Role of Weeds in Persistence of Corky Ringspot in Crop Rotations

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Corky ringspot (CRS) is a serious disease of potato caused by tobacco rattle virus (TRV) and vectored by stubby root nematode (*Paratrichodorus allius*) in the western U.S. CRS causes necrotic arcs, rings, or spots in potato tubers, which can result in crop rejection. The nematode vector is more prevalent than the virus in Washington and Oregon (Mojtahedi and Santo 1999; Mojtahedi, et al. 2000). The incidence of CRS in the Pacific Northwest (PNW) potato growing regions has increased since first reported in Washington State in 1976 (Thomas et al. 1993). Soil fumigation with 1,3-dichloropropene prior to planting potatoes, in-furrow placement of aldicarb, and postemergence applications of oxamyl are the only tools currently available to growers to control or suppress *P. allius* populations.

Like other plant-parasitic nematodes, *P. allius* molts several times during its life cycle and sheds the virus with each molt (Robinson and Harrison, 1989). If young viruliferous nematodes are allowed to develop on a plant that is a non-host of TRV, the population becomes virus free after several generations. Several researchers (Rowe 1993 and Thomas et al. 1999) have suggested that incidence of CRS in potato is reduced when the crop follows alfalfa, implying that alfalfa was not a host of TRV. We have demonstrated in greenhouse trials that viruliferous stubby root nematode populations were no longer able to transmit TRV to the susceptible indicator plant tobacco, after growing for several generations on alfalfa, var. Vernema, or Scotch spearmint, var. 770 (Mojtahedi et al. 2002b and Boydston et al. in press), suggesting that CRS could be cleansed from fields by growing these two rotation crops. Although these crops may help reduce CRS in soils, the presence of weeds that are hosts of TRV and *P. allius* may contribute to disease persistence. Other rotation crops such as winter wheat, corn, and peppermint have been shown to act as virus reservoirs and stubby root nematode populations are not cleansed of TRV (Mojtahedi et al. 2002a).

Data compiled by Brunt et al. (1996) indicated that TRV has a wide host range including numerous weeds. Studies conducted in the PNW (Allen and Davis 1982; Davis and Allen 1975; and Locatelli et al. 1978) demonstrated that several weed species were naturally infected with TRV in commercial fields with CRS history. The primary interests on the impact of weeds on CRS are two fold. First, the weeds may serve as hosts for both TRV and *P. allius* to maintain the viruliferous vector population in the problem fields. Second, the virus may become seedborne in susceptible weeds, which could disseminate and introduce TRV to potato fields that already contain the more widespread stubby root nematode. In order to confirm the role of specific weeds in maintaining CRS in fields, it must be demonstrated that TRV can be acquired by a known nematode vector and transferred to potato or other susceptible hosts.

This report summarizes two studies that are published in their entirety in the American Journal of Potato Research (Mojtahedi et al. 2003 and Boydston et al. in press). The objectives of these studies were to 1) evaluate host status of 37 common weeds present in the Pacific Northwest for stubby root nematode and TRV, 2) determine the ability of selected weeds to serve as a reservoir of TRV when present in mixed cultures with alfalfa or spearmint, and 3) to determine their role in the CRS disease cycle of subsequent potato.

MATERIALS AND METHODS

Host suitability of various weeds to *P. allius* and TRV. Host suitability studies were conducted on 37 weed species commonly found in the Pacific Northwest (Table 1). Selected weeds were planted in pots containing sterilized sandy soil. Two weeks after planting, pots were inoculated with 100 viruliferous *P. allius* obtained from isolates collected from a CRS problem field near Pasco, WA and maintained on tobacco. Host suitability of weeds to stubby root nematode was determined by measuring changes in nematode populations 55 days after nematode introduction. At 4 weeks after introducing nematodes, roots were sampled for presence of TRV using ELISA. Roots were then thoroughly washed to remove any nematodes and weed seedlings were transplanted into sterilized soil. Nonviruliferous *P. allius* were then introduced to pots to determine if they could acquire TRV from infected weeds. After 4 weeks, host weed plants were removed and tobacco transplanted into pots to bioassay transmissibility of TRV. Tobacco was then observed for presence of TRV by visual symptoms and confirmed using ELISA.

