Journal of Nematology 39(4):313–316. 2007.

Establishing a Corky Ringspot Disease Plot for Research Purposes

H. Mojtahedi,[1] R. A. Boydston,[1] J. M. Crosslin,[1] C. R. Brown,[1] E. Riga,[2] T. L. Anderson,[1] D. Spellman,[1] R. A. Quick[1]

Abstract: A method to establish two experimental corky ringspot disease (CRS) plots that had no prior CRS history is described. CRS is a serious disease of potato in the Pacific Northwest caused by tobacco rattle virus (TRV) and transmitted primarily by *Paratrichodorus allius*. 'Samsun NN' tobacco seedlings were inoculated with viruliferous *P. allius* in the greenhouse before they were transplanted into the field soil at the rate of 3,000 plus seedlings/ha. Care was taken to keep soil around plants in the greenhouse and transplants in the field moist to avoid vector mortality. The vector population in the soil of one of the fields was monitored by extraction, examination under microscope and bioassay on tobacco seedlings to ascertain that they were virus carriers. Presence of virus in tobacco bioassay plants was determined by visual symptoms on tobacco leaves and by testing leaves and roots using ELISA. Although TRV transmission was rapid, there was loss of infectivity in the first winter which necessitated a re-inoculation. After two years of planting infected tobacco seedlings, 100% of soil samples collected from this field contained viruliferous *P. allius*. In the second field, all five commercial potato cultivars, known to be susceptible, expressed symptoms of CRS disease indicating that the procedure was successful.

Key words: method, *Paratrichodorus allius*, potato, *Solanum tuberosum*, tobacco rattle virus

Corky ringspot disease (CRS) of potato, caused by tobacco rattle virus (TRV) and vectored by trichodorid nematodes, is an important potato disease in the Pacific Northwest. *Paratrichodorus allius* (Jensen) Siddiqi is the most prevalent trichodorid vector of TRV in the region, and TRV was present in 10% of fields containing that nematode (Mojtahedi et al., 2000). Nevertheless, CRS increases in importance every year (Pelter, 1997). The disease is characterized by distinct necrotic concentric rings and diffuse brown blemishes in tubers that render the crop unmarketable. The research program at the Irrigated Agriculture Research and Extension Center (IAREC) Washington State University (WSU) and USDA-ARS in Prosser, WA, addresses diverse issues related to this disease: (i) population dynamics of the vector (Mojtahedi and Santo, 1999), (ii) control of vector nematode with soil fumigants and non-fumigant chemicals (Santo et al., 1997), (iii) control of vector nematode with cultural methods (Thomas et al., 1999; Riga and Collins, 2004), and (iv) selection of TRV-resistant potato germplasm (Crosslin et al., 1999; Brown et al., 2000). Previous collaborations with growers who had fields with CRS history have been very successful, especially for short-term research. However, seeking cooperation for multi-year projects involving crop rotation, field testing of breeding lines and evaluating the alternative control measures requires a local, accessible population. To avoid inconveniences to growers and researchers, two 0.5 ha CRS disease plots were established at IAREC field units where multi-year CRS research projects could be initiated. One site is at the WSU Pear Acres research farming unit and the other is at the WSU Roza unit. Currently, these plots are used to select CRS-resistant breeding lines and evaluate non-chemical control measures to alleviate the CRS problem on potato. This report describes the procedure and details of our successful establishment of CRS research plots, in particular, the field at the WSU Roza unit.

Materials and Methods

Field plot: In the spring of 2005, a 0.5 ha field plot (110 m × 49 m) at WSU Roza unit was allocated for CRS studies. The soil was sandy loam (70% sand, 24% silt, 6% clay; 1.05% organic matter; pH 6.6). Alfalfa variety 'Perfect' had been grown the previous 2 yr under sprinkler irrigation.

