



Addressing root threats in pulse crops The Journal of the International Legume Society Issue 25 • July 2024

Front cover:

Exploring new sources of resistance to root rot in pea Provided by Tom Warkentin

Image p.10: Pea seedlings sprouting in jiffys AdobeStock[™] image

Image p.26: UAV image of the 2023 Campus Field pea/lentil root rot nursery, University of Saskatchewan Bright green plots are soybean used as a marker between populations Provided by Steve Shirtliffe (University of Saskatchewan)

IMPRESSUM

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LEGUME PERSPECTIVES

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EDITORIAL



This special issue of Legume Perspectives focuses on legume root diseases. The root rot disease complex has proven especially challenging in pulse crops but continued efforts in improved agricultural practices and resistance breeding are providing a more positive outlook in terms of managing this disease complex. In this issue various authors have contributed to provide a concise overview of current research on legume root diseases, specifically root rot, highlighting constraints and prospects. On behalf of the International Legume Society, we wish to thank the authors for their contributions to this special issue.

Tom Warkentin



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oot rot has become the key disease complex of legume crops internationally. In the 1990s and first decade of the 2000s, several international workshops were convened to discuss research on the ascochyta complex of pulse crops, but since then those

meetings have faded and in essence have been replaced by workshops on legume root rots. This issue of Legume Perspectives arises from the 9th International Legume Root Disease workshop held in Granada, Spain on September 18, 2023. Articles have been prepared to highlight key current research on genetic resistance to Aphanomyces, breeding for root rot resistance, field management of root rots including Aphanomyces and various Fusariums, and decision support systems to advise farmers on root rot risks. Specific articles address research on nematodes and parasitic weeds that also infect roots. A valuable view from two funding organizations provides farmer perspectives on the urgency of reaching solutions to legume root rots. Overall, it is critical that the legume research community makes solid progress on improving resistance and management of root rots in the coming 5-10 years, so that the benefits of legumes in rotation are maintained and expanded. As we all know, legumes provide valuable nutrition to people and animals while fixing nitrogen, which reduces the carbon footprint of agriculture. We hope you enjoy this special issue! Let's keep up our collaborations on this important topic.

Tom Warkentin



Managing Fusarium root rots in pea

by Lyndon D. PORTER^{1*}

Abstract: Peas are a critical rotational crop in dryland cereal production systems in the United States. Fusarium root rot caused by several *Fusarium* species can limit pea production and yields. *Fusarium avenaceum* is one of the primary *Fusarium* species impacting production. Integrated pest management practices associated with cultural, chemical, and biological practices, as well as genetic resistance, are recommended to limit Fusarium root rot in pea production areas.

Key words: *Fusarium*, integrated pest management, pea, root rot

Dry pea production in the United States in 2023 covered approximately 404,686 h and was centered in Montana (230,671 h), North Dakota (117,359 h), Washington (28,328 h), Nebraska (12,950 h), Idaho (12,141 h) and South Dakota (3,237 h) [1]. Peas are planted as an economic rotation crop with wheat and other cereals to break up disease cycles, fix nitrogen in the soil for the following crop and have low water use [2] making them an excellent rotational crop. Peas can be very prone to root rot pathogens. There are multiple *Fusarium* species that contribute to Fusarium root rot on pea [3]. These include *Fusarium avenaceum*, F. solani, F. redolens, F. acuminatum, F. graminearum and even F. oxysporum,

¹USDA-ARS, Grain Legume Genetics and Physiology Research Unit, 24106 N. Bunn Rd. Prosser, WA 99350, USA which is normally associated with Fusarium wilt but has been implicated in causing root rot. A root rot disease survey in Montana from 2016 to 2017 on chickpea, dry pea and lentil determined F. avenaceum (Figure 1) as the most abundant root rotting species isolated from diseased roots of these pulses and as highly aggressive on pea and chickpea [4]. Similarly, root rot disease surveys conducted in North Dakota in 2004 and 2007-2009, identified F. avenaceum as the most common root rot pathogen on pea in 3 out of the 4 years and as the most aggressive of the Fusarium pathogens isolated, making it the number one root rotting pathogen of concern in North Dakota [3]. In addition, in Canada, researchers found F. avenaceum isolated from pea to be very aggressive on pea as well as causing significant disease symptoms on chickpea and faba bean, with moderate symptoms on red lentil [5]. A root rot survey on edible dry winter peas in Washington State in 2018, determined the principle root-rotting pathogen prior to entering the winter dormancy period as F. redolens. Extensive root rot disease surveys on spring dry peas have not been conducted in other states outside of Montana and North Dakota, however, F. avenaceum has been identified in Washington [6,7], Nebraska [8], Idaho [9], and South Dakota [10] as either a common root rot and head blight pathogen on barley and wheat, or as a root rot on lentil. Isolates of F. avenaceum from cereals have been shown to cause root rot on both pulse and cereal crops [4].

In addition to infecting roots, it is known that F. *avenaceum* is a common contaminant on lentil seed from the Palouse region of eastern Washington and northern Idaho [6] and may be

a potential risk as a seed contaminant on dry pea seed throughout the major pea production regions of the USA as well. Since F. avenaceum is a pathogen on dryland barley, chickpea, wheat, pea and lentil, the likelihood of building up high soil inoculum levels of this pathogen in dryland soils is a high risk since these crops make up the common pulse/cereal rotations in the primary pea production areas previously mentioned. It has also been observed that Fusarium species tend to infect the same root areas rhizobia prefer to colonize, particularly right around the seed attachment zone, which are needed to form important nitrogen-fixing relationships with legume plants. Therefore, identifying control measures to manage Fusarium root rot in pea is critical to the sustainability of pea production and the benefits they provide.

What do we know and what needs to be done to manage Fusarium root rots on pea?

Cultural practices: It has been well established that reducing soil compaction can limit problems caused by Fusarium root rot [11,12]. Pea growers in the Palouse region of Washington



Figure 1. Fusarium avenaceum on pea roots.

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continue to roll the soil after planting to increase soil contact with the seed and produce a level field that improves harvestability. Continuing to develop plants with upright architecture, excellent anti-lodging characteristics and pod set toward the top of the plant can help growers move away from rolling their fields, especially when soil moisture levels are high in the spring, which can promote compaction. In addition, limiting the total number of ground rig passes over a field for management purposes can also reduce compaction issues in the fields. Promoting seed treatments or aerial applications that can reduce the number of pesticide applications applied by ground rig can also help limit compaction issues in a field.

