Cereal Cyst Nematode Tolerance and Resistance in Spring Wheat

Richard Smiley (OSU), Juliet Marshall (UI) and Timothy Paulitz (USDA-ARS)
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SYNOPSIS

The cereal cyst nematode (CCN) reduces wheat yield in the Pacific Northwest. Previous studies of varietal resistance had been conducted in the greenhouse. Resistance and tolerance of spring wheat varieties had not been evaluated in the field. Resistance and tolerance traits are independent in that they are not genetically linked. The most profitable variety for production on CCN-infested fields will be a variety that combines tolerance and resistance to CCN. Seven spring wheat trials were conducted on commercial farms in three states over two years. Five trials evaluated both resistance and tolerance and two trials evaluated resistance alone. This tri-state research clearly demonstrated potential benefits to be derived by developing varieties with resistance plus tolerance to CCN. Buck Pronto and UI Stone (ID0599) were susceptible but very tolerant, meaning that CCN multiplied profusely on roots but the grain yield was only slightly diminished by the nematode. Ouyen was resistant but only moderately tolerant, meaning that the nematode did not multiply on roots of that variety (resistance) but still caused substantial reduction in grain yield. Louise was consistently more tolerant than Alpowa and Ouyen. WB-Rockland was both resistant and tolerant. Additional resistance was also identified in several advanced breeding lines. We identified potential parents to cross for developing resistant plus tolerant varieties of soft white spring wheat and hard red spring wheat.

The cereal cyst nematode (CCN) *Heterodera avenae* reduces yields of wheat, barley and oats in localized areas throughout the Pacific Northwest (PNW). The root injury by this nematode consists of a proliferation of short adventitious roots at points where the nematode has established a feeding site. This results in a short, shallow root system with reduced ability to extract water and nutrients, and an increased susceptibility to attack by root-infecting fungi. Close examination of invaded roots reveals the presence of swollen egg-filled females that are loosely embedded in the root. These females contain hundreds of eggs. After the wheat plant dies the female body transforms into a very tough cyst that protects eggs during periods between susceptible crops. Eggs hatch during the spring over a period of several years. A juvenile stage emerges from the cyst and migrates through soil to invade young roots of a subsequent crop of wheat, barley or oat.

Practices recommended for managing the impact of CCN are currently limited mostly to long rotations of a cereal grain with multiple years of broadleaf crops or bare fallow. No nematicides are commercially available for managing CCN. Varieties with tolerance <u>plus</u> resistance have been advocated internationally for many decades but neither of these traits had been fully examined in the PNW. Tolerance and resistance are genetically independent, and both are required for optimal performance in existing plantings (e.g., tolerance) as well as to reduce the level of risk to future plantings (e.g., resistance). Definitions of these traits are explained more fully in the following paragraphs.

Tolerance is defined as the ability of a variety to withstand or recover from nematode invasion and to yield well in comparison with non-invaded plants in the same field or region. In contrast, when yield is reduced substantially by the nematode the variety is characterized as being intolerant because it exhibits a sensitive response. The response of an individual variety can be over a continuous range from tolerant to intolerant. Because grain yield is the unit of measure to define tolerance, essentially all reports of tolerance are based upon research in fields that are naturally-infested by the nematode. Plants are grown in plots that are either left untreated (e.g., this measures what the farmer would experience) or are treated with one or more nematicides to reduce the impact of the existing nematode population. Alternatively, it is possible but technically impractical to attempt to screen for tolerance by inoculating portions of fields to compare growth in side-by-side plots that are non-infested or highly infested by the nematode.

Resistance is defined as the ability of a variety to greatly suppress or prevent reproduction of the nematode. In contrast, when the nematode is capable of multiplying the variety is considered susceptible. The range of response by an individual variety can be continuous from resistant to susceptible. Because nematode reproduction is the critical measure for these tests, most reports of resistance are based upon research under controlled conditions in the greenhouse. These tests require that plants are placed in soil with a known number of nematodes at the beginning of the test and then grown to anthesis (flowering stage). The number of nematodes is re-evaluated at the end of the test to determine a reproductive index. Resistance can also be estimated by digging plants from the field and quantifying the number of egg-filled females embedded in roots. It is also possible to estimate resistance by collecting soil samples to measure the initial and final densities of CCN in field plots. We used both of the field-based methods in studies reported here.