In July 2005, alfalfa was killed with glyphosate, rototilled shallow, disc-plowed and packed. One week later, 15 composite soil samples (10 probes/each) for nematode analysis were collected randomly across the field by using a 7.5-cm-diam. soil auger 25-cm deep. Nematodes were extracted from 250 cm^3 sub-samples (Jenkins, 1964), and their specific identities determined and recorded. The trichodorid nematode in Roza proved to be *Paratrichodorus allius*. The nematode-infested soil samples were placed in 10-cm-diam. clay pots; 3-wk-old 'Samsun NN' tobacco (*Nicotiana tabacum* L.) seedlings were transplanted into pots, and previously extracted nematodes from Roza soil were added around the root system. 'Samsun NN' tobacco is a suitable host for *P. allius* and TRV (Mojtahedi and Santo, 1999), and the virus causes diagnostic symptoms on tobacco leaves (Robinson and Harrison, 1989). The presence of TRV in roots and leaves of tobacco plants was further tested by ELISA (Converse and Martin, 1990).

Vector and virus isolates and preparing infested tobacco seedlings: A population of viruliferous *P. allius* was isolated from a potato field with CRS history near Pasco, WA, and was maintained in pots on 'Samsun NN' tobacco. This population transmitted TRV and caused

Received for publication May 25, 2007.

[1] USDA-ARS, 24106 N. Bunn Rd., Prosser, WA 99350

[2] Washington State University, IAREC, 24106 N. Bunn Rd., Prosser, WA 99350. E. Riga is supported by the College of Agricultural, Human, and Natural Resource Sciences Agricultural Research Centre, PPNS No. 0452, Department of Plant Pathology and IAREC WSU-Prosser, Project No.WNP00542, Washington State University, Pullman, WA 99164–6430, USA.

E-mail: rick.boydston@ars.usda.gov

This paper was edited by Gregor Yeates.

typical TRV symptoms on tobacco. Leaf samples from these tobacco plants were tested by ELISA and found to be TRV-positive. Soil was removed from tobacco pots, *P. allius* extracted and 15 nematodes/tobacco seedling added around tobacco seedlings planted in 72-cell tray inserts filled with sandy loam soil previously fumigated with methyl bromide. The tobacco seedlings were maintained in the greenhouse for 3 wk before moving them outdoors to acclimate for 1–2 d. Due to extreme sensitivity of *P. allius* to drought (Mojtahedi and Santo, 1999), care was taken in the greenhouse and during acclimation to keep the soil moist. The tobacco seedlings were then transplanted in the field and watered abundantly to keep seedlings and nematodes alive.

Conduct of field trials: Between 3 and 24 August 2005, 1,700 tobacco seedlings infested with viruliferous *P. allius* (described previously) were planted 1.5 m apart in rows spaced 3 m at the WSU Roza site. The ground was kept wet to avoid vector mortality due to drought and in late September plants were fertilized with 20 kg N/ha in the form of ammonium sulfate. The soil was sampled for nematodes in early November 2005 before freezing temperatures killed the tobacco plants. The soil was tilled and prepared for winter wheat *Triticum aestivum* L. "Stephens" seeding, but cold weather and snow prevented seeding until mid-January 2006. The ground was colonized by some annual and perennial weeds between the decline of tobacco plants and tilling the ground. It was also observed that weeds were present in early spring of 2006 before wheat began to tiller and cover the ground. The wheat was mowed to a height of 15 cm once in late May and then again in late June 2006. To boost nematode population in the field, on 30 June, 5 and 12 July and 10 August 2006, 1,800 infested new tobacco seedlings were planted in strips (1.5 m wide spaced every 3 m) where wheat had been removed by rototilling. Wheat between the tobacco strips was mowed periodically to maintain growth, prevent seed production and suppress weeds.

After tobacco seedlings were introduced, the soil was sampled for nematode and TRV assays five times between 9 November 2005 and 14 March 2007 (Table 1). The field was sampled along two diagonal lines with an auger (3-cm-diam.) 30 cm deep. One core was taken every meter, and 10 cores were combined and mixed to comprise one sample. Part of this soil sample (250 cm^3) was used to extract and tally the nematodes, and the other part (500 cm^3) was placed in 10-cm-diam. clay pot and bioassayed for TRV on tobacco as described previously. The presence of TRV on tobacco was confirmed by ELISA.