Chemical control: Use of seed treatments to control seedborne and soilborne Fusaria is important. The surface or subsurface of pea seed coats are naturally infested or infected with Fusarium spp. This has been made very apparent in greenhouse studies where we have attempted to grow non-Fusarium-inoculated pea seed free from Fusaria, but unless an extensive seed surface sterilization technique is used, Fusaria are abundantly associated with the non-inoculated seed. Therefore, an effective contact fungicide to manage Fusarium spp. on the seed is paramount, and there is a need to develop or use seed treatments, such as pydiflumetofen, that have systemic or locally systemic action to manage soilborne Fusaria.

Biological control: In 2022, while digging and observing roots on multiple winter pea varieties in research plots in Moscow, ID, USA we came across plants with extremely large spherical nitrogen-fixing nodules which we referred to as mega-nodules (Figure 2). The plants associated with these mega-nodules had extremely healthy root systems that were unusually devoid of root rot symptoms and plants were larger and healthier than adjacent plants not colonized with these nodules. These mega-nodules were found forming on 10 different varieties of winter peas. A strain of Rhizobium leguminosarum was isolated from these nodules and an effort is underway to determine if other endophytes are associated with the rhizobium involved. Identifying different strains of rhizobium that act individually

or in synergistic relationships with other endophytes to promote root health may be a future means of managing root rot pathogens associated with not only Fusarium root rot but other root rotting pathogens as well.

Genetic resistance: Resistance to Eusarium root rotting pathogens have not been tied to single genes that confer complete disease resistance to these pathogens [13]. Instead, multiple genes appear to be involved. Despite multiple genes being involved, only partial resistance has been observed in hundreds of pea lines screened for resistance to F. solani and F. avenaceum, despite the lines representing diverse germplasm collected worldwide. This indicates genetic resistance alone will not be sufficient to completely manage these pathogens. In addition, it appears from initial research that different genes are likely conferring resistance to the different Fusarium species, since disease severity responses of pea lines independently inoculated with F. solani or F. avenaceum under greenhouse conditions varied greatly in resistance responses. Identifying pea lines with the best partial resistance to Fusarium root rot pathogens and identifying lines that are "larger-rooted" that were shown by Kraft and Boge [11] to help the plant respond better to Fusarium root rot infections, would help to dramatically improve our integrated management



Figure 2. Mega-nodule on a winter pea root grown under field conditions and the associated clean root system.

approach by pyramiding multiple resistance genes to combat Fusarium root rots in the future.

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Faba bean in North America — status and potential

by Nicholas J. LARKAN^{1*}

Abstract: Research into the molecular mechanisms underlying the *A. euteiches* infection process is difficult due to a number of factors, including the quantitative nature of the interaction and a lack of clear phenotypic differentials. However, recent progress in both pathogen and host genomics promise to open the door to fresh insights and help facilitate progress towards mitigation strategies for producers worldwide.

Keywords: molecular diagnostics, plant pathogen, resistance, *Vicia faba*

As a newcomer to the legume field and longtime resident of the Canadian prairies, I am both excited and somewhat daunted by the challenges faced by producers, particularly those growing pea and lentil crops, by the soil-borne oomycete pathogen *Aphanomyces euteiches*. Previous to starting the Pulse Crop Genomics group at the Saskatoon Research & Development Centre, I spent over 15 years researching the molecular interactions of blackleg disease in canola (*Brassica napus*; oilseed rape), caused by the fungus *Leptosphaeria maculans*. Our group was able

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to take advantage of the rapid development in genomics on both the host and pathogen, international collaborations and collections, the rich diversity of resistance sources within the available germplasm pool, the relative ease and robustness of available pathology techniques, and the ability to transform both host and pathogen. With these tools at our disposal, we were able to precisely characterize the presence of, and the sometimes complex interactions between, resistance genes (R) in the host and avirulence genes (Avr) in the pathogen [1], to produce robust molecular diagnostics for pathotype determination and pathogen surveillance, and to deliver valuable resistance genetics to breeding programs around the world.

Moving into researching Aphanomyces and its interaction with various pulse crop hosts, I'm definitely appreciating how spoiled I was working on the Brassica-Leptosphaeria pathosystem for all those years. There are many hurdles currently hampering efforts to characterize the molecular interactions of the system. The apparent sparseness of available resistance sources from which to tease out resistance alleles, the lack of transformation protocols for both pulse crops and A. euteiches to validate those interactions we can define, the complexity of the pathology systems, the challenge of maintaining long-term viability of isolates in the lab, differential pathotype interactions with multiple host species, the quantitative nature of the infection process, and the limited characterization of pathogen populations, all pose major issues for the development and deployment of effective management practices in pulse crops.

While these issues currently limit the progress

of the research, several recent developments are opening new doors. Genomic resources for the pathogen are available [2,3] and my own group hope to soon add several high-quality genome assemblies from Canadian A. euteiches isolates to this effort. We are building on the pathotyping of Canadian isolates that has already been performed [4] by defining the secretome of various isolates and correlating the effector complement to host specificity and aggressiveness. Establishing a system for the efficient transformation of A. euteiches would be of great benefit, to both our lab and several other labs around the world, for determining the molecular interactions governing development of the disease and the interplay between pathogen effectors and host resistance genetics, and we hope to see progress in this effort in the coming years. We are also contributing to efforts to use molecular diagnostic tools in deciphering the diversity of pathogen populations in Canadian prairie farmland soils. In doing so we hope to devise methods to make soil testing by producers more specific and insightful, and allow for comprehensive molecular surveys across the prairies and beyond. Having a proper grasp of the pathogen diversity, host specificity and distribution of individual pathogen genotypes, will also enhance efforts to both screen germplasm for effective resistance, especially in setting up relevant disease nurseries and to deploy the genetics in an effective and responsible manner. The A. euteiches genomic information will also form the basis for research into the next generation of pesticides based on RNA interference (RNAi).

Pulse genomics has made crucial advances in the last few years, providing us genomic resources for the extremely challenging genomes of pea, faba, and lentil [5-7], which will aid genetic research into these crops immensely. High-density marker systems enable efficient Genome-Wide Association Studies (GWAS) for large populations of plants and mining the diversity of germplasm available in international collections. While I am also involved in molecular genetic studies of both dry bean and pea, my main interest lies in faba, which has enormous potential in the Canadian agricultural system for many reasons, one of which is its apparent sturdiness against root rots, which are limiting traditional pea and lentil pulse production in many areas. However, we know that not all accessions of faba are resistant to Aphanomyces infection [8,9], and understanding the genetics controlling resistance in faba is important for maintaining those genetics in breeding programs to protect its current status. With support through the recent AAFC Pulse Cluster funding initiative, we are developing a faba diversity set of around 250 accessions for use in GWAS analysis of many phenotypic characteristics, including Aphanomyces resistance. To overcome the lack of genetic manipulation techniques in pulse crops we will be working with the model legume species Medicago truncatula, for which Aphanomyces-susceptible and tolerant accessions have already been identified [10], to provide a platform through which genes of interest, identified through GWAS or other molecular genetic studies, can be validated via transformation or gene editing techniques. However, recent exciting developments in pulse crop transformation [11] may soon open the door to direct testing in the host crop of interest.

