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Review

Low input no-till cereal production in the Pacific Northwest of the U.S.: the challenges of root diseases

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Abstract

Direct-seeding or no-till is defined as planting directly into residue of the previous crop without tillage that mixes or stirs soil prior to planting. No-till reduces soil erosion, improves soil structure and organic matter, and reduces fuel inputs. No-till is widely used in cereal production in Australia, Canada, Argentina, and Brazil, but has not been widely adopted in Europe and the Pacific Northwest of the U.S. One of the limitations is that root diseases may increase with a reduction in tillage. This paper discusses the importance and management of take-all, Fusarium dryland foot rot, Rhizoctonia bare patch and root rot, and Pythium root rot in dryland cereal production systems, and how they are influenced by changes in tillage practices. To address this challenge, specifically with *Rhizoctonia* and *Pythium*, our research group has (1) developed classical and molecular techniques to detect and quantify *Rhizoctonia* and *Pythium* spp. from the soil to assess disease risk; (2) studied the disease dynamics of root disease during the transition from conventional to no-till; (3) developed greenhouse methods to screen germplasm for tolerance or resistance to *Pythium* and *Rhizoctonia*, and (4) using GPS and geostatistics, has examined the spatial distribution of *R. solani* and *R. oryzae* at a field scale up to 36 ha, across a number of crop rotations and years. By a combination of ecological, epidemiological, field, and laboratory studies, we hope to provide growers with a set of disease management tools to permit the economical and sustainable production of dryland cereals without degradation of the soil resource.

Introduction

Over the last 20 years, there has been a shift toward reducing inputs and increasing the long-term sustainability of cereal (small grain) production throughout the world. Part of the reason for this shift is economics: to reduce the inputs and costs in order to be competitive on the world market. Another part is environmental: to reduce or eliminate (in the case of organic production) the use of pesticide and inorganic fertilizer inputs. Organic cereal production is becoming increasingly popular in European countries, with a strong consumer demand. In Europe, 3.4% (6.3 million ha) of the agricultural land area is in organic production, 1.5% of the farms (Willer and Yussefi,

2005). In Nordic countries, 27% of the organic production area is in cereals, but only 15% in southern European countries (Duchateau, 2003). In the U.S., only 0.2% of wheat is organically produced, an increase of 31% during the late 1990s (Greene and Dobbs, 2001). But the area of organic wheat production (50,500 ha) is more than any other organic crop in the U.S. European producers typically have higher inputs and subsidies than other wheat producing regions, but the yields of intensively managed cereals are also higher, and are produced on smaller-sized farms. In contrast, farm sizes are larger in the U.S., but average yields are lower, so the economic value of the crop per ha is lower. To reduce production costs and improve environmental sustainability, U.S. growers are increasingly turning to no-till or direct-seeding. But this shift in cultural management practices has led to a new set of root and crown disease problems.

This review will discuss how our research group has approached this challenge, and what lessons may be applied to cereal pathology research in Europe. It will begin with a brief introduction to the small grain (wheat and barley) cropping systems of the Pacific Northwest (PNW) of the U.S. and the use of no-till or direct-seeding. Four major diseases caused by soilborne pathogens affect grains in this region-Rhizoctonia bare patch and root rot, Pythium root rot, Fusarium crown rot, and take-all. These diseases are presently managed by cultural techniques, but no-till can exacerbate some of these pathogens. To address this problem, we have taken five approaches: (1) pathogen surveys to ascertain inter- and intra-specific diversity; (2) development of methods for the quantification and detection of specific pathogens; (3) understanding the population dynamics of pathogens during the transition from conventional tillage to no-till; (4) development of methods to identify resistant or tolerant germplasm and (5) spatial analysis of the pathogen at a scale relevant to the grower.

Cereal production in the Pacific Northwest of the U.S.