Effect of weeds in maintaining CRS in alfalfa or spearmint mixtures. Five previously reported (Allen and Davis 1982; Jensen et al. 1974; Lister and Murant 1967; Locatelli et al. 1978) weed hosts of TRV, henbit, green foxtail, prickly lettuce, Powell amaranth, and hairy nightshade were grown separately or with alfalfa or Scotch spearmint in greenhouse pots over a three- or four-month period. A control treatment of tobacco, a host of *P. allius* and TRV, and indicator plant, was also included (Mojtahedi and Santo, 1999). Soil was sterilized with methyl bromide prior to each experiment to kill any existing nematodes.

Two weeks after planting, pots were inoculated with stubby root nematodes containing TRV. Viruliferous nematodes were obtained from isolates collected from a CRS problem field near Pasco, WA and maintained on tobacco. At 1, 2, 3, and 4 months after addition of viruliferous nematodes, soil was sampled and nematodes were isolated and counted to determine nematode populations in each pot.

Nematodes were then bioassayed for TRV by placing in sterilized soil containing a tobacco seedling. After 3 weeks, visual TRV symptoms on tobacco were recorded and TRV presence on tobacco roots was confirmed by ELISA. Root and shoot tissue of each weed and crop species growing alone were also tested for presence of TRV at each sampling date by ELISA and (or) RT-PCR (Crosslin and Thomas 1995).

As a final test, each pot was bioassayed with potato following the last sampling date in each experiment, to determine if TRV could be transmitted from the host plants by *P. allius* and cause CRS symptoms on potato. Plant roots and shoots were removed from the soil and certified seed of potato, var. Russet Burbank tubers were planted. All newly formed potato tubers were harvested after growing for 10 to14 weeks in the greenhouse. Tubers were sectioned longitudinally into four wedges and each cut surface examined for CRS symptoms.

RESULTS

I. Host suitability of various weeds to *P. allius* and TRV.

Twenty-four weed species served as suitable hosts of the nematode, but only 11 of those were infected with TRV (Table 1). The host status of a given weed species was not changed whether the viruliferous nematode population originated from CRS problem field in WA, OR, or ID. Several weeds that served as hosts for both the vector and virus included three nightshade species (black, hairy, and cutleaf), kochia, green foxtail, downy brome, prickly lettuce, henbit, common chickweed, annual sowthistle, and Canada thistle.

Four weeds, redstem filaree, flixweed, shepherd's purse, and western salsify were suitable hosts of TRV, but poor hosts of stubby root nematode (Table 1). These weeds could act as TRV reservoirs, where nematodes might acquire the virus during incidental feeding in plant mixtures.

Virus-free stubby root nematodes acquired TRV from the three nightshade species, volunteer potato grown from TRV-infected tubers, and prickly lettuce, and subsequently transmitted the virus to tobacco. Thus, some weeds may play a role in the epidemiology of CRS by perpetuating TRV and its vector in a problem field.

II. Role of five weeds in CRS persistence in alfalfa and Scotch spearmint.

Alfalfa and Scotch spearmint maintained *P. allius* populations and nematodes extracted from pure stands of these crops were unable to transmit TRV to indicator tobacco seedlings or potato. These results are in agreement with Mojtahedi et al. (2002b), suggesting alfalfa and 770 Scotch spearmint as rotation crops to suppress CRS.

Henbit. Henbit plants grown alone often tested positive for TRV throughout the experiment. TRV detected by ELISA was usually confined to root tissue, but virus was also located in shoot tissue occasionally. *Paratrichodorus allius* extracted from henbit was occasionally able to transmit TRV to tobacco. However, no TRV was detected in tobacco that received nematodes extracted from pots containing a mix of henbit and alfalfa or henbit and Scotch spearmint at any sampling date. **Similarly, no CRS symptoms were present on potato tubers that were grown in soil containing *P. allius* from pots containing mixes of henbit and alfalfa or spearmint.** The difficultly in maintaining live henbit plants in mixes with alfalfa spearmint throughout the duration of the experiment may have contributed to the lack of henbit acting as a virus reservoir in mixes with alfalfa and spearmint. Henbit may be particularly important in epidemiology of CRS as TRV was transmitted via seed to new seedlings when henbit plants were artificially inoculated with TRV (Lister and Murant, 1967).