A huge positive for root rot research is the passionate group of researchers around the globe who are investigating all aspects of the issue, many of whom I was able to meet at the recent 9th International Legume Root Disease Workshop, hosted immediately before the 4th International Legume Society meeting in Granada, Spain. As a member of the Scientific Advisory Committee, I was unsure about how much interaction there would be from the group during the scheduled discussion session, but boy was I wrong! It was a great meeting and I hope to see even more people at the next meeting (virtual), early in 2025.



Figure 1. Dr. Nicholas Larkan inspecting a faba plant from the worldwide faba diversity collection assembled at AAFC Saskatoon, Canada.

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Joining hands against the common foe

by Jagroop Gill KAHLON¹, Jenn WALKER¹, Meagen REED² and Sherrilyn PHELPS²

Abstract: The article discusses the challenges of growing pulse crops in western Canada especially the root rots and how the provincial commissions are coming together to cope with the disease in creative ways such are targeted funding calls to attract the best researchers and technology to mitigate the disease and hence improve profitability for pulse farmers. Details about inception of the Pulse Root Rot Network (PRRN) and Root Rot Task Force (RRTF) are provided.

Key words: *Aphanomyces euteiches*, Alberta, pulses, pathogens, root rot

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Root rot complex has become the leading production risk to pea and lentil acres in western Canada. Root rot complex consists of *Fusarium* spp., *Pythium, Rhizoctinia* and the pathogen of most concern, *Aphanomyces euteiches*. *Aphanomyces euteiches* was identified in Alberta and Saskatchewan in the early 2010's [1,2]. Since its identification, root rot complex and *A. euteiches* has caused a rapid reduction in pea and lentil acres and growers have been forced to rethink production strategies [3].

In 2021 Canada's provincial pulse grower associations developed a National Pulse Research Strategy to identify research priorities and coordinate pulse research across the country. Controlling root rots in pea and lentil, in particular *Aphanomyces* and *Fusarium* spp., were identified as a top priority. In 2022 the Root Rot Task Force (RRTF) was established consisting of the three prairie pulse crop commissions, Saskatchewan Pulse Growers (SPG), Alberta Pulse Growers (APG), and Manitoba Pulse and Soybean Growers (MPSG), to coordinate provincial efforts to maintain profitable and sustainable pea and lentil production and eliminate the risk of A. euteiches and root rot complex. Since these three organizations are producer led, and farmers are at the forefront, their voice becomes the priority. The first initiative of the RRTF was to bring together researchers, agronomists, and industry from across Canada through participation in the first Root Rot Rodeo event. From this event the Pulse Root Rot Network (PRRN) was established as a collaborative approach to root rot research and management focused on agronomy, breeding, and pathology in peas and lentils. Since the root rot rodeo, the RRTF has developed a strategic action plan to address root rots with a mission to eradicate the risk of root rots through a coordinated, collaborative effort across the pulse industry. This strategy was released at the second Root Rot focused session during the Canadian Pulse Research Workshop in February 2023 (Figure 1, 2).

The root rot strategy identifies three actionable areas of research, funding, and communi-

cation. Under research, three subcategories of agronomy, breeding, and pathology were prioritized, with targeted outcomes identified in each. Agronomic research is to provide growers with recommendations and tools for effective onfarm root rot risk management, and assessment. The focus in pea and lentil breeding is to develop robust genetic resistance to root rot pathogens, including both traditional and novel techniques. Pathology research aims to deepen our understanding of the biology, pathogenicity, and interactions of the root rot pathogens in pulses in the Canadian prairies. To achieve our research goals access to consistent funding, managing research funding cycles and investments in research capacity were identified as major hurdles to finding answers to the root rot issue facing producers. By collaboratively addressing these issues surrounding research funding and capacity, the RRTF aims to develop funding mechanisms for root rot that are collaborative, coordinated, and accessible, by establishing reliable funding sources and streamlined processes for ongoing research initiatives. Communication was identified as the third actionable area by the RRTF in the root rot complex strategy. Unified, consistent, science-based messages on root rot recommendations is vital information for growers, agronomists, and industry and foundational to meet the objective of eradicating risk.

Development of a full-scale website with detailed pathology, breeding, agronomy, and research pages is due to be launched later in 2024 (rootrot.ca), as part of RRTF project. Additionally, several fact sheets have been developed addressing root rot in peas and lentils that are currently available at saskpulse.com and will also be accessible through the new website (rootrot.ca). The Aphanomyces Risk Evaluation Application (AREA) app uses satellite imagery, crop inventory data, seeded acreage reports from crop insurance data, and weather information to determine the risk of developing an Aphanomyces infection the following growing season based on crop rotation. Growers can navigate to their field and the app will evaluate whether it is at low, intermediate, or high risk of an A.s euteiches infection.

All members of the RRTF identified root rot complex, specifically *A. euteiches*, as a serious issue to pea and lentil production and developed a long-term strategy to aid ongoing efforts by the PRRN in mitigating this risk. Currently, SPG is in the 4th year of a 10-year long-term strategy to mitigate root rot in peas and lentils. Within SPG's strategy, key initiatives have been identified for targeted action towards this goal including developing predictive tools for growers and agronomists, continued investments in research projects targeting solutions for root rot and understanding the pathogens, supporting continued survey, and monitoring efforts, investing in breeding programs focused on developing genetic resistance to root rot complex, and continued collaboration with RRTF. Since 2014, when root rot complex became a serious pulse issue, additional investments have also been made in pea and lentil breeding programs, where one of the main objectives is breeding for resistance to root rot complex pathogens.

The RRTF continues to invest in root rot research with several new fully collaborative projects that will drive advancements into the future. The Saskatchewan Ministry of Agriculture's Strategic Research Initiative (SRI) identified root rot complex as a major issue for Saskatchewan producers in 2023. In January of 2024, the funding for a new pulse-specific research program through the Saskatchewan Ministry of Agriculture's Strategic Research Initiative (SRI) under the Sustainable Canadian Agriculture Partnership (Sustainable CAP) was announced. Funding from commissions and other partners was leveraged for a total investment in pulse root rot research of over \$4.4 million. This fiveyear, multi-disciplinary program, with 15 collab-



Figure 2. Strategy to eradicate root rot in pulses.

orators and co-investigators from the University of Saskatchewan, the National Research Council of Canada - Saskatoon, and Agriculture & Agri-Food Canada (AAFC), will be led by Dr. Sabine Banniza, Professor of Plant Pathology at the Crop Development Centre, University of Saskatchewan. A second multifaceted project titled "PEA (Pea Climate-Efficient): Developing climate-resilient, low carbon footprint field pea as a preferred rotation crop through inter-disciplinary integration of genomic technologies" led by Dr. Sateesh Kagale from the National Research Council and Dr. Marcus Samuel from the University of Calgary was approved for funding through Genome Canada by both APG and SPG in 2023 and has a strong focus on finding genomic solutions for root rots. The interdisciplinary team includes 30 collaborators and co-investigators and spans both private and public research institutions across Canada, and internationally with a total project cost of \$6M. In total, so far, SPG and APG's have contributed \$35+million in root rot research with several projects to be invested in, currently in the pipeline.