Field tolerance and resistance of wheat to CCN had not been evaluated in North America. The potential benefit of growing a tolerant and/or resistant variety in naturally-infested fields had therefore not been demonstrated. We evaluated spring wheat varieties in seven field trials during 2011 and 2012.

METHODS

Spring wheat trials were performed over a two-year period on seven commercial fields at six locations. Tolerance and resistance were each evaluated in five trials in Idaho, Oregon and Washington. Two additional resistance trials were performed in Washington.

Five tolerance and resistance trials: Trials during 2011 were performed near St. Anthony, ID (Fremont County), Cove, OR (Union County) and Steptoe, WA (Whitman County). The trial near St. Anthony was on a dryland wheat/fallow rotation with an initial density of about 3,000 CCN eggs/lb of soil. Our trial followed a crop of spring wheat. The trial near Cove was on an irrigated field recently cropped annually to winter wheat. It contained 2,600 CCN eggs/lb. The trial near Steptoe was a dryland field recently cropped annually to spring wheat. It had 1,000 CCN eggs/lb. Trials during 2012 were near St. Anthony, ID and Cashup, WA (Whitman County). The trial near St. Anthony was on the dryland triangle between two irrigation circles. It was managed as a wheat/potato rotation and our experiment followed potato. The trial area had 2,600 CCN eggs/lb. The trial near Cashup was on a dryland field recently cropped annually with winter wheat. It had 1,300 CCN eggs/lb.

All five trials had six replicates of each wheat entry. Each 5 × 30 ft plot had four drill rows at 14-inch spacing. Over the length of the trial area drill rows were alternated so that every other drill row was treated with nematicides. Within each replicate each variety was paired to allow side-by-side comparisons of each variety with or without nematicides.

During 2011, four spring wheat varieties were evaluated; Alpowa, Louise, Ouyen and Sönmez. We knew that Alpowa and Louise were susceptible to CCN. Ouyen is an Australian variety that has the *Cre1* gene for resistance to CCN and performed well in our previous greenhouse tests with soils from all three states. Ouyen is resistant but intolerant to CCN in Australia. Turkish scientists reported that Sönmez was resistant to *H. filipjevi*, which is another CCN species that occurs in Oregon. Sönmez had not been evaluated for tolerance or resistance to *H. avenae*. During 2012, we evaluated Ouyen plus 19 PNW varieties selected by Juliet Marshall (UI), Mike Flowers (OSU) and Mike Pumphrey (WSU). Each trial occupied one acre.

Seed was treated with standard fungicides and insecticide. Seed was planted at each location with a no-till drill equipped with a cone-seeder and two Gandy distributors, one for fertilizer and another for nematicide. Fluted opening coulters mounted on a front tool bar were followed by a sweep-type deep-bander for dispensing fertilizer 2-inches below and 1.5-inches to each side of the seed. A second toolbar had double-disk openers to dispense seed in line with the opening coulter and deep bander. Seed was dispensed through the cone-seeder. In alternate drill strips we dispensed the nematicide Temik 15G into the seed row. To extend the protective effect in treated drill rows, we also applied the insecticide/nematicide Movento to drill rows that had been treated with Temik. Since these nematicides are not registered for commercial use we destroyed all grain after harvest. Weeds, insects and stripe rust were managed using standard practices.

Data during 2011 included symptoms of root injury from CCN, grain yield, test weight, and post-harvest density of CCN eggs in soil. During 2012 we also evaluated numbers of swollen white egg-bearing CCN females on roots of all varieties in the control drill strips and for five representative varieties in the nematicide-treated drill strips. A variety was rated resistant to CCN if there were fewer than three white females per root system, or susceptible if there were more than three. Varietal tolerance was measured as the percentage increase in grain yield due to application of nematicides, as compared to the untreated control. Tolerance ratings were VT = very tolerant (<5% yield response to nematicide), T = tolerant (5 to 10%), MT = moderately tolerant (10 to 15%), MI = moderately intolerant (15 to 30%), I = intolerant (30 to 50%), or VI = very intolerant (>50%). Soil samples were collected from each plot immediately after harvest to determine the post-harvest density of CCN eggs. Samples were processed by Western Laboratories.