The Pacific Northwest of the U.S. encompasses three states—Washington, Oregon, and Idaho. Most of the cereal production is dryland or non-irrigated, and is located in the drier areas east of the Cascade Mountains, in eastern Washington, northeast Oregon, and western and southern Idaho (Papendick, 1996). This area has a climate with warm, dry summers with most of the precipitation occurring in late fall, winter and early spring as rain or snow. Most of the precipitation falls outside of the growing season, so the plants rely on stored soil moisture. There are three major rainfall zones—low (150–300 mm) in central Washington and Oregon just east of the Cascade Mountains; intermediate (300–400 mm) and high (400–600 mm) in eastern Washington and northern Idaho. Yields range from 0.6 t ha^{-1} to 9 t ha^{-1} under irrigated conditions. The average yield of wheat in the PNW is 4.2 t ha^{-1}, compared to the U.S. average of 2.8 t ha^{-1}. Most of the wheat production is soft white winter wheat (75%, 1.41 million ha), and most of this (85%) is exported to Asian markets for noodle and flat breads (Washington Wheat Commission, 2004). In 2002, Washington was the 5th largest wheat producing state in the U.S., with a value of $537 million U.S. In the higher rainfall areas, crops are grown every year, and winter wheat is commonly in a 3-year rotation with spring wheat/spring barley in the second year and pulse crops (pea, lentils and chickpeas) or brassica crops (canola, mustard) in the third year. In the low and intermediate rainfall areas, winter wheat is grown every other year in rotation with a fallow to retain moisture for the following crop. In the dry to intermediate rainfall areas, wind erosion can be a major problem, especially on the fallow land (Papendick, 2004). The fallow has very little crop residue or cover and is often maintained with a dust mulch, which is a fine layer of powdered soil on the surface to prevent evaporation of moisture from lower soil levels due to capillary action (Lindstrom et al., 1974). In the most productive high rainfall areas of eastern Washington, soils are deep and fertile wind-deposited loess, a silty-loam derived from volcanic basalt. These soils were deposited by wind over thousands of years in hills 50–75 m high with slopes up to 40%, and very deep topsoil (3–4 m deep). These steep hillsides are subject to severe water erosion. Most of the farms are still family owned (over 80%), but the number of farmers has decreased over the last 40 years, and farm size has increased to 1000–2000 ha in size. These farms are highly mechanized with large

combines and tractors. Harrows, cultivators, and seeders can be up to 20 m in width.

Direct-seeding or no-till

In direct-seeding or no-till, the soil is left undisturbed from harvest to planting. The crop is seeded directly into the previous stubble, using a no-till drill equipped with disk openers, hoe openers, or coulters. Planting is usually done in one operation, with no seedbed preparation. According to the USDA-Natural Resource and Conservation Service (NRCS) definition (cited in Smiley et al., 2005b), this method leaves more than 30% of the residue after planting, or more than 1,120 kg ha^{-1} of residue. No-till offers several economic and environmental advantages for growers. Because of the residue cover and standing crop stubble, soil loss from wind erosion is significantly reduced (Papendick, 2004). This also reduces the atmospheric levels of PM_{10}, particles with a diameter less than 10 μm. These particles have adverse effects on human health (Ostro and Chestnut, 1998; Schwartz and Neas, 2000), can be blown to populated urban areas to the east, and are regulated as a pollutant by the Environmental Protection Agency. Fuel, labour and machine costs are reduced. Conventional tillage in summer fallow may require an additional 7 tillage or cultivation operations, compared to a minimum tillage system that relies on herbicides (Papendick, 2004). Organic matter increases with lack of tillage, improving soil structure and water infiltration (Douglas and Goss, 1982; Dao, 1993;). No-till may also result in more water storage, since water is lost from soil with tillage, and crop residues trap more snow and allow for better infiltration. This may allow for more intensive rotations and eliminate the need for summer fallow. No-till allows for more carbon sequestration in the soil, and no-till farmers in the PNW have sold carbon credits in 2002 to offset greenhouse gas emissions.

No-till is widely used in soybean and corn production in the U.S. and has increased from 6% in 1990 to 22.6% in 2004 (Conservation Technology Information Center, 2004). When combined with mulch tillage, 40% of the crop area of the U.S. uses some kind of conservation tillage. In other countries, the adoption of no-till is even higher. In Alberta, Canada, 63% of the crop land was no-till in 2001. Argentina and Brazil also have a high level of no-till adoption. In Europe, however, the adoption of no-till is much less. Between 0 and 3% of agricultural land in European countries is no-tilled, while in Germany and France, approximately 20% is under conservation tillage (European Conservation Agriculture Federation). The adoption rate of no-till in the PNW is also low, although it is increasing. No-till wheat production increased from 1% in 1996 to 17% in 2004 in Oregon; and from 3% to 13% in Washington (Smiley et al., 2005b). One of the reasons for the low adoption rate is the threat of increased soilborne diseases.