Diseases in pulses have always attracted research focus and hence funding, but it is notable that since the detection of *A. euteiches* and root rot complex, APG and SPG have invested in 16 and 19 root rot complex or *A. euteiches* projects respectively, with many more proposals under review for future implementation. From these research projects APG and SPG has published the project results which can be found at Alberta Pulse website (www.albertapulse.com), SPG's Resource Library (www.saskpulse.com) or www.rootrot.ca.

To date, no one practice, or management tool eliminates or controls *A. euteiches* in peas and lentils. Current research findings show that rotation management reducing the frequency of pea and lentil crops in rotation, agronomic best management, and integrated pest management practices (IPM) like balanced fertility, using seed treatments, disease-free seed lots, soil testing, and evaluating your risk for *A. euteiches* can help reduce the impact of root rot complex infections [4-6]. The lack of effective chemical, biological, and cultural controls and lack of genetic resistance leave only one management option: avoidance [7]. Apart from the RRTF teams, Result Driven Agriculture Research (RDAR),

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Western Grains Research Foundation (WGRF) and the Agriculture Development Fund (ADF), also identify the issue and are prioritizing their research funding commitment towards this as well. Provincial producer organizations such as SPG and APG continue to support the annual provincial pulse disease survey where root rot complex pathogens are visually identified and rated based on both disease incidence and severity and are supporting the provincial and federal breeding programs. APG and SPG's work as members of the RRTF, and in conjunction with the PRRN, focus on conducting actionable, applied research that can be easily implemented at the farm gate by investigating IPM practices that will either increase resistance to Aphanomyces infections or eliminate treatment combinations from the list of unanswered questions in the search for solutions to root rot mitigation.

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In search of a decision support system for pea root rot: Assessment of multiple methods for accurate quantification of Aphanomyces euteiches in soil

by Syama CHATTERTON¹ and Shimaila ALI¹

Abstract: Aphanomyces root rot causes severe damage to field pea and lentil, leading to high yield loss. Fungicides and/or genetic resistant cultivars are not available. An accurate and consistent DNA quantification method to assess pathogen levels in soil and relate to disease severity would be ideal for risk prediction prior to planting pea or lentil. However, in a largescale test of soils from infested fields across the Canadian Prairies, several fields tested negative using DNA quantification methods despite high Aphanomyces root rot levels observed in the crop. Understanding the germination dynamics of A. euteiches in soil after planting pea could lead to the development of a better decision support system. Using quantitative polymerase chain reaction (qPCR) methods, 100 field soils that caused varying root rot levels were tested for pre-plant and post-harvest concentrations of

A. euteiches. These two numbers were significantly different for soils containing viable oospores. However, counts from soils that did not cause any disease remained the same before and after pea growth. To understand oospore germination dynamics, 5 pea seeds were planted in ~25 g of soil from eight fields and changes in quantifiable levels of A. euteiches in soils were monitored every other day using qPCR. The earliest detectable surge occurred 7 to 9 days after planting, and the soil DNA concentration at this time point was significantly correlated to observed root rot severity. More soils are being tested to understand how these dynamics can be linked to the risk associated with growing a susceptible crop in any given soil.

Keywords: Aphanomyces root rot, lentil, oospores, pea, quantitative PCR



Aphanomyces euteiches was first detected in Saskatchewan and Alberta pea fields in 2012 and 2013, respectively. Affected fields show large yellowing and stunted patches, with dying plants throughout. In severely infected fields, complete yield loss was observed. A wide-scale surveillance effort was then undertaken from 2014 to 2019, where it was found that 40 - 50% of pea and lentil fields across the three Canadian prairie provinces (Alberta, Saskatchewan, and Manitoba) were positive for A. euteiches [1]. The prevalence was as high as 70% in wet years and as low as 0% in lentils in dry years in Alberta. In areas where A. euteiches is endemic, the primary recommendation is disease avoidance. Primary inoculum exists as dormant oospores that remain in the field for up to 20 years [2]. Given that the longevity of oospores in any given field is unknown, pulse crop producers are left with the difficult task of deciding when or if it is safe to plant a pea or lentil crop in a particular field.



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Figure 1. Linear regression model of the quantifiable amount of ITS gene copies of Aphanomyces with respect to disease severity rating in pre- and post-bioassay soils. A. Pre-bioassay soil, B. Post-bioassay soil.

To aid with this decision, inoculum potential can be determined by growing a susceptible pea cultivar in field-collected soil in a greenhouse, under conditions that are conducive to disease development [3]. There is a strong correlation between disease severity in greenhouse assays and in the field, but the tests are labor- and time-intensive and have generally failed to gain acceptance with stakeholders. Quantitative molecular techniques such as real-time PCR (qPCR) or droplet digital PCR (ddPCR) have the potential to be more efficient methods for determining the presence and quantity of A. euteiches in soil [4-6]. Many assays have been developed for such purposes but need to be tested on Canadian field soils for validation.

The pea and lentil production area in Canada stretches across the Canadian Prairies (51M ha), with approximately 5M ha cropped annually, in three biogeographic zones. The biogeographic zones are also defined by different soil zones; brown, dark brown, and black chernozemic soils [7]. Recent research demonstrated that the relationship between oospore inoculum dose to Aphanomyces root rot severity response varied between soil zones [6]. This large variation in climate and soil properties across the Canadian prairies makes application of a soil-based molecular test for *A. enteiches* levels in the soil challenging. With this bio-climatic variation in mind, soil samples were collected in the fall from fields in Alberta and Saskatchewan that had just been cropped to pea or lentil in the summer from 2015 - 2017 and were tested using a standard bioassay and ddPCR to determine the relationship between disease severity and inoculum quantity. In this study, the correlation between the internal transcribed spacer region (ITS) copy number of *A. enteiches* and disease severity in the greenhouse bioassay was low. There were a number of false negatives, where *A. enteiches* was not detected .in the soil but Aphanomyces root rot severity was high. Studying the germination dynamics of *A. enteiches* in soil before and after planting pea could help to understand why the accuracy of measuring *A. enteiches* levels in soil was so low.