Two resistance trials: Resistance was evaluated at two sites in Washington. Both experiments followed spring wheat in dryland fields maintained as a rotation of winter wheat, spring wheat, and spring legume. A trial near Pullman in 2011 was on a field with 1,700 CCN eggs/lb. A trial near Colton during 2012 was on a field with 1,900 CCN eggs/lb. During 2011, there were 24 varieties that included 9 PNW varieties and 14 entries from CIMMYT and other providers. During 2012, there were 40 varieties from the Western Regional Hard and Soft Spring Wheat Nurseries, including 7 commercial varieties and 33 advanced breeding lines. Experiments were planted as 4-row head rows in 3-ft plots and 12-inch row spacing. Each 4-row plot had 2 rows of the entry and 2 rows planted with Louise, which served as the susceptible check. Each plot was replicated eight times. White females were counted on roots after plants had headed.

FINDINGS

Plant growth – Seedling growth in the nematicide treatment during 2011 was more rapid and plants became larger and had more tillers (Fig. 1). Little difference was detected during 2012. Differences in responses to application of nematicides are likely to vary from year to year.

Figure 1. Spring wheat varieties in a CCN-infested field at Steptoe, WA during 2011; A = Alpowa (intolerant & susceptible), L = Louise (tolerant & susceptible), O = Ouyen (moderately tolerant & resistant). The 4-row drill strip on the left is 'what the farmer would experience.' Drill strip on the right was treated with two non-registered nematicides.

Root injury – Nearly all plants exhibited the root injury symptom in all five tolerance plus resistance trials in 2011. Disease incidence was therefore about 100%. The severity of the root injury symptom was always high for all varieties in the control drill strips; ratings ranged from 3.0 to 4.7 on a 5-point scale. The nematicide slightly reduced the amount of abnormal root branching. During 2012, disease severity in the controls as well as the nematicide treatment was least for Ouyen and WB-Rockland, but they too expressed moderate to severe root injury (ratings of 3.0) without nematicide treatment. Invasive CCN juveniles penetrate cells behind the root cap and move toward the growth zone. This occurs equally well in resistant and susceptible varieties. The females then reprogram root cells to induce the formation of specialized feeding cells called syncytia. Cells of the syncytium develop but then quickly deteriorate in resistant varieties, reducing or stopping the reproductive capacity of the female. Resistance is therefore expressed by a reduced ability of the nematode to produce viable eggs and is unrelated to the ability of the nematode to cause initial injury to the root. Resistant varieties may therefore reduce the density of CCN in soil but those varieties are still sensitive to root injury that typically results in a reduction of grain yield.

Tolerance – CCN eggs hatch throughout the spring. The juveniles emerge from the protective cyst and can survive in soil and can invade roots for up to several months after emerging. Temik was banded with the seed and has a half-life as long as five weeks. Movento is a nematicide that is applied to foliage and is translocated to the roots. Our goal was to expand the protective activity of nematicides over a longer period of the growing season by applying Movento to drill strips that were also previously treated with Temik. These nematicides did not greatly reduce the amount of root injury but were clearly helpful in differentiating tolerance levels in spring wheat.