Soilborne pathogens and diseases of wheat and barley in PNW

Rhizoctonia bare patch and root rot

This disease is caused by *Rhizoctonia solani* AG-8 and was first observed in Australia in the 1930s. It became a major disease problem in Australia with the adoption of no-till in the 1980s and was first observed in the PNW in 1986 (Weller et al., 1986). The pathogen causes a root rot and stunting of wheat and barley plants. These stunted or killed plants are often present in the field in patches several metres in diameter. However, this disease has a chronic phase in which plants are stunted, resulting in uneven stands, but without patches (Paulitz et al., 2002a). Rhizoctonia diseases can be increased in direct-seed systems that lack tillage (McNish, 1985; Rovira, 1986; Pumphrey et al., 1987). Tillage may break up hyphal networks in the soil, or change the microbial activity in the soil to suppress the pathogen. Rhizoctonia can survive on living and killed roots of volunteer and grassy weeds, and can 'greenbridge' to the crop following killing of weeds with glyphosate (Smiley et al., 1992) (See *Present strategies for the management of root diseases* below for an explanation of the greenbridge effect).

Pythium root rot

Called the 'chronic cold' of wheat roots (Cook and Veseth, 1991), *Pythium* is ubiquitous in most soils. It can rapidly attack emerging seedlings and embryos within 24–48 h after planting, resulting in

stunted seedlings, although damping-off is not common in cereals. The pathogen will also attack root tips and root hairs, resulting in a loss of fine feeder roots. The impact of this disease was not appreciated until work by Cook et al. (1980) showed that metalaxyl increased yields 2.2–4.4 t ha^{-1}. In over 10 years of trials with soil fumigation, methyl bromide or telone increased yields 3–36% (Cook et al., 1987). But unless there is a healthy fungicide-treated control in a field, most growers would be unaware of the stunting and yield loss caused by these pathogens. Although not widely studied in no-till systems, there are some reports that *Pythium* increases with a reduction in tillage (Cook et al., 1980; Pankhurst et al., 1995). *Pythium* can survive for long periods as oospores formed in the roots.

Take-all

Caused by *Gaeumannomyces graminis* var. *tritici*, take-all is probably the most important disease of wheat in intensive systems/higher rainfall areas of Europe. A recent survey of the PNW found low levels in most fields (Ramsey, 2001). This disease is favoured by higher moisture levels, so high disease levels are typically found in irrigated wheat or in higher rainfall areas of western Washington and Oregon. The pathogen infects the roots and moves into the crown, producing runner hyphae on the root surface that can spread to other roots. By destroying the lower stem and blocking the flow of nutrients to the head, whiteheads are formed, which contain fewer, shrunken seeds. The pathogen survives in infested plant debris and on the roots of grassy weeds. There is conflicting evidence on the effect of no-till on this disease. Moore and Cook (1984) found more take-all in no-till plots in the PNW, but a more recent study (Ramsey, 2001) did not detect any differences between conventional and no-till farms. Some studies have found lower take-all with no-till (Bailey et al., 1992; Pankhurst et al., 2002).

Fusarium crown and foot rot

Foot rots or crown rots can be caused by a complex of pathogens, including *Fusarium pseudograminearum*, *F. culmorum* and *Bipolaris sorokiniana*. Based on surveys in the PNW (Smiley and Patterson, 1996), *Fusarium pseudograminearum* and *F. culmorum* are the predominant pathogens, with *F. culmorum* in the higher rainfall areas. These pathogens infect the roots but move into the crown, causing whiteheads and reduced seed size. Plants are predisposed by drought, and disease is increased by overfertilization with nitrogen (Papendick and Cook, 1974). In low rainfall areas, plants with excess N fertilizer will produce lush growth early in the season but will outstrip the water supply in the soil profile, resulting in drought stress and the pathogen moving up 1–3 nodes of the crown. Based on recent surveys in northern Oregon, this complex causes an average of 9% yield loss (Smiley et al., 2005a). The pathogens survive in crop residue and stubble as chlamydospores or macroconidia. Because of the survival on crop residues, many studies have reported more *Fusarium* in no-till systems (Smiley et al., 1996; Bailey et al., 2001). However, this disease is very common in conventional tillage systems, and disease levels may be less under direct-seed systems that have higher levels of soil water retention.