Methods and results

1) Comparison of *A. euteiches* DNA quantification in soil before and after performing a bioassay with field soils

One-hundred field soils that caused varying levels of root rot severity were selected for measuring pre- and post-bioassay amounts of *A. euteiches* in the soil using qPCR. A standard greenhouse bioassay was performed with disease severity measured at 28-days after planting on a scale of 1 (healthy) to 7 (dead) [1]. The pre- and post-bioassay concentrations of *A. euteiches* in the soil were significantly different for soils that caused Aphanomyces root rot, whereas counts from soils that did not cause any disease did not



Figure 2. Changes in the quantifiable amount of ITS gene copies of A phanomyces per gram of soil in eight field soils at 5 – 11 days after planting pea. DAP = Days after planting.

Table 1. Correlations between root rot severityandAphanomycesquantityinrootandrhizosphere soil.

Days after planting	Roots	Rhizosphere	
5	0.4416	0.7805	
7	0.6831	0.6486	
9	0.5624	0.1753	
11	0.3613	0.2706	
13	0.298	0.6563	
15	0.6694	0.4582	
17	0.5116	0.3807	
19	0.5691	0.316	
21	0.392	-0.0798	
23	0.2487	0.1625	

change. The ITS gene copies per gram of soil in the pre-bioassay soil was very poorly correlated with disease severity observed in the bioassays (R2 = 0.093) (Figure 1A). Furthermore, the quantities measured were below 100 oospores/g soil, which is the threshold level for moderate to severe disease to occur [8]. The correlation improved when *A. enteiches* was measured in the soils after the bioassay (R2 = 0.388), and the quantities increased by approximately 100-fold from pre-bioassay levels (Figure 1B).

2) Determination of the change in *A. enteiches* DNA levels in soils and roots after planting in field soils

To determine the best time to sample soil and roots from a modified bioassay, five pea seeds were planted in 25 g of soil from eight select fields with varying root rot severity levels. The quantifiable amounts of *A. euteiches* in soils and in roots were measured using qPCR every other day for 23 days after planting. Disease severity at the end was also measured in the standard 250 g bioassay. For all eight fields, the earliest detectable surge of *A. euteiches* in soil occurred

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5-9 days after planting (Figure 2) and generally plateaued at this level, until declining around 24 - 30 days after planting. The soil DNA concentration at 5 and 7 days in relation to disease severity at 28 days had R2 values of 0.7805 and 0.6486, respectively (Table 1). The root DNA concentrations had the highest R2 values at day 7 and day 15. However, our own personal observations were that it was harder to interpret root quantification values as sometimes the counts were extremely high, when root rot levels were still low. This could be due to the multi-copy nature of the ITS gene, and a single copy gene may be a better indicator of actual root infection since quantities are so much higher. On the other hand, for soil quantification, a multi-copy gene such as ITS is a better fit.

Interpretation of results and future directions

The results from these studies suggest that direct DNA quantification of soils from fields, collected either in the spring before planting or in the fall directly after a pea or lentil crop, do not provide a reliable estimate of oospore levels in the soils and subsequent disease risk. This seemed to occur more often with soils collected during a dry year or from a drier bioclimatic zone. This could be due to the dormancy properties of oospores that make DNA extraction difficult, although this hypothesis requires further testing. The surge observed at 5 - 9 days after planting a susceptible crop would, however, indicate germination of oospores leading to the exponential increase in zoospores, which are more easily quantified after DNA extraction. Furthermore, the gradual decline in the quantifiable amount of A. euteiches in the soil over time also suggests that oospores are more difficult to quantify. A quantification technique that is a hybrid between the traditional soil bait assay and direct DNA quantification from soils or roots at an earlier time point may provide the most accurate results. We also hypothesize that the amplitude of the surge is dependent on soil physio-chemical parameters, A. euteiches isolate, and initial concentration of dormant oospores. More soils are being tested to understand how these dynamics are linked to the risk associated with growing a susceptible crop in any given soil.

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Integrating early planting and fungicide seed treatment to mitigate yield loss due to root rot of field pea in the U.S. northern great plains

by Audrey K. KALIL¹ and Michael J. WUNSCH²

Abstract: Root rot caused by Fusarium spp. and Aphanomyces euteiches is a yield limiting disease of field pea in North Dakota and Montana. Management strategies are limited. Extended crop rotation intervals reduce root rot pressure but is insufficient as a stand-alone management tool, and resistant varieties are not available. Agronomic management strategies are needed to mitigate losses. In multi-location, multi-year studies conducted in North Dakota from 2017 - 2022, yield loss in fields infested with Fusarium spp. and A. euteiches was reduced by integrating early planting with the use of a seed applied fungicide. Root rot was less severe in early planted treatments, and seed applied fungicides improved establishment. In combination with extended crop rotations, early planting and fungicide seed treatment may provide growers with agronomically acceptable yields in infested fields.

Keywords: field pea, fungicide seed treatment, planting date, root rot

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North Dakota is a top producer of field pea (Pisum sativum L.) in the U.S. with 270,000 acres in 2023 [1]. Field pea is typically grown in semi-arid regions of the state in rotation with hard red spring or durum wheat under reduced tillage production systems. Production of field pea, as well as other pulse crops such as lentil (Lens culinaris Medik.), has gained in popularity in North Dakota due to high prices, good market access, compatibility with existing management practices and low input costs due to biological nitrogen fixation. Low wheat prices have driven short rotations for field pea and lentil production, resulting in the accumulation of soilborne root rot pathogens which impact both crops [2,3].

Surveys of grower fields have shown that the predominant root rot diseases of field pea in North Dakota are Fusarium and Aphanomyces root rot [4-7]. While Rhizoctonia solani Kühn and Pythium spp. are associated with the root rot complex of field pea, these organisms are generally well controlled with seed applied fungicides, perhaps explaining their relatively low abundance in survey efforts [7,8]. Fusarium root rot is caused by several Fusarium species, however the predominant species associated with root rot in North Dakota are F. oxysporum Schlecht. and F. avenaceum (Fr.) Sacc. [9]. These pathogens overwinter in soil and on infected crop debris. Fusarium avenaceum is also pathogenic on wheat so the combination of reduced tillage and wheat-pea rotation practices provides ample inoculum for Fusarium root rot [10]. Conditions which cause plant stress, drought and soil compaction, and warm soil temperatures facilitate disease development [4]. Symptoms include

brown to black necrosis beginning at the seed attachment site and spreading up the epicotyl and down the tap root [11]. When infection is severe, necrosis spreads out into the lateral roots and plants will appear stunted and chlorotic.