Prickly lettuce. No TRV was detected by ELISA in prickly lettuce. However, TRV was often detected in tobacco that received nematodes extracted from pots containing prickly lettuce. These data suggest that prickly lettuce grown alone remained a reservoir for TRV even though levels were too low to detect with ELISA. TRV was detected in *Lactuca* sp., by ELISA in studies by Allen and Davis (1982). TRV was seldom detected in tobacco that was inoculated with *P. allius* extracted from pots containing mixes of prickly lettuce and alfalfa or prickly lettuce and spearmint. **However, slight CRS symptoms were present on potato tubers that were grown in soil containing *P. allius* from pots containing mixes of prickly lettuce and alfalfa,** indicating that low levels of TRV were maintained in pots containing prickly lettuce, and nematodes were able to acquire and transmit the virus to potato in a very low frequency. However, no CRS symptoms were present on potato tubers that were grown in soil harboring *P. allius* from pots containing mixes of prickly lettuce and Scotch spearmint.

Powell amaranth. No TRV was detected by ELISA in Powell amaranth. No TRV was detected in tobacco that received *P. allius* extracted from pots containing Powell amaranth (alone or in mixes with alfalfa or Scotch spearmint). **However, slight CRS symptoms were present on 1 potato tuber that was grown in soil containing *P. allius* from mixes of Powell amaranth and alfalfa, indicating that low levels of TRV were maintained in pots containing Powell amaranth with alfalfa.** Stubby root nematodes were able to acquire and transmit TRV from Powell amaranth to potato and cause slight symptoms on tubers.

Although nematode populations declined in pure cultures of Powell amaranth, nematodes thrived in mixtures of alfalfa and Powell amaranth and *P. allius* may have acquired TRV by incidental probing of Powell amaranth roots. Although a poor host for stubby root nematode in these studies, Powell amaranth may act as a TRV reservoir and incidental feeding by *P. allius* on the weed may transfer the virus to other hosts including potato.

Green foxtail. No TRV was detected by ELISA in green foxtail. TRV was rarely detected in tobacco that received *P. allius* extracted from pots containing green foxtail alone, and was never detected in tobacco that received *P. allius* extracted from mixes of green foxtail with alfalfa or spearmint. **However, slight CRS symptoms were sometimes present on potato tubers that were grown in soil containing *P. allius* reared on mixes of green foxtail and alfalfa or green foxtail and Scotch spearmint, indicating that low levels of TRV were maintained in pots containing green foxtail with alfalfa or spearmint.** Green foxtail was found to be a weak host of TRV in the previous host suitability study (see table 1). However, TRV was detected in green foxtail collected from fields with known CRS history (Allen and Davis 1982).

Hairy nightshade. Sixty to one hundred percent of hairy nightshade root tissue tested positive for TRV. Hairy nightshade root, shoot, and fruit tested positive for TRV in previous and current studies (Allen and Davis 1982, Boydston et al. unpublished), but TRV (as detected by ELISA) was confined to root tissue of hairy nightshade in this study.

P. allius isolated from pots containing hairy nightshade alone or in mixtures with Scotch spearmint or alfalfa were able to transmit TRV to tobacco at all sampling periods. **Potato planted in pots containing nematodes previously grown in the presence of hairy nightshade or mixes of hairy nightshade with alfalfa or Scotch spearmint exhibited severe CRS symptoms on tubers (Figure 2).** Hairy nightshade was reported a good host of TRV and the nematode vector (Allen and Davis 1982; Jensen et al. 1974; Mojtahedi et al. 2003) and may be particularly important for growers to target in weed control programs in fields with a history of CRS.

III. Implications.

Utilizing crop rotation to suppress CRS disease from soil is a desirable strategy that in theory could reduce the need for costly soil fumigation. Crops like alfalfa and Scotch spearmint can be utilized as rotational crops with potato to suppress CRS disease. However, these results illustrate that specific weeds can act as TRV reservoirs and nullify the beneficial effect of these rotational crops. We have demonstrated that *P. allius* maintained on weed hosts of TRV can retain the virus and transmit it to potato resulting in CRS symptoms.

In order to utilize crop rotation to suppress CRS, specific weeds that serve as reservoirs of TRV should be identified and controlled. In these studies hairy and black nightshade were particularly good hosts of both the nematode and the virus. Hairy nightshade is often difficult to control in potatoes and is a common weed in potato rotations in the Western U.S. Nightshade often infests potato crops late in the season when potato vines have begun to senesce. These late season infestations could further increase TRV levels in nematode populations and increase CRS persistence in problem fields.