Application of nematicides increased the yield of all varieties. In 2011 the average increase was 47% at Cove (15.9 bu/A), 93% at Steptoe (26.9 bu/A), and 25% at St. Anthony (10.1 bu/A). During 2012 the average increase was 9% at St. Anthony (2.4 bu/A) and 10% at Cashup (7.0 bu/A). Yield increase following application of

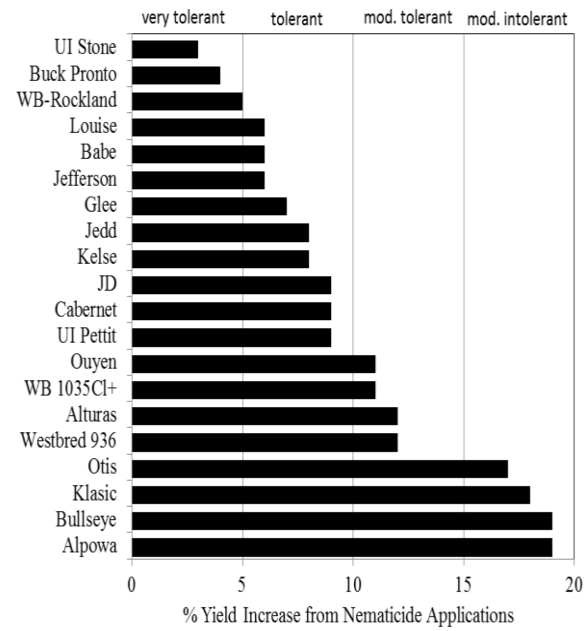

Figure 2. Percentage increase in spring wheat yield when nematicides were applied in CCN-infested fields during 2012; averaged for trials near St. Anthony, ID and Cashup, WA.

nematicides (Fig. 2) was less than 5% for Buck Pronto, UI Stone and WB-Rockland, indicating tolerance to CCN, and was greater than 15% for Alpowa, Bullseye, Klasic and Otis, indicating intolerance. For instance, the average increase in yield was 9.1 and 1.8 bu/A for Alpowa and UI Stone, respectively. Using a wheat price of $8.70/bu, the nematode injury in these two trials during 2012 was responsible for a $63 greater profitability of UI Stone compared to Alpowa. Louise was also consistently more tolerant than Alpowa. This was the first demonstration in North America that commercial spring wheat varieties differ in tolerance to CCN. Unfortunately, Ouyen was intolerant to this nematode, as had been shown previously for both Ouyen and Chara in Australia.

Tolerance does not necessarily relate directly to absolute yield potential. This was true in these trials. Highest yielding varieties in the control treatment during 2012 included Alturas, Babe, Otis, UI Pettit and UI Stone, a group which spanned the range from tolerant to intolerant.

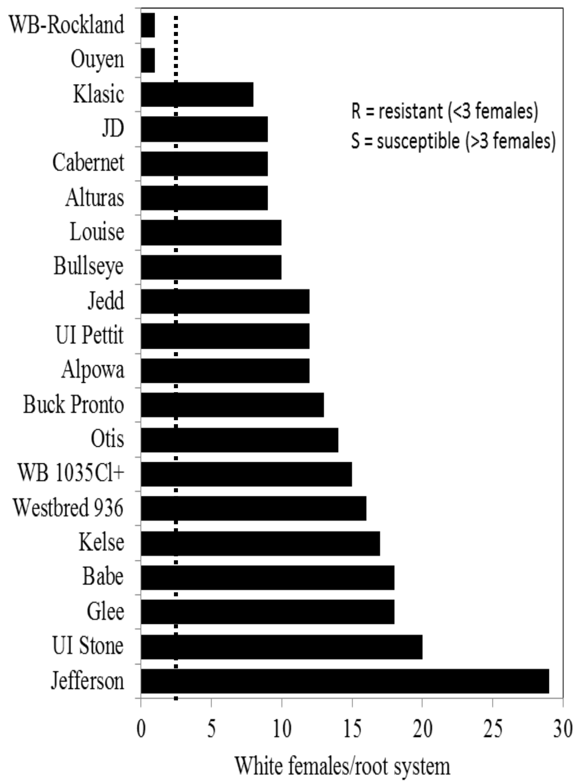

Figure 3. Number of egg-bearing CCN females on roots of spring wheat during 2012; averaged for trials near St. Anthony, ID and Cashup, WA.