Aphanomyces root rot is caused by the oomycete Aphanomyces euteiches Dreschler, which overwinters in the soil as oospores. This pathogen is particularly problematic due to the long-lived nature of the oospores, which can persist in soils for up to 10 years [12]. Severe disease occurs in seasons where soils are warm (22 - 28°C) and moisture is high during the early part of the growing season just after crop emergence [12]. Symptoms associated with Aphanomyces root rot include golden-brown discoloration of the roots associated with degradation and sloughing off of the root epidermis. Severe infection may lead to bare patches in the field due to seedling death [13] (Figure 1). Where plants survive, they will be stunted and chlorotic.

Fusarium and Aphanomyces root rot typically co-occur in grower fields. Control tactics are limited as these pathogens can infect throughout the season, beyond the time frame that seed applied fungicides will provide protection. While efforts are being made to generate resistant varieties, even a variety that has performed well in recent North Dakota State University trials is described as moderately susceptible to Fusarium root rot [14]. Long crop rotations, where growers avoid host crops for five or more years before planting pea again, are recommended to reduce risk when planting into infested fields. This strategy, while effective, greatly limits the ability of growers to capitalize on market highs. Agronomic management strategies are needed

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Figure 1. Symptoms of Aphanomyces root rot in a grower pea field in northwestern Noth Dakota.

to allow growers greater flexibility in field pea production.

Collaborative research was conducted from 2017 - 2022 at the North Dakota State University Carrington and Williston Research Extension Centers and at on-farm locations in west central and northwestern North Dakota, with a history of root rot, to determine if the integrated use of planting date and seed applied fungicides would reduce soil-borne disease and improve yield under root rot pressure. Trials used a split plot design with planting date as the main plot effect and seed applied fungicide as the sub-plot effect. 'DS Admiral' yellow-cotyledon peas (2017, 2018, 2019) or 'LG Sunrise' vellow-cotyledon peas (2020) were planted on three dates 10 - 14 days apart, generally mid/ late April, early May, and mid-May. Soil temperatures were monitored with data-logging sensors placed at seeding depth (5 cm) which recorded temperatures every 2 hours over the first 7 days of planting.

Root rot severity (assessed at mid-vegetative growth) increased sharply with delays in planting (Figure 2). Root rot severity was minimized when peas were planted into soils that averaged (across day and night) less than 10°C at seeding depth (5 cm) in the 7 days after planting. Emergence suffered in very cold soils (< 7°C), and yield was maximized at soil temperatures of 7 to 10°C. Under root rot pressure, early planting conferred average yield gains of 4 - 8 bu./ac

(269 - 538 kg/ha). Planting within these target soil temperatures is possible with knowledge of current daytime and nighttime soil temperatures combined with the current 7 to 10-day forecast.

Seed applied fungicides improved yield consistently when soil temperatures averaged less than 13°C (average, day and night, at a 5 cm depth) over the first 7 days after planting, and seed treatments mitigated the emergence problems associated with planting into very cold soils (< 7°C) (Figure 3). Use of the commercial fungicide seed treatment Obvius/Insure Pulse (pyraclostrobin, 16.7 g/L + fluxapyroxad, 16.7 g/L + metalaxyl, 13.3 g/L; applied at 3.0 ml/kg seed) or Evergol Xtend C (penflufen, 154 g/L + trifloxystrobin, 154 g/L; applied at 0.25 ml/kg seed) + Proline (prothioconazole, 480 g/L; applied at 0.17 ml/kg seed) + Allegiance (metalaxyl, 312 g/L; applied at 0.16 ml/kg) fungicide seed treatment conferred average gains of 4-6 bu./ ac (269 - 404 kg/ha). Quantitative PCR analysis performed on root samples by the National Agricultural Genotyping Center (Fargo, ND) for A. euteiches and F. avenaceum and F. oxysporum confirmed the contribution of these pathogens to root rot symptoms observed when assessed at mid-vegetative growth. Testing was not conducted for Pythium spp. or Rhizoctonia solani, but the strong response to seed treatment observed when soils were cool suggests that both pathogens may be contributing to the root rot complex in problem fields.

Combining the practice of early seeding with a fungicide seed treatment for the control of Pythium spp. and R. solani has the potential to partially mitigate yield loss to root rot caused by Fusarium and A. euteiches. Planting at soil temperatures of 7.5 - 10°C (45.5 - 50°F, average day and night at 5-cm seeding depth in the first 7 days after planting) versus planting at soil temperatures \geq 12.5°C, conferred average yield gains of 8 - 14 bu/ac (538 - 942 kg/ha). Growers in the Northern Plains have reported unsatisfactory root rot pressure even with the use of extended crop rotations. The use of early planting in conjunction with extended crop rotation intervals and resistant varieties (when available) may allow growers to achieve commercially acceptable field pea yields under root rot pressure.

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Figure 2. Impact of soil temperature on root rot severity (A), field pea establishment (B) and yield of field peas (C) in fields with severe root rot pressure. Data represents the average across non-treated seed and fungicide-treated seed from 13 field trials conducted from 2017 to 2020 in fields with elevated Fusarium and Aphanomyces root rot across four locations in central and western North Dakota. Bar graphs show the average response relative to soil temperature observed across all studies. Scatter plots show the relative performance of peas planted at a given soil temperature relative to the two other planting dates conducted in each study.



Figure 3. Field pea yield response to fungicide seed treatment relative to soil temperature in fields with severe root rot pressure. Performance of non-treated seed versus seed treated with (A) XtendC + Proline + Allegiance (0.25 + 0.17 + 0.16 ml/kg seed) or (B) Obvius/Insure Pulse (3.0 ml/kg seed) or on field pea yield relative to soil temperature (at 5 cm deep, day and night, in the 7 days after planting). Data are from field trials conducted from 2019 to 2022 in fields with elevated Fusarium and Aphanomyces root rot across four locations in central and western North Dakota. Bar graphs show the average response relative to soil temperature observed across all studies. Scatter plots show the results from individual replicated studies or replicated planting dates within a planting date study.

Genetics and breeding for quantitative resistance to Aphanomyces root rot in pea (Pisum sativum)

by Marie-Laure PILET-NAYEL*, Théo LEPRÉVOST¹ and Clément LAVAUD¹

Abstract: Aphanomyces root rot is a significant threat to global pea cultivation. The development of resistant varieties is crucial for managing the disease in the absence of highly effective methods of control. Pea genetic resistance to Aphanomyces euteiches is partial and polygenically inherited, making the breeding of resistant varieties challenging for many years. Recent advances in pea genomics and breeding have enabled the identification of consistent genetic regions on the pea genome that are associated with partial resistance. These advances include the fine mapping of two of these regions showing major effects and the evaluation of combinations of these regions for their impact on increasing resistance levels. The results provide valuable markers and knowledge to support breeding programs, which have already led to the registration of the first tolerant varieties in France.

