

APPENDIX B

Soilborne Pathogens and Root Diseases - Wilke Farm

Soilborne Pathogens and Root Diseases - Wilke Farm

No disease or pathogen measurements were made over the course of these rotation studies at the Wilke farm. However, in Fall 2003, soil samples were taken from the rotations at the end of the experiment. These samples were sent to Australia for DNA analysis by SARDI (South Australian Research and Development Institute) (Ophel-Keller 2003). This test provides a quantitative analysis of the pathogen populations in the soil, based on picograms DNA/g of soil. With this method, DNA is extracted from soil, and PCR primers specific to each pathogen are used to amplify the DNA. The following pathogens are detected by this test: *Gaeumannomyces graminis* var. *tritici* (take-all), *Rhizoctonia solani* AG8, (*Rhizoctonia* root rot), *Heterodera avenae* (cereal cyst nematode), *Pratylenchus neglectus*, and *P. thornei* (lesion nematode); *Fusarium pseudograminearum* and *F. culmorum* (*Fusarium* crown rot), and *Bipolaris sorokiniana* (common root rot).

We hypothesized that root diseases result from inoculum in the soil and residue, which are produced during the previous year(s) of cropping. These pathogens produce resistant spores (*Fusarium*, *Bipolaris*) or survive as mycelium in decaying roots (*Rhizoctonia*, *Gaeumannomyces*). This test also detects 3 important nematodes, cereal cyst nematode (*Heterodera*) and 2 species of lesion nematode (*Pratylenchus*).

It is well known that crop rotation will influence certain root pathogens, if one of the crops is a non-host or if there is no crop (fallow). During this unfavorable period for the pathogen, inoculum in the soil will decline over time due to microbial attack, feeding by soil invertebrates, or loss of energy through respiration. In any case, the inoculum level of certain pathogens will be reduced following crop rotation and fallow, and we hypothesized this test would detect these differences.

Methods and Materials

Soil samples were taken from 3 locations within each rotation strip of 8-10 acres. These locations were randomly chosen within the north, middle, and southern sections of the rotation strips. Three samples were taken from every rotation strip, and 9 replicated strips were sampled for the 3-year rotations and 12 replicated strips from the 4-year rotations. Approximately 1 kg of soil was dug from the upper 6 inches. Soil was air-dried and frozen at -20°C for 2 weeks before being sent to Australia. The freezing was a quarantine requirement to kill any insects in the soil.

Results were received in a spreadsheet as picograms DNA/g soil. DNA levels were log transformed after adding 1 to each of the values, in order to make the data normally distributed. ANOVA was performed using rotation and strips as main factors. Means were separated with LSD at $P=0.05$. If data was not normally distributed after transformation, means were separated with Kruskal-Wallis One-Way non-parametric ANOVA. Risk levels were also assigned to the values, based on Australian conditions, but may not be applicable to our conditions.

Results and Discussion

The take-all pathogen, *Gaumannomyces graminis* var *tritici*, was below detectable levels in all samples taken. This pathogen is normally not a problem in the normal 3- or 4-year rotations in eastern Washington that contain a non-host broadleaf crop (peas, chickpeas, lentils, canola, sunflower, safflower, buckwheat, flax), a fallow, or a non-host monocot (corn, oats, millet). This data fits the observations of numerous studies of this disease over the past 50 years. The cereal cyst nematode was not detected in any samples. This nematode is a problem in northeast Oregon and the Willamette Valley, but has not been detected in the dryland areas of Washington. *Pratylenchus neglectus*, the root lesion nematode, was only detected in 12 out of 63 samples, and was in low levels at those sites. *P. thornei*, the more virulent of the 2 species, was found in only 1 sample.

These nematodes have been implicated in yield losses on cereals in Oregon (Smiley, personal communication and submitted manuscripts). They have a wide host range, with wheat being the most preferred host, followed by barley. They can also reproduce on broadleaf crops, but there is variability among species and cultivars in terms of reproductive success. These rotation crops may have limited the buildup of these nematodes, but we cannot identify the effect of specific broadleaf crops in this experiment.

Surprisingly, *Rhizoctonia solani* AG-8 was not detected in most of the samples. It was only detected in 6 out of 63 samples, in low levels in 5 of those samples. This finding was surprising. Of all the soilborne pathogens, *Rhizoctonia solani* has the strongest evidence for increasing under direct-seeding conditions. This increase is often found early in the transition from conventional to direct-seeding (years 3-4 in studies by Schroeder, 2004, in Garfield, WA). But, in longterm direct-seeded fields, he did not find any differences, when compared with conventional tillage.

The Wilke farm may have transitioned to this more suppressive state regarding *Rhizoctonia*. However, this test detects only one AG group, AG-8. We have found other AG groups, specifically AG 2-1, which is pathogenic to brassicas. *R. oryzae* also causes root rot in the PNW, many isolates are highly virulent (Paulitz et al. 2003), and this pathogen would not be detected by this test.