Present strategies for the management of root diseases

The effect of different management techniques on root diseases of cereals is summarized in Table 1. Presently, there are no sources of genetic resistance for any of these pathogens, despite recent attempts to identify resistance in locally adapted cultivars (Smith et al., 2002). This is in contrast to the widespread use of genetic resistance to control foliar pathogens such as stripe rust caused by *Puccinia striiformis*. Chemical control methods are also not feasible or economical for control of soilborne pathogens. Most growers use protectant and systemic seed treatments such as tebuconazole and difenoconazole to control smuts and bunts. Chemicals such as metalaxyl and mefanoxam are often mixed with other protectant chemicals to protect against Pythium seedling rots. These chemicals will protect the germinating seed and young seedling, but are not translocated in the root and do not protect the root tips. Nevertheless, seed treatments are fairly inexpensive and sometimes give a slight yield increase (Cook et al., 2002b). Biocontrol seed treatments have shown success in field trials (Cook et al., 2002b) but are

Table 1. Effect of management techniques on root diseases of cereals

Management technique						
Disease	No-till or minimum tillage	Greenbridge control	Crop rotation	Later planting date	Increased fertilizer and better placement	Chemical seed treatments
Rhizoctonia root rot and bare patch	↑[a]	↓	–	ND[c]	↓	↓[–e]
Pythium root rot	↑[b]	↓	–	↓	↓	↓[f]
Fusarium crown rot	↑↓	–	–	↓	↑	–
Take-all	↑[–]↓	↓	↓	↓	↓[d]	↓[g]

↑ = increased disease, ↓ = decreased disease, [–] = no effect.
[a] Rhizoctonia root rot is also found in conventional tillage, but bare-patch is mostly confined to no-till systems.
[b] Increased infiltration rates from better soil structure in long-term no-till soils may reduce *Pythium*.
[c] Not determined.
[d] NH_4^+–N reduces take-all, Mn, Cu and Cl deficiencies increase disease.
[e] Seed treatments have no effect on bare-patch, but will improve early seedling health under high Rhizoctonia root rot conditions.
[f] Metalaxyl and mefanoxam will improve early seedling health and emergence, but cannot protect against root rot in mature plants.
[g] Take-all can be reduced by silthiofam and fluquinconazole, two new chemicals registered in EU and Australia, but not in North America.

not commercially available. This leaves the grower with only cultural management options.

Crop rotation is used by growers in the higher rainfall areas, who often grow a pulse crop in the 3rd year. This controls take-all, since broadleaf crops are not a host for *G. graminis* var. *tritici*. However, this does not control *Rhizoctonia* or *Pythium*, which have wide host ranges. In addition, oospores of *Pythium* can survive for more than one year. Crop rotation does not control Fusarium crown rot, since these fungi form macroconidia and chlamydospores which can survive for many years (Inglis and Cook, 1986). One of the most successful ways to reduce *Rhizoctonia*, besides tillage, is elimination of the 'greenbridge' effect (Smiley et al., 1992). It is recommended that growers kill weeds with herbicides at least 3 weeks before planting. Most growers use the non-specific herbicide glyphosate because of the low cost, ability to control most weeds, and quick breakdown in the soil. However, glyphosate inhibits the shikimic acid pathway in plants, which is needed for plant defence against pathogens. Dying grassy weeds and crop volunteers (re-seeded from the previous crop) treated with glyphosate become colonized by *Rhizoctonia* and *Pythium*, which serve as a source of inoculum (greenbridge) for the following crop planted in the field (Lévesque and Rahe, 1992). By allowing time for pathogen inoculum to be reduced by substrate depletion or microbial breakdown before planting, less plant damage results. Growers will often apply a starter fertilizer in the seed row right above the seed, to provide the plant with instant access to nutrients, especially phosphorus, even if some of the roots are damaged by pathogens. Planting fresh seed will also reduce *Pythium* damage, since older seed takes longer to germinate and is susceptible to embryo damage for a longer period. Fallow may be useful for reducing pathogens that only survive in roots and on volunteers, such as *Rhizoctonia* and *G. graminis* var. *tritici*, which do not form resistant spores. To manage Fusarium crown rot, growers use N fertilizer management and planting date. The amount of N applied to a crop is based on the water in the soil, average precipitation, and the yield potential. Preventing overfertilization reduces the drought stress on plants that occurs when they outstrip their water supply by vigorous vegetative growth.