Resistance - As expected, Alpowa, Louise and many other varieties allowed the nematode to multiply very effectively. WB-Rockland was the only commercial variety that was resistant (Fig. 3). This is the first report of a commercial spring wheat variety that is resistant to CCN in North America. The Australian varieties Ouyen and Chara were also resistant (Figs. 3 & 4) and both of those varieties inherited the *Cre1* gene for resistance to *H. avenae* from the line AUS10894. This was the first field research that demonstrated the value of *Cre1* resistance in naturally-infested field environments in the PNW. We also identified resistance in four advanced breeding lines from the Western Regional Nurseries; SY-97621-05 and UC1711 were highly resistant and SO900163 and SY-B041418 exhibited useful levels of resistance (Fig. 4). The nature and origin of the resistance in WB-Rockland and the breeding lines is unknown.

The mean number of white females was 20-times greater in the control than in the nematicide treated plots during 2011 and 2012. The nematode density after harvest was much lower following Ouyen or WB-Rockland compared to all other varieties (Fig. 5). The nematode density following those two varieties was as low as when they or the susceptible varieties were grown in soil that was treated with nematicides. The post-harvest CCN density in plots of Ouyen and WB-Rockland therefore appeared to identify the natural background level remaining from eggs that were remaining from cysts that were produced by previous crops. This natural background level occurs because CCN juveniles emerge from cysts over a period of two or more years. About 60% of the eggs in a cyst hatch during a single season; the range can be 40% to 90%. Individual cysts can contain from a few to as many as 600 eggs. Hatching from individual cysts is therefore spread over many years. This means that a reduction of nematode density to a non-damaging level requires multiple years of planting a resistant variety or a non-host (broadleaf) crop, or of fallowing the field. Since the nematode could not effectively multiply on Ouyen we concluded that the average density of eggs remaining from older cysts produced on previous crops was about 1,000/lb of soil for fields we used during 2011 and about 2,000/lb for fields during 2012.

Sönmez did not reduce post-harvest density of *H. avenae* at three locations during 2011 (Fig. 5) and was also intolerant. Since Sönmez is resistant to *H. filipjevi* in Turkey it may become a useful resource for regions where both *H. filipjevi* and *H. avenae* are present in the PNW, as is known to occur in eastern Oregon.

We identified a number of potential parents for crosses to develop varieties with resistance plus tolerance. For example, a resistant and tolerant soft white spring wheat might be achievable using a cross between Ouyen and either Louise or UI Stone. Likewise, a resistant and tolerant hard red spring wheat could be explored using a cross such as Buck Pronto × WB-Rockland.