Several effective herbicides are available for hairy nightshade control in alfalfa (bromoxynil, 2,4D-B, diuron, EPTC, hexazinone, imazethapyr, norflurazon, and terbacil) and Scotch spearmint (bentazon, bromoxynil, diuron, sulfentrazone, terbacil, pyridate). Multiple hay cuttings and the lack of soil disturbance by cultivation would also serve to suppress hairy nightshade in alfalfa and Scotch spearmint.

Although prickly lettuce, green foxtail, and Powell amaranth grown in mixtures with alfalfa and/or Scotch spearmint were able to maintain sufficient quantities of TRV that was transmitted by *P. allius* to potato resulting in slight CRS symptoms on tubers, their importance was far less than hairy nightshade. Volunteer potato may also be an important host of TRV and stubby root nematode and play a role in CRS persistence in problem fields.

Spread of TRV through weed seed could be important in epidemiology of CRS disease (Cooper and Harrison 1973; Lister and Murant 1967; Locatelli et al. 1978). Studies are in progress to determine seed transmission of TRV in several nightshade species.

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Table 1. Host status of 37 common weeds to stubby root nematode (*P. allius*) and TRV.

Weed tested as suitable hosts of both *P. allius* and TRV	
Black nightshade	*Solanum nigrum*
Hairy nightshade	*Solanum sarrachoides*
Cutleaf nightshade	*Solanum triflorum*
Common chickweed	*Stellaria media*
Green foxtail	*Setaria viridis*
Henbit	*Lamium amplexicaule*
Kochia	*Kochia scoparia*
Prickly lettuce	*Latuca serriola*
Annual sowthistle	*Sonchus oleraceus*
Downy brome	*Bromus tectorum*
Canada thistle	*Circium arvense*

Weeds tested suitable hosts of *P. allius*, but **not** TRV	
Lambsquarters	*Chenopodium album*
Russian thistle	*Salsola kali*
Field bindweed	*Convolvulus arvensis*
Common mallow	*Malva neglecta*
Dandelion	*Taraxacum officinale*
Yellow foxtail	*Seteria lutescens*
Barnyard grass	*Echinochloa crus-galli*
Quackgrass	*Agropyron repens*
Burmudagrass	*Cynodon dactylon*
Common purslane	*Portulaca oleracea*
Spiny sowthistle	*Sonchus asper*
Tumble mustard	*Sisymbrium altissimum*
Blue mustard	*Chorispora tennella*

Weeds tested suitable hosts of TRV, but poor host of *P. allius*	
Flixweed	*Descurainia sophia*
Shepherd's purse	*Capsella bursa-pastoris*
Western salsify	*Tragopogon dubius*
Redstem filaree	*Erodium cicutarium*

Weeds tested poor host of *P. allius* and TRV	
Powell amaranth	*Amaranthus powelli*
Redroot pigweed	*Amaranthus retroflexus*
Prostrate pigweed	*Amaranthus blitoides*
Common groundsel	*Senecio vulgaris*
Longspine sandbur	*Cenchrus longispinus*
Jagged chickweed	*Stellaria umbillatum*
Horseweed	*Conyza canadensis*
Umbrella spurry	*Holosteum umbellatum*
Coast fiddleneck	*Amsinckia intermedia*

Figure 1. CRS symptoms on potato tubers grown in soil containing *P. allius*, which was maintained for 3 months on host plants of Scotch spearmint, hairy nightshade, a mix of Scotch spearmint and hairy nightshade, alfalfa, a mix of alfalfa and hairy nightshade, and tobacco.

Table 2. Detection of tobacco rattle virus (TRV) by ELISA in alfalfa, Scotch spearmint, and hairy nightshade after inoculating with 150 viruliferous *Paratrichodorus allius* and detection of TRV by ELISA in tobacco that received nematodes extracted from pots containing alfalfa, Scotch spearmint, hairy nightshade, or mixtures of crops with hairy nightshade.

	---2 month---		---3 month---	
	No. TRV (+)/no. tested		No. TRV (+)/no. tested	
Host plant(s)	Host plant	Indicator tobacco	Host plant	Indicator tobacco
Tobacco	**5/5**	**5/5**	**4/5**	**5/5**
Alfalfa	0/5	0/5	0/5	0/5
S. Spearmint	0/5	0/5	0/5	0/4
H. Nightshade	**5/5**	**5/5**	**3/5**	**5/5**
Alfalfa + H. nightshade	*	**4/5**	*	**5/5**
Spearmint + H. nightshade	*	**3/5**	*	**3/5**

* no data obtained