Research strategies

Given the constraints that diseases impose on the adoption of this sustainable, low-input technology, how can research provide some answers and tools to overcome these obstacles? My research programme, which concentrates on *Pythium* and *Rhizoctonia* diseases, has followed a strategy of investigations to fill in the gaps of knowledge, and then to use this information to target one of the three principal factors influencing disease–either the environment (cultural and agronomic disease

management techniques), the plant host (identifying resistance or tolerance) or the pathogen (biological control and suppressive soils). Our research unit has worked on this last aspect for the last 25 years, and it is beyond the scope of this paper, but much of this work has been reviewed by Weller et al. (2002). Chemical control, except for seed treatments, is not an option in these low-input systems.

Identifying pathogen diversity at the inter- and intra-species level

The first step in any disease management programme is to identify pathogen diversity in the field. This is an ever-evolving process, as advances in technology allow us to define a greater degree of complexity. *Pythium* spp. in the PNW are a good example. Although the work of Cook et al. (1987) showed the importance of *Pythium* spp. in yield loss, we did not have a good idea of which species were causing the loss, and which species were the most virulent. Work in the mid 80s identified *P. ultimum*, *irregulare*, *torulosum* and *heterothallicum*, from wheat, barley, peas and lentils (Ingram and Cook, 1990). Chamswarng and Cook (1985) identified 10 species of *Pythium*, including *P. ultimum*, *P. irregulare, P. torulosum, P. volutum* and *P. heterothallicum*. But this survey was only from a few sites in Whitman County, WA. In 2000, we surveyed 80 sites throughout eastern Washington, and baited *Pythium* spp. from soil using grass leaves. Species were identified by classical morphology. This can be difficult because some of the species are heterothallic, requiring two mating types to form oospores, which are required for identification, and some isolates are completely asexual. To overcome this problem, a molecular approach based on sequencing the ITS region of the rDNA, and comparing the data to a library of sequences from over 1200 authenticated cultures was also used (Lévesque and de Cock, 2004). Using this approach, we identified 13 species, including *P. ultimum, P. irregulare, P. heterothallicum, P. oligandrum, P. sylvaticum, P. dissimile, P. dissoticum*, and *P. paroecandrum*. Among these was a new species which was described as *P. abappressorium*, based on the unique formation of chlamydospores and oospores from remnant appressoria and a unique ITS sequence (Paulitz et al., 2003a). In surveys, *P. abappressorium* was the most predominant, followed by *P. rostratum, P. debaryanum* (later re-identified as *P. irregulare* group IV), *P. heterothallicum* and *P. oligandrum* (Paulitz and Adams, 2003). Using canonical correspondence analysis, we found an association between the species composition and gradients in rainfall and soil type across a 27,000 km^2 area of eastern Washington. Two of the species found in this survey were later classified as new species—*P. intermedium* as *P. attrantheridium* (Allain-Boule et al., 2004) and *P. rostratum* as *P. rostratifingens* (de Cock and Lévesque, 2004). These examples demonstrate the power of molecular techniques in combination with classical techniques. The molecular data alerted us to the fact that diversity may be broader and that unique, previously undescribed species exist. Further morphological studies identified these distinctions. Without the molecular data, however, we would have lumped these species together with similar-looking species. The situation with *Rhizoctonia* spp. was similar. Five years ago, we assumed that *R. solani* AG-8 was the primary pathogen on cereals, based on previous work (Weller et al., 1986). However, we had a difficult time isolating this anastomosis group from roots or soils in surveys. Instead, we found a predominance of *R. oryzae* which had been described from the PNW in the early 1990s, but was thought to be a minor pathogen and not highly virulent (Ogoshi et al., 1990; Smiley and Uddin, 1993). However, pathogenicity studies showed many of the isolates of *R. oryzae* were highly virulent (Paulitz et al., 2002b; Schroeder and Paulitz, 2002) and pathogenic on other rotation crops such as pea (Paulitz, 2002). Recent work with ITS sequencing (Okubara and Paulitz, unpublished) has suggested that there are two or three different groups within *R. oryzae* and we have found two other anastomosis groups of *R. solani* besides AG-8, namely AG-2-1 and AG-10. A binucleate group, *Ceratobasidium* spp., has also been identified, which is close to AG-I based on ITS sequence data (Okubara and Paulitz, unpublished). Isolates of *R. oryzae* have shown a range of virulence, suggesting that intraspecific diversity may be important (Paulitz et al., 2002b). To examine intraspecific diversity, our group has developed AFLP markers for *R. solani*, and are presently evaluating a collection of over 200 isolates from a location with