Keywords: Aphanomyces euteiches, fine-mapping, Near-Isogenic Lines, Quantitative Trait Loci

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Aphanomyces root rot, caused by the soilborne oomycete Aphanomyces euteiches, is a major disease affecting peas worldwide. Aphanomyces euteiches has a broad host-spectrum among legumes including lentil, alfalfa, common bean, clover, vetch and faba bean. The pathogen can produce oospores that display a high level of resistance to extreme environmental conditions, leading to an extended life-time of over 10 years in soils or root residue. The primary symptoms of the disease are necrosis of the roots and softening of the epicotyl, resulting in wilting and chlorosis of plant shoots. Two main pathotypes of A. euteiches are reported in pea, including pathotype I detected in several countries around the world and pathotype III which has only been reported in the United States and Canada [1-3].

Long rotations of pea crops, alternated with non-host species, are recommended to limit the development of the pathogen in cultivated soils. In addition, predictive tests have been developed to detect and quantify the presence of *A. euteiches* in contaminated soils, aiming to prevent the cultivation of pea crops [4,5]. However, no highly effective method of control is currently available. Partially resistant germplasm have been released, particularly in the United States and France, over the past 30 years [6].

Pea breeding for resistant varieties to Aphanomyces root rot has been a major challenge worldwide for several decades. This difficulty is attributed to several factors, including (i) the polygenic inheritance and partial level of the resistance, along with linkages with undesirable traits, (ii) the limited availability of resistance sources in *Pisum sativum* and related species that are intercrossable with peas, (iii) the difficulty of simultaneously breeding for partial resistance and polygenic agronomic traits, such as yield, and (iv) the limited understanding of the biology of the pathogen and the populations present in infested soils. However, significant research progress achieved over the past decade is now paving the way for new strategies involving the diversification, fine selection, and combination of genetic resistance loci, for breeding pea varieties with high and durable levels of resistance.

An overview of the diversity of genetic regions associated with partial resistance to A. euteiches in peas was recently reported [7]. Using 1,850 common bridge Single Nucleotide Polymorphism (SNP) markers, this study integrated Quantitative Trait Loci (QTL) mapping studies from four Recombinant Inbred Line (RIL) [8] and two Advanced Backcross (AB) populations, along with a Genome-Wide Association Study (GWAS) conducted from a pea-Aphanomyces collection [9]. Ten main "Ae-Ps" genetic regions were consistently identified across various environments, isolates and/or sources of resistance. These regions were then mapped onto a high-density consensus marker map (Figure 1). Resistance alleles at these QTL were derived from four resistance sources previously identified in the United States., i.e. 90-2079, 90-2131 [10], PI180693 [11] and 552 [12], and two new exotic sources of resistance, E11 and LISA, identified through a large germplasm screening program conducted at INRAE in the 2000s [9,13]. This study provides new QTL closely linked SNP markers, potential new alleles from previously unexplored sources of resistance, and confirmed allele combinations associated with an increased level of resistance, which



Figure 1. Schematic representation illustrating the 10 consistent Ae-Ps genetic regions associated with partial resistance to Aphanomyces root rot, mapped onto a high-density consensus marker map. These regions were identified by linkage mapping in Recombinant Inbred Lines (RIL) or Advanced Backcross (AB) populations, or by Genome-Wide Association Study (GWAS). At the top of the bars: linkage groups in Roman numerals and corresponding pea chromosome numbers; name of Ae-Ps in bold corresponding to the seven main regions identified in Hamon et al. (2013) [8]; with two sub-regions (a and b) at Ae-Ps7.6. Photo: symptom of Aphanomyces root rot on pea (Source: INRAE). Figure adapted from Figure 2 in Leprévost et al. (2023) [7].

will be useful for breeding pea resistant varieties. The fine mapping of two resistance QTL, Ae-Ps7.6a and Ae-Ps4.5, which exhibit major-effects against reference strains of A. euteiches belonging to the pathotype I and III, respectively, was conducted. This work aimed to identify candidate genes and diagnostic markers that could be useful in QTL pyramiding schemes, allowing to broaden the spectrum of efficacy of the resistance. Screening with SNPs of large plant populations (>200 for Ae-Ps4.5 and >3000 for Ae-Ps7.6a) derived from NILs (Near-Isogenic Lines) carrying each QTL, led to the identification of recombinant plants. Analysis of the marker-phenotype association in these recombinants enabled the reduction of the Ae-Ps4.5 size to a physical interval of 3 Mb, containing 50 annotated genes on the Caméor pea reference genome v1a [14]. Among these genes, three putative candidate genes were identified, encoding DEAH helicase, C2 domain and subtilase family proteins [15]. The characterization of recombinant individuals at Ae-Ps7.6a resulted in the reduction of the QTL size to a physical interval of 1.9 Mb. The analysis of annotated genes within this interval on the second version of the pea reference genome and the identification of candidate genes underlying the QTL are currently underway using additional sequencing data (from RNA and whole genome of the progenitor). Quillévéré-Hamard *et al.* [16] reported that the *Ae-Ps7.6a* genetic region could exhibit a moderate spectrum of efficacy on the French pathogen populations, suggesting the potential benefit of combining *Ae-Ps7.6a* with other QTL to enhance and preserve the efficacy and durability of the partial resistance.

The effect of combining QTL on increasing resistance levels was investigated using >300 NILs. These NILs combined up to five QTL and were generated from marker-assisted back-crossing followed by intercrossing within spring and winter pea agronomic lines [17]. The NIL series were evaluated for resistance under controlled conditions and across a multi-location/ year French Aphanomyces network of highly contaminated nurseries and plots. Several combinations of two to three QTL confirmed their increased effect on resistance, particularly when the *Ae-Ps7.6a* genetic region was included either alone or in combinations of minor-effect QTL. Some combinations of minor-effect QTL also

demonstrated increased resistance in NILs, but also undesired linkages, notably with late flowering and colored flowers. Pyramided NILs, combining resistance alleles from different sources, exhibited a reduced impact of the disease on both vegetative and reproductive plant development. The effect of combining resistance QTL in NILs was also demonstrated in reducing the colonization and reproduction of *A. euteiches* [18], and will be explored on the first stages of the plant-pathogen interaction within the soil.

Resistance QTL for Aphanomyces root rot have been successfully introduced into pea breeding programs in France. This resulted in the registration of the first tolerant varieties in 2019, characterized by a yield preservation score in highly contaminated field conditions [19; Figure 2]. However, additional efforts are required to durably increase the levels of partial resistance by diversifying and combining resistance QTL in future pea varieties. Strategies, such as gene discovery at major-effect QTL and the implementation of new genomic-assisted pea breeding schemes, are promising for combining resistance and yield alleles. A deeper understanding of QTL-associated defense mechanisms in plant roots and the rhizosphere, coupled with that of the conservation of QTL-associated resistance genes among legumes, will provide new targets and breeding methods for QTL diversification, to effectively manage Aphanomyces root rot in legume-rich cropping systems.