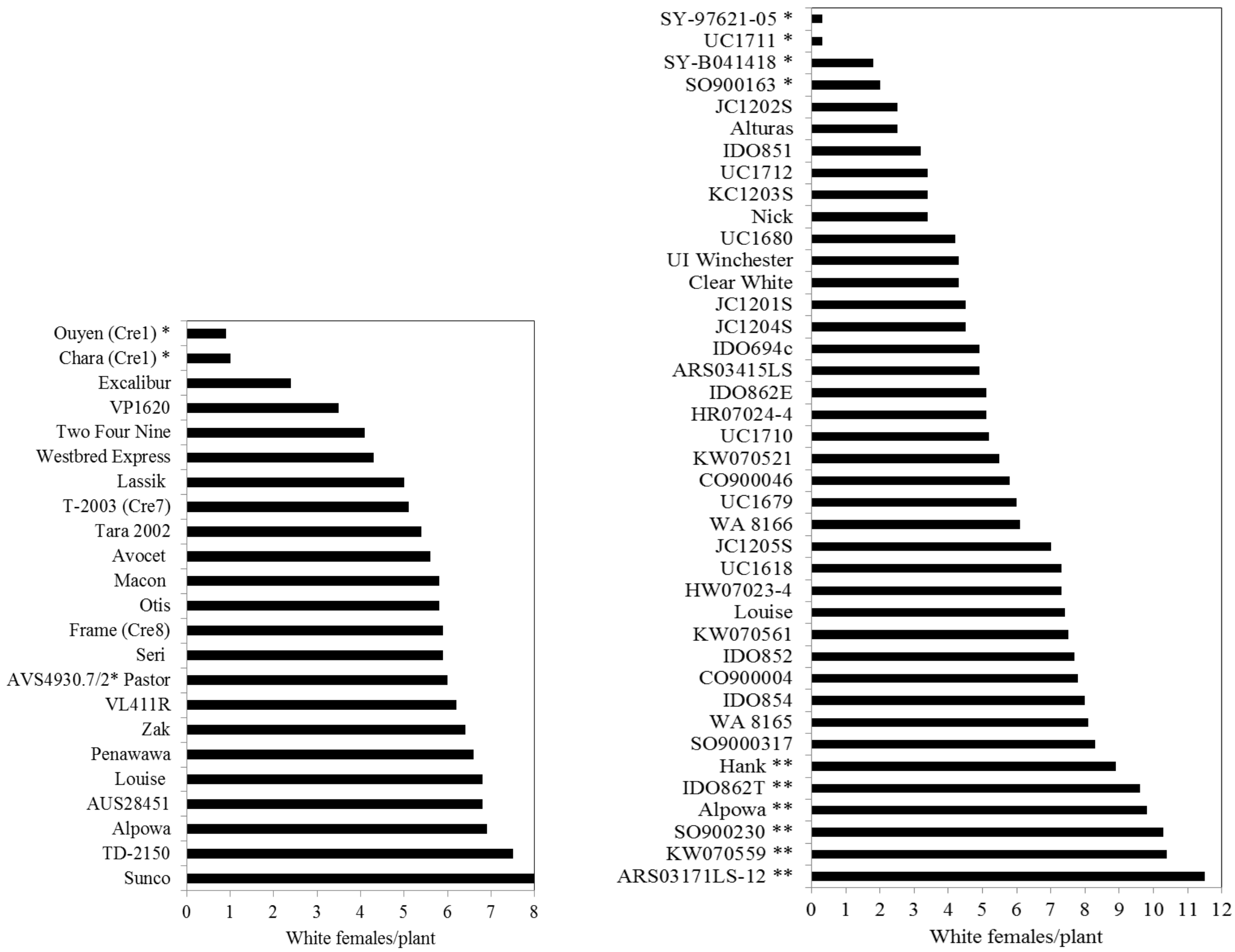

Figure 4. Number of egg-bearing CCN females produced on spring wheat near Pullman, WA during 2011 (left) and near Colton, WA during 2012 (right). Varieties with * or ** are significantly more or less resistant than the check variety Louise.

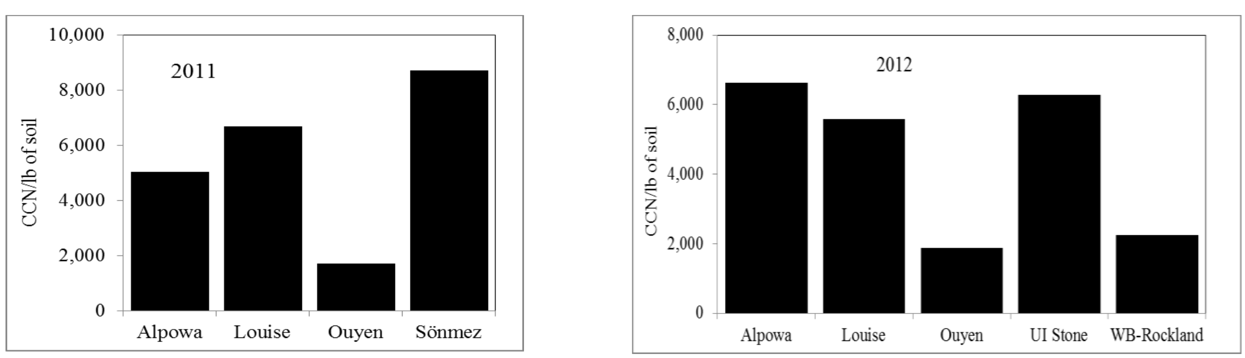

Figure 5. CCN egg density in soil after harvesting spring wheat; averages of trials near St. Anthony, ID, Cove, OR, and Steptoe, WA in 2011 (left) and St. Anthony, ID and Cashup, WA in 2012 (right).

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TECHNICAL PUBLICATION:

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