Rhizoctonia bare patch. We found a high degree of concordance between AFLP groups and AGs based on ITS sequences.

Development of methods for detection and quantification of pathogens from soil using classical and molecular techniques

One of the limitations in the study of soilborne pathogens has been accurate quantification at the species and subspecies levels. Dilution plating has been used in the past, but this cannot be used to quantify individual species of *Pythium*, since the morphologies are similar on the selective medium used for isolation (Mircetich and Kraft, 1972). Individual colonies can be sub-cultured and identified, but this is very laborious and expensive. In addition, slower-growing species of *Pythium* may not be detected on a selective medium. For *Rhizoctonia*, the problem is even more acute, since dilution plating cannot be used because of the low population densities of this fungus. Instead, baiting, elutriation of the organic fraction and plating, or isolations from soil pellets have been used. Because these methods are indirect, quantification of the original number of propagules is difficult.

Our laboratory has focused on the development of molecular methods of detection to overcome these two constraints—specificity and speed of identification. Our long-term goal is to develop these methods so that growers can determine their risk before planting and make the proper management decision, whether it be tillage, crop rotation, cultural practice, or cultivar.

To achieve this goal, we developed specific primers for nine species of *Pythium* (Schroeder, 2004; Schroeder et al., 2006). These were developed from the ITS region, and designed using GeneRunner v.3.05 (Hastings Software, Inc., Hudson, NY) and Oligo v.6.65 (Molecular Biology Insights, Cascade, CO) to prevent dimer formation. These primers are used with a capillary quantitative real-time PCR system called Light Cycler (Roche Applied Science, Indianapolis, IN), using the SYBR green dye. Real-time PCR quantifies the starting DNA, based on the cycle at which amplification becomes logarithmic. The primer sets from each species were tested against the other *Pythium* spp., and no cross-reactivity was found. Standard curves were developed by using known amounts of DNA, and also by adding known concentrations of inoculum to pasteurized soil and extracting DNA from soil. Using this method, we were able to quantify up to seven different species from a single soil sample. This technique will be used in future ecological studies to look at how management practices such as tillage and crop rotation influence the species composition of *Pythium*. A similar approach is being used to develop primers specific to individual anastomosis groups of *R. solani*, based on ITS sequences.

However, quantification methods do not have to be based on molecular methods. We developed a semi-quantitative method for *Rhizoctonia* based on a toothpick assay in the soil (Paulitz and Schroeder, 2005). Soil samples are placed in 10 cm pots, and watered to field capacity. After 2 days, five non-sterile birchwood toothpicks are inserted into the soil. After 48 h, the toothpicks are placed on a selective medium consisting of water agar, 1 ppm benomyl, and 100 ppm chloramphenicol. After 24 h, hyphae of *Rhizoctonia* grow out into the agar, and can be recognized under a dissecting scope with transmitted light. Using a 5 mm grid, the number of grids containing hyphae can be counted. *Rhizoctonia solani* can be distinguished from *R. oryzae* by differences in hyphal morphology and the angle of branching. We developed standard curves by adding known concentrations of inoculum to the soil, and quantifying the number of colonies from the toothpicks. This method has been used to develop a large culture collection, for spatial analysis studies, and to diagnose *Rhizoctonia* from grower fields. However, this assay only detects active hyphae that can contact the surface of the toothpick within 48 h. It cannot detect dormant sclerotia.