In October 2006, wheat and tobacco were discplowed and packed. Wheat was planted in late October and became established through the winter. In mid-

TABLE 1. Number of *Paratrichodoris allius* (Pa) per 250 cm^3 soil sample collected at different times from the WSU Roza site during 2005–07 and transmission (+/−) of tobacco rattle virus (TRV) to 'Samsun NN' tobacco seedlings by Pa as detected by ELISA test on tobacco leaves and (or) roots[1]. Data are for individual soil and plant samples.

Sample no.[1]	2 Aug 2005 (before tobacco)		9 Nov 2005 (after tobacco)		5 Apr 2006 (before tobacco)		3 May 2006 (before tobacco)		12 Oct 2006 (after tobacco)		14 Mar 2007 (after overwintering)	
	Pa	TRV	Pa	TRV	Pa	TRV	Pa	TRV	Pa	TRV	Pa	TRV
1	36	–	10	+	0	–	0	–	10	+	10	+
2	2	–	36	+	14	–	14	–	60	+	15	+
3	1	–	12	+	13	–	16	–	60	+	0	+
4	22	–	12	+	60	–	26	–	120	+	40	+
5	3	–	24	+	0	–	64	–	120	+	130	+
6	1	–	0	+	12	–	8	–	1	+	5	+
7	6	–	6	+	20	–	32	–	10	+	24	+
8	2	–	24	+	2	–	8	–	5	+	4	+
9	10	–	2	+	14	–	5	–	50	+	6	+
10	0	–	6	+	10	–	1	–	120	+	10	+
11	10	–	5	+	5	–	2	–	5	+	180	+
12	1	–	10	–	1	–	3	–	60	+	40	+
13	15	–	30	+	10	–	12	–	10	+	52	+
14	6	–	24	–	9	–	4	–	40	+	20	+
15	10	–	1	+	1	–	7	–	20	+	40	+
16					3	–	14	–	40	+	10	+
17					1	–	16	–	10	+	5	+
18					2	+	25	+	10	+	25	+
19					2	–	3	–			10	+
20					36	–	40	–			25	+
21											26	+
22											96	+
23											12	+
24											2	+
25											60	+

[1] Each data point represents a single observation per nematode count and bioassay results on tobacco.

March 2007, the soil was sampled again and bioassayed for nematodes and virus.

The Pear Acres site was infested with TRV and *P. allius* in 2001 by transplanting tobacco previously inoculated with viruliferous *P. allius* in strips for two consecutive years, but the nematode and viral infestation of ground was not monitored as regularly as the Roza field. After establishing the nematode and vector, this field was partitioned between potato and wheat in 2003 and rotated between these two crops each year. This site has been used to select resistant potato breeding lines and to study the nature of resistance to CRS (Brown et al., 2007).

Results and Discussion

The native plant-parasitic nematode fauna in the alfalfa field at the Roza in August 2005 included *P. allius* (8 ± 3/250 cm^3), *Pratylenchus penetrans* (166 ± 21/250 cm^3) and *Meloidogyne hapla* (323 ± 76/250 cm^3). All three species are parasitic on potato. However, the symptoms the latter two species of nematodes cause on potato are distinctly different from TRV and would not interfere with assessment of CRS symptoms on potato in the future. The native *P. allius* population was free from TRV at the onset of the experiment (Table 1), which simplified the evaluation of success in establishing viruliferous nematodes at the Roza site.

As a TRV vector, the damage threshold of *P. allius* is 3 nematodes/250 cm^3 soil (Mojtahedi et al., 2000). Based on TRV symptoms developed on leaves and stems of tobacco bioassay plants grown in TRV-infested soil containing 2 to180 *P. allius*/250 cm^3 (Table 1), we are confident that the Roza field contains adequate vector and virus to provide suitable CRS disease pressure and symptomology on susceptible potato for future research, comparable to the Pear Acres plot established earlier (Table 2).

