as point source and its spread in the field is restricted to the rate of movement of the nematode vector - a few meters per year. This produces circular hot spots in the field, but it takes years for the entire field to become contaminated. The widespread and uniform occurrence often observed in the first year in the Columbia Basin suggests that the virus is being introduced in lots of infected seed potatoes.

Reliable Diagnosis of Tobacco Rattle Virus in Plant Tissues, Nematodes and Soil. Development of reliable methods to detect tobacco rattle virus in soil, nematode, and plant tissue samples was required, not only to identify the problem when we have it, but to implement a broad range of control strategies including the development of a corky ringspot disease prediction assay, seed certification, development of resistance, and basic studies on the biology of the disease.

We (Crosslin and Thomas 1995) adapted and developed three methods for detection of tobacco rattle virus. These methods are in place now for use by the industry in Washington. The methods are as follows:

1. **Bait Plant Method:** This is a method to detect whether or not the virus is present in the soil. The assay involves growing a bait plant in the soil to be assayed. Nematodes in the soil feed on and transmit the virus to roots of the bait plant. After a suitable period of time for virus to multiply in the roots of the bait plants, the virus is detected in the roots of the bait plant by the PCR method (Crosslin and Thomas), ELISA (see below) or a local lesion assay method. This bait plant method can detect the virus in samples containing so few nematodes that the nematodes often go undetected. Although it is an excellent assay, the bait plant assay requires several weeks to complete, is very laborious, and requires a lot of greenhouse space.

2. **PCR Method:** This is an extremely sensitive clinical method (Robinson, 1992) that is based on experimentally replicating the nucleic acid of the virus in a sample until quantities are present that are easily detected. In theory, it could detect the virus if but a single particle were present in a sample. It detects all strains of the virus.

3. **ELISA Method:** This is a more easily applied clinical method than is PCR. It is based on serological detection of the viral coat protein. A problem in this method is that all isolates of TRV do not produce coat protein, therefore, are not detectable by ELISA. However, it is rapid and convenient and has been useful in many routine studies.

Corky Ringspot Disease Prediction Assay. Using the bait plant method to detect TRV, we can now detect the virus in soils prior to planting. Thus, growers can identify fields that are at risk to corky ringspot disease before a decision about applying chemical soil treatment to control the disease. This could save the cost of the treatment where treatment is not necessary, and it could save the devastating losses that certainly occur when treatment is not applied where it is needed.

Eradication of Corky Ringspot Disease Potential from Soils by Cropping System. Historically, contamination of soil with TRV took the land out of potato production. The only legal alternative today, annual fumigation with Telone II, is very expensive. Since the virus is rarely introduced to new soils under careful sanitation, eradication of the virus from contaminated soils could provide good long-term control of the disease. By logic, it should be possible to eradicate the virus from soils by growing crops that are immune to either the virus or the nematode vector. The first requirement is to find an immune crop. Even then the capacity of the nematode vectors to survive and harbor the virus for years in the absence of a viral host plant (Van Hoof, 1971) requires that an immune crop be grown for several years. Furthermore, the extensive host range of the virus among both crop and weed species requires that the crop be grown completely free of weed hosts of the virus. These considerations have made eradication of the virus by appropriate cropping very difficult in the past. However, with modern weed control methods, it might be possible to eliminate the virus from soils if an immune host could be found.

The susceptibility of major cultivars of the major crop species of the Columbia Basin that are used in rotations with potatoes was assayed both in the greenhouse and field. The plants were grown in replicated plots in the field and in the greenhouse in soils collected from contaminated fields. Virus was routinely detected in the roots of all cultivars of wheat, sweet corn, field corn, and popular potato cultivars in both the greenhouse and field. The virus could not be detected in the roots of Sudan grass cultivars in the greenhouse, but was detected in the field. Virus was not detected in the foliage of any of these crops except, at times, in Russet Burbank and Norkotah potatoes. The virus was rarely detected in the roots of some resistant potato cultivars.

TRV was not detected in the roots or foliage of eight cultivars of alfalfa grown in contaminated field soil in a greenhouse. It was detected rarely and in extremely small quantities by the PCR procedure in roots of young alfalfa in the field. Quantities detected in alfalfa were so low as to suggest either that TRV does not multiply in alfalfa roots, in which case the virus detected was that injected by feeding nematodes, or that infection may occur only in the cell at the feeding site. In either case, it would be extremely unlikely that a second nematode would pick up the virus and carry it to another plant.

The eradication of TRV was monitored in a commercial circle that had sustained heavy corky ringspot losses in a potato crop and then was planted to alfalfa and maintained free of weeds. In the spring, following the potato crop, TRV was isolated from each of 50 soil samples taken from the field using the bait plant method. After two years in alfalfa, the virus was isolated from only one of 67 samples taken from the field. TRV could not be isolated from the field after 3 years of alfalfa.

Field results were confirmed by greenhouse studies. When viruliferous nematodes were propagated on alfalfa in the greenhouse, the nematodes lost their capacity to transmit the virus.

Weeds that were found infected in the field were Shepherds Purse, Lambsquarter, Chick-Weed, Pigweed and Prickly Lettuce. Infection of Chickweed and Shepherds Purse is of grave concern because seed transmission has been demonstrated in these hosts (Harrison and Robinson, 1978). Weeds found free of virus in limited testing were Dandelion, Crabgrass, Sowthistle, Barnyard Grass, and Tumble Mustard.

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