Population dynamics of pathogens in the transition from conventional to no-till

Once the methods have been developed, important experimental questions concerning management and cropping systems can be addressed. One of these questions is how diseases and pathogens change in the transition from conventional to no-till. As mentioned previously, there is evidence that *Rhizoctonia* may increase with the lack of tillage. Schroeder (2004) followed pathogen populations and disease in field plots established on land that had been in long-term conventional tillage. Half of

the plots were no-till and half remained in tillage. Plots were planted with a spring barley-spring wheat rotation. Conversely, another field experiment was set up on long-term no-till land about 1 km away, and half of the plots were ploughed. Over the next 4 years, disease and pathogens were monitored using non-molecular methods, primarily root symptoms, dilution plating for *Pythium* spp. and the toothpick assay for *Rhizoctonia*. Levels of take-all remained low in all plots, and there were no differences in total populations of *Pythium* spp. For the first 2 years, there were no significant differences between the conventionally tilled and no-till plots in the experiment on the land that was originally conventionally-tilled. However, in the 3rd and 4th year, the no-till plots had significantly lower yields than the conventionally tilled plots, and more symptoms of *Rhizoctonia* on the root system. During the last two years of the experiment, the activity of *R. solani* and *R. oryzae* was monitored over the season with the toothpick assay. *Rhizoctonia solani*, but not *R. oryzae*, was consistently higher in the no-till plots. However, in the no-till plots that were converted back to conventional tillage, no yield increase or change in disease level was measured. This result indicates that bringing back the plough to long-term no-tilled land is not advantageous, and that *R. solani* is the major pathogen responsible for the yield losses in the 3rd and 4th year of the conversion. However, in disease surveys of farms that had been in no-till longer than 12 years, there were no consistent differences in disease, compared to adjacent conventionally-tilled fields (Schroeder, 2004). It appears that disease levels may decline after more years of no-till, but the mechanism for this disease suppression or pathogen reduction is unknown. The molecular techniques described above will provide a greater level of resolution for future studies on changes in management systems.

Development of methods to identify tolerant or resistant germplasm and to evaluate pathogen virulence

Probably the most cost-effective and sustainable method of management of root diseases is genetic tolerance or resistance. However, efforts to identify resistance to necrotrophic root pathogens such as *Rhizoctonia* and *Pythium* in wheat have not been successful, partially because of the difficulty of working with these pathogens and lack of tolerance in agronomically adapted cultivars (Smith et al., 2002). For a successful screening programme, reproducible methods are needed to score the disease reaction of the plant. For foliar pathogens, this is relatively easy, but most root pathogens do not have distinctive symptoms that can be measured above or below ground. *Rhizoctonia* symptoms can be quantified on the root because of distinct brown lesions, but *Pythium* usually does not cause any root discolouration. For a proper screening programme, one must have an idea of the range of virulence among pathogen populations, and strains should be selected to reflect this diversity. The use of root scanning software has enabled our group to accomplish these objectives and make progress in this area. Washed root systems are scanned with a flatbed scanner, and the image is analyzed with the WinRhizo software, (Regent Instruments Inc, Québec, Canada), which calculates the total root length, number of root tips, and average root diameter, as well as a number of other variables. A greenhouse bioassay was developed using small pine seedling containers and pasteurized soil inoculated with a soil-rolled oat inoculum of *Pythium* spp. Among *Pythium* species, *P. ultimum* and *P. debaryanum* (later identified as *P. irregulare* group IV), were the most virulent species (Higginbotham et al., 2004a). *Pythium ultimum* and *P. debaryanum* were then used to screen a collection of spring wheat lines from the U.S., and three lines were found to have a reproducibly higher level of tolerance (Higgingbotham et al., 2004b). A similar technique was used to evaluate the virulence of isolates of *R. oryzae* on wheat and barley (Paulitz et al., 2002b). All isolates caused significant stunting and reduction in roots, barley was more susceptible than wheat, and there was a significant cultivar × isolate interaction, indicating that the species level may not be the taxonomic level at which these studies need to be done in the field. This same technique has been used to identify a chemically-induced ethyl methane sulfonate (EMS) mutant of a spring wheat cultivar that shows a heritable resistance (Steber, Kidwell, and Okubara, unpublished).