Almost 87% of nematode samples collected in November 2005 were found to be positive for TRV (Table 1). These nematodes presumably acquired TRV from the 1,700 infected tobacco plants transplanted into the field that year. However, most of the samples (95%) did not test positive for TRV in spring 2006 (Table 1). The nematodes appeared to have lost TRV during winter 2005 and early spring 2006. It is plausible that, in the absence of a suitable TRV host like tobacco or wheat, the nematodes fed on weeds such as common lambsquarters (*Chenopodium album* L.), common mallow (*Malva neglecta* Wallr.), tumble mustard (*Sisymbrium altissimum* L.) and spiny sowthistle (*Sonchus asper* (L.) Hill), which are hosts of *P. allius* but not of TRV (Mojtahedi et al., 2003). Consequently, the *P. allius* population may have lost its virus load by feeding and molting on these weeds (Cooper and Harrison, 1973; Boydston et al., 2004). Additionally, some mature stages may have died and been replaced by recently hatched individuals. The highest surge of *P. allius* population has been observed in March to April in Columbia Basin (Mojtahedi and Santo, 1999). Due to the late planting of wheat in January, wheat seedlings were still relatively small in March to April of 2006, and non-TRV host weeds were present in the plot. In 2006, we planted winter wheat, a suitable host of vector and TRV (Mojtahedi et al., 2002), early enough (October) for nematodes to thrive and retain their virus load in November. The nematodes continued to remain viruliferous until March 2007, when 100% of samples were TRV-positive (Table 1).

We intend to utilize the WSU Roza site to determine if growing weed-free alfalfa will cleanse the *P. allius* population of TRV sufficiently so that a crop of potato can be grown without soil fumigation. Soil fumigation is costly and is an environmental concern.

The WSU Pear Acres field has also been infested with viruliferous *P. allius* by sequential transplanting of TRV-infected tobacco seedlings in a similar fashion to that described for Roza unit. Presently, this site is used to screen the resistant potato lines. High incidence of disease on susceptible lines is essential to ascertain that a lack of symptoms on a resistant line is not due to a chance escape, but rather due to inherent resistance to TRV infection. On an annual basis for the past 4 years, we have scored 12 to 82% of disease incidence (43% on the average) on susceptible varieties like 'Russet Nor-

Table 2. Incidence (%) of corky ringspot (CRS) and disease severity ± standard error on selected susceptible potato cultivars planted in four successive years (2003–06) in manually established CRS disease field at WSU Pear Acres site.

	2003 (6 reps × 10 tubers/rep)		2004[3] (4 reps × 10 tubers/experiment)		2005 (6 reps × 10 tubers/rep)		2006 (6 reps × 10 tubers/rep)	
Potato cultivars	Incidence[1]	Severity[2]	Incidence	Severity	Incidence	Severity	Incidence	Severity
Russet Norkotah	14 ± 8	1.0 ± 0.6	33 ± 10	1.8 ± 0.5	20 ± 14	1.5 ± 1.0	23 ± 11	1.4 ± 0.8
Ranger Russet	—	—	82 ± 7	5.4 ± 0.5	36 ± 22	2.8 ± 1.8	50 ± 15	3.0 ± 1.3
Red La Soda	—	—	60 ± 7	3.1 ± 0.4	23 ± 19	0.9 ± 0.8	50 ± 7	2.6 ± 0.5
Russet Burbank	38 ± 12	2.0 ± 0.8	74 ± 7	4.1 ± 0.8	12 ± 8	0.6 ± 0.3	55 ± 9	2.5 ± 0.5
Dark Red Norland	45 ± 15	2.7 ± 1.0	75 ± 8	4.8 ± 0.6	38 ± 14	2.0 ± 0.7	43 ± 13	2.7 ± 0.8

[1] Presence of any corky ringspot symptom on tuber flesh rendered it as an infected tuber.
[2] Severity of CRS disease was based on coverage of tuber flesh with symptoms. Tubers were cut into 4 wedges, and presence of CRS symptoms on any of the 8 facets was given a numeric value of 1, thus the intensity of CRS ranged between 0 (no symptoms) to 8 (all 8 sites symptomatic).
[3] Average of two experiments.

kotah', 'Ranger Russet', 'Red Lasoda', 'Russet Burbank' and 'Dark Red Norland' in Pear Acres with disease severity (explained in Table 2) ranging between 0.6 to 5.4 (on average 2.5) (Table 2). Importantly, CRS-susceptible potato cultivars interspersed with resistant breeding lines in the WSU Pear Acres field always developed symptoms of CRS.