The 3 pathogens, *F. culmorum*, *F. pseudograminearum*, and *Bipolaris sorokiniana*, cause a crown, foot, and root rot complex. Most of the research in the PNW has focused on *Fusarium*, which is increased by plant drought stress and excessive nitrogen. *F. pseudograminearum* is more predominant in the low rainfall areas, and *F. culmorum* in the higher rainfall areas. This was supported by the data at the Wilke farm (Table 1), which agrees with other surveys in the Palouse area. This data indicates that *F. culmorum* is responsible for *Fusarium* crown rot at this site. It was found in low levels in 38 samples, medium levels in 15 samples, and high levels in 9 samples.

Table 1B. DNA levels of crown and foot rot pathogens in the soil of 3-year and 4-year rotations at the Wilke Farm, Fall 2003.

Rotation	<i>Fusarium pseudograminearum</i> (log pg DNA/g soil)	Risk	<i>Fusarium culmorum</i> (log pg DNA/g soil)	Risk	<i>Bipolaris sorokiniana</i> (log pg DNA/g soil)	Risk
3-year	0.41	Below detection	1.92	low	1.22	low
4-year	0.17	Below detection	1.66	low	0.39	Below detection
P value	0.03		NS		0.001	

On the other hand, *F. pseudogramineum* was below the detection limit in 54 out of 63 samples. *Bipolaris sorokiniana* was detected in many of the samples. Although little research has been done on this pathogen in the PNW, surveys from 1994 indicated it was important in Adams, Lincoln, and Whitman counties (Smiley and Patterson 1996). It was also one of the predominant pathogens isolated from the roots in a survey in Idaho (Strausbaugh et al. 2004). At the Wilke farm, it was primarily associated with the 3-year rotation, found in 13 out of 27 samples, but only in 1 out of 36 samples in the 4-year rotation. Of the 13 positive samples in the 3-year rotation, 7 contained high levels of *Bipolaris*, and 2 contained medium levels.

The DNA concentration of *Bipolaris* was significantly higher in the 3-year rotation. This pathogen is found primarily on wheat, barley, and other temperate grasses, but corn and millet are also hosts. However, corn and millet may support lower populations of this pathogen on the crowns and stalks, which may be preferentially colonized by other fungi. This rotation effect will have to be examined further.

There was also a significant rotation effect on *F. pseudograminearum*, but not *F. culmorum*. *F. culmorum* produces thick-walled chlamydozoospores that can survive in the soil for longer periods than *F. pseudograminearum*, which survives in crop residue. This longevity would negate any rotation effect, since the pathogen could survive in the absence of a host. For *F. pseudograminearum*, higher levels were found in the 3-year rotation, which could be due to the effect of millet. However, the levels of this pathogen were so low that it would be unwise to speculate without data from fields with higher levels of the pathogen.

In conclusion, these results indicate that crown and foot rot caused by *Fusarium culmorum* and *Bipolaris sorokiniana* was the major disease on the Wilke farm. It was probably not in epidemic proportions that would be noticeable to growers and researchers. Above ground, this disease

may cause whiteheads and reduced seed size, but only when the plant is heavily infected, as may occur during a drought year.

The symptoms of *Bipolaris* may be mistaken for *Fusarium* or *Rhizoctonia*, and this pathogen may be underestimated in the total disease complex of wheat in the PNW. *Bipolaris* was higher in the 3-year rotation, an interesting result which suggests the warm season grasses may support lower populations of this pathogen. The other pathogens (take-all, *Rhizoctonia solani* AG8, and root lesion nematodes) probably were not major yield-limiting factors. One should not discount the effects of pathogens that were not measured by these tests; *Rhizoctonia oryzae* and *Pythium* spp. With the exception of *Bipolaris*, there did not appear to be any major pathogen differences between the 3-year and 4-year rotation.

Literature Cited

Ophel-Keller, K., McKay, A., Hartley, D., Driver, F., Wanjura, W., Heap, J., Herdina, Dumitrescu, I., and Curran, J. 2003. Quantitative detection of soil-borne plant pathogens. 8th International Congress of Plant Pathology, Christchurch, New Zealand. Vol. 1, p. 122 (abstract).

Paulitz, T. C., Smith, J. D., and Kidwell, K. K. 2003. Virulence of *Rhizoctonia oryzae* on wheat and barley cultivars from the Pacific Northwest. *Plant Disease* 87:51-55.

Schroeder, K. L. 2004. The Dynamics of Root Diseases of Wheat and Barley and Transition from Conventional Tillage and Direct Seeding. Ph.D. Dissertation. Washington State University, Dec. 2004.

Smiley, R. W. and Patterson, L. M. 1996. Pathogen fungi associated with *Fusarium* foot rot of winter wheat in the semiarid Pacific Northwest. *Plant Disease* 80: 944-949.

Strausbaugh, C. A., Bradley, C.A., Koehn, A.C., and Forster, R.L. 2004. Survey of root diseases of wheat and barley in southeastern Idaho. *Canadian Journal of Plant Pathology* 26: 167-176.