Spatial analysis of Rhizoctonia *spp.*

The spatial analysis of soilborne pathogens has been hampered by many of the same limitations in

methods for ecological studies. Foliar disease can be quantified based on disease severity or disease incidence, and airborne pathogens can be sampled with a variety of spore traps. However, most root diseases do not produce a readily quantifiable symptom above the ground, and determination of propagule density in soils is laborious and inexact. One disease that is more tractable for this type of analysis is Rhizoctonia bare patch, which produces measurable patches in a field. With the advent of low-cost GPS technologies with sub-metre accuracy, these patches can be mapped and quantified. Cook et al. (2002a) mapped Rhizoctonia bare patches across a number of different rotations during the first five years after conversion to no-till. They concluded that broadleaf rotations did not reduce patch area, because the broadleaf crops were also susceptible to *R. solani* AG-8. However, in a later study in years 6–8 of the rotation, patch area was reduced in wheat following spring barley, an unexpected result since barley is highly susceptible to *Rhizoctonia* (Schillinger and Paulitz, 2006).

How can spatial analysis be performed on the more chronic phase of *Rhizoctonia* that does not cause distinctive patching? The use of GPS provides a powerful tool for spatial analysis and the use of geostatistical techniques. Three hundred and sixty-nine GPS-located sites were established on a 36 ha farm north of Pullman WA, and sampled over two years for *R. oryzae*. Disease levels were assessed by visual ratings of root samples, and root segments were plated on a selective medium. Frequency distributions of root ratings and incidence of colonized roots were highly skewed, that is, most of the sites had zero or low levels of the disease. This distribution fitted a beta binomial distribution, indicating an aggregated or clustered distribution. Geostatistical techniques were used, but no spatial correlation was detected because of the skewed samples. Spatial correlation can be visualized with a semivariogram, which expresses variance as a function of the distance between points and indicates that samples that are close together are more similar than those further apart (Isaaks and Srivastava, 1989). However, a generalized linear mixed model was used to establish that spatial correlation existed, and kriging (a spatial interpolation method) was used to develop disease maps of the farm (Paulitz et al., 2003b). With the development of the toothpick assay for *Rhizoctonia*, more extensive sampling was done across a number of different rotations and at different hierarchical sampling scales, ranging from 30 m to 0.3 m. Maps were derived with an inverse distance weighted interpolation, and SADIE (Spatial Analysis by Distance Indices) analysis was used to describe the degree of aggregation of the pathogen foci. *Rhizoctonia solani* showed a more aggregated distribution than *R. oryzae*, but surprisingly, the same spatial structure was maintained across all the scales (Paulitz and Rossi, 2004). This indicates that the pathogen exists as patches at a large scale (30–100 m), but there are smaller patches within the larger patches. This analysis also showed that *R. solani* was primarily found on rotation strips previously cropped with canola. Further analysis of these isolates found that they were *R. solani* AG 2-1, which caused both pre-emergence and post-emergence damping-off of canola in greenhouse testing. These are examples of how spatial analysis can provide important insights into the biology of pathogens that may not be apparent with single-site sampling.

Conclusion

Shifts in management systems toward low-input, sustainable practices can cause shifts in crop disease profiles. This is often reflected in changes in pathogen diversity, both at the interspecies and intraspecies level, but to detect these changes requires higher resolution DNA-based methods. These methods can also accurately detect and quantify pathogens without culturing from soil. If commercialized, this would enable growers to establish disease risks before planting, allowing them to base management decisions on this risk. Another utility of a quantitative DNA-based technique is for plant breeders to screen germplasm based on the biomass of the pathogen in the host tissue, or for breeders to establish a pathogen profile at variety-testing sites. Sites with high pathogen pressure could then be selected for cultivar evaluation. In the absence of disease resistance, insights gained from ecological and epidemiological studies can be used to develop and test cultural and agronomic methods that will minimize pathogen damage. These include crop rotation, fallow, residue management with

minimum tillage, control of weeds and crop volunteers, row spacing, and row openers that provide more disturbance in the seed row.

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