These results suggest that the procedure described above involving planting infected tobacco spaced 1.5 × 3 m is an effective technique to establish a CRS disease plot with high disease pressure. To maintain levels of virus and vector nematodes, potato is alternated with winter wheat and field corn (Mojtahedi et al., 2002).

Literature Cited

Boydston, R. A., Mojtahedi, H., Crosslin, J. M., Thomas, P. E., Anderson, T. L., and Riga, E. 2004. Evidence for the influence of weeds on corky ringspot persistence in alfalfa and Scotch spearmint rotations. American Journal of Potato Research 81:215–225.

Brown, C. R., Crosslin, J. M., Mojtahedi, H., James, S., and Charlton, B. 2007. Stability and nature of resistance to corky ringspot disease in an advanced tetraploid breeding population of potato. American Journal of Potato Research. 84:79.

Brown, C. R., Mojtahedi, H., Santo, G. S., Hamm, P., Pavek, J. J., Corsini, D., Love, S., Crosslin, J. M., and Thomas, P. E. 2000. Potato germplasm resistant to corky ringspot disease. American Journal of Potato Research 77:23–27.

Converse, R. H. and Martin, R. R. 1990. ELISA methods for plant viruses. Pp. 179–196 *in* R. Hampton, E. Ball, and S. De Boer, eds. Serological methods for Detection and Identification of viral and bacterial Plant Pathogens. St Paul, Minnesota: American Phytopathological Society Press.

Cooper, J. I., and Harrison, B. D. 1973. The role of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. Annals of Applied Biology 73:53–66.

Crosslin, J. M., Thomas, P. E., and Brown, C. R. 1999. Distribution of tobacco rattle virus in tubers of resistant and susceptible potatoes and systemic movement of virus into daughter plants. American Journal of Potato Research 76:191–197.

Jenkins, W. R. 1964. A rapid centrifugal-floatation technique for separating nematodes from soil. Plant Disease Reporter 48:692.

Mojtahedi, H., Boydston, R. A., Thomas, P. E., Crosslin, J. M., Santo, G. S., Riga, E., and Anderson, T. L. 2003. Weed hosts of *Paratrichodorus allius* and tobacco rattle virus in the Pacific Northwest. American Journal of Potato Research 80:379–385.

Mojtahedi, H., Crosslin, J. M., Thomas, P. E., Santo, G. S., Brown, C. R., and Wilson, J. H. 2002. Impact of wheat and corn as rotational crops on corky ringspot disease of Russet Norkotah potato. American Journal of Potato Research 79:339–344.

Mojtahedi, H., and Santo, G. S. 1999. Ecology of *Paratrichodorus allius* and its relationship to the corky ringspot disease of potato in the Pacific Northwest. American Journal of Potato Research 76:273–280.

Mojtahedi, H., Santo, G. S., Crosslin, J. M., Brown, C. R., and Thomas, P. E. 2000. Corky ringspot: Review of the current situation. Pp. 9–13 *in* Proceedings 39th Washington State Potato Conference, Moses Lake, WA. http://www.potatoes.com/

Pelter, G. 1997. Corky ringspot disease of potatoes. Washington Potato Commission Spud Topics 43:1.

Riga, E., and Collins, H. 2004. Green manure effects on *Meloidogyne chitwoodi* and *Paratrichodorus allius*, economically important nematodes of potato in the Pacific Northwest of the USA. Agroindustria 3:321–322.

Robinson, D. J., and Harrison, B. D. 1989. Tobacco rattle virus. CAB description of Plant Viruses No. 346 (no. 12 revised).

Santo, G. S., Wilson, J. H., and Mojtahedi, H. 1997. Management of Columbia root-knot nematode and corky ringspot disease on potato. Pp.17–25 *in* Proceedings 36th Annual Washington State Potato Conference. Moses Lake, WA. pp 17–25. http://www.potatoes.com/

Thomas, P. E., Mojtahedi, H., Crosslin, J. M., and Santo, G. S. 1999. Eradication of tobacco rattle virus from soils by growing weed-free alfalfa. Pp. 164–65 *in* Proceedings of VII International Plant Virus Epidemiology Symposium. Aguadulce (Almeria), Spain (abst).