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Figure 2. Tolerant pea variety (on the left) and susceptible pea variety (on the right) to Aphanomyces root rot, cultivated in a highly contaminated field (Source: Terres Inovia).

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LEGUME PERSPECTIVES

19

Breeding pea for improved root rot resistance

by Kishore GALI, Shivappa HUKKERI, Devini DE SILVA, Sivachandra KUMAR NIMLLASH, Stephen O. AWODELE, Sabine BANNIZA* and Tom WARKENTIN*

Abstract: The emergence of root rot diseases is a recent threat to pea production in western Canada. We targeted the rapid development of pea cultivars with improved Aphanomyces root rot resistance using marker-assisted introgression of resistance QTLs. This approach resulted in lines with improved field level root rot resistance. A root rot nursery was developed to screen pea and lentil breeding lines for resistance to the root rot complex. We continue our pea breeding efforts to achieve an optimum combination of disease resistance and agronomic performance.

Keywords: Aphanomyces, Fusarium, pea, Pisum, root rot

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Root rot background in western Canada

Western Canada contributes approximately a quarter of global pea and lentil production. These crops account for a substantial portion of the region's agricultural economy and are integral to crop rotation practices which maintain soil health and reduce greenhouse gas emissions. The area devoted to peas and lentils in western Canada averaged 3.10 million ha in the most recent five-year period (2019-2023) [1]. However, the last decade has seen root rot diseases becoming a serious threat that has disrupted production and poses a risk to the sustainability of farming in the region. The spread of root rots can have a profound economic impact on farmers and the agricultural sector as a whole. Managing root rot diseases is challenging and requires urgent and sustained efforts. Among the root rot diseases of pea and lentil, Aphanomyces root rot caused by Aphanomyes euteiches is the most important. One of the recommended strategies to combat the spread of Aphanomyces root rot is altering crop rotation patterns to have a six to eight-year gap between planting two susceptible crops, due to the resilience of A. euteiches in field soils for extended periods without host plants. Such practices would alter the crop composition of the region, potentially affecting the overall agricultural output, economic returns, soil health, and climate-smart agricultural practices. As a strategic approach to maintaining the delicate balance between disease management, economic stability, and environmental sustainability in agriculture, we have focused on the rapid development of pea cultivars with improved Aphanomyces root rot resistance using marker-assisted introgression of resistance quantitative trait loci (QTLs) identified by colleagues in France and USA [2,3].

Rapid introgression of Aphanomyces resistance

An intensive backcrossing program was initiated to introgress resistance from the reported main sources of Aphanomyces root rot resistance into yellow and green pea varieties well suited to western Canada. Three lines PI 660729, PI 660733 and PI 660736 [4] were used as sources of major-effect QTLs Ae-Ps7.6a, Ae-Ps7.6b and minor-effect QTLs including Ae-Ps1.2, Ae-Ps3.1a and Ae-Ps5.2. Line 90-2079 [5] was used as a source of the major-effect QTL Ae-Ps4.5. The three PI lines and 90-2079 were crossed with six elite Crop Development Centre, University of Saskatchewan (CDC) cultivars and backcrossed twice using the CDC cultivar as the recurrent parent. The F1/F2 seeds of each cross were tested using Kompetitive Allele Specific PCR (KASP) assays to select seeds with the introgressed QTLs. The BC2F3 seeds of each cross were phenotyped for Aphanomyces root



Figure 1. Aphanomyces oospore counts based on samples collected at the root rot nursery, University of Saskatchewan from 2017-2022. Data shown are in oospores per 50 views.

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Figure 2. Root rot nursery developed at University of Saskatchewan to screen germplasms against root rot complex. The chlorotic area is the section with drip irrigation, while the green area was not irrigated in Summer 2022. The smaller rectangle is the small plot trial.

rot resistance in controlled environment conditions, and the selected resistant plants were intercrossed to pyramid Ae-Ps7.6a, Ae-Ps7.6b, and Ae-Ps4.5. The F2 seeds of these intercrosses were again genotyped for the presence of the three major QTLs and phenotyped for Aphanomyces root rot resistance. Seeds harvested from the best partially resistant plants were bulked and tested for agronomic traits including yield differences in field trials in the University of Saskatchewan pea breeding program, and subsequently in registration trials. In the course of three years, molecular markers were developed to trace QTLs, a total of 75 crosses were made, and more than 9,000 plant tissue or seed samples were screened with molecular markers in addition to pathogenicity testing of close to 100 lines.

Establishment of root rot nursery

In 2017, we established a field root rot nursery at the University of Saskatchewan to facilitate root rot testing of pea and lentil breeding lines under conditions mimicking their natural growing environments. Since then, root samples collected from the nursery have been used for monitoring soil pathogens using microscopic and Polymerase Chain Reaction (PCR) tests. The data indicate a consistent rise in the concentration of *A. enteiches* oospores since 2017, as illustrated in Figure 1. In 2022, the introduction of a drip-line irrigation system significantly enhanced the severity of root rot. The overview of the root rot nursery presented in Figure 2 clearly illustrates the impact of irrigation. Over the years, our investigations have revealed that the nursery harbors a diverse complex of root-rotting pathogens, including *A. euteiches*, *Berkeleyomyces basicola, Fusarium avenaceum, F. redolens* and *F. solani*. This diversity underscores the ongoing value of the nursery for evaluating the overall 'root rot' susceptibility of breeding lines under field conditions.

Root rot resistance in QTL pyramided lines - perspectives

The backcross-derived lines pyramided with Aphanomyces root rot QTLs were identified to be resistant to Aphanomyces root rot in controlled environment tests. The level of resistance is comparable to the resistant lines used as trait donors. For example, CDC Inca introgressed with the major-effect QTLs (cross No. 70901) showed an average disease severity of 32.5% in comparison to 71.8% in CDC Inca, 37.8% in PI 660733 and 40.9% in 90-2079 (Figure 3). PI 660733 and 90-2079 were the sources of Aphanomyces root rot QTLs in cross 7090. Four breeding lines from the molecular breeding experiments with improved resistance to Aphanomyces were selected for their agronomic performance in the first year of field evaluation. These lines have shown reasonable overall performance in registration trials thus far, with lower disease severity than other elite lines in three different root rot nurseries, but are somewhat lower yielding than the best checks. This is not surprising as our first cohort of lines with improved root rot resistance was quite small. As part of our ongoing breeding efforts, these lines have been crossed with many elite breeding lines to select the next cohort of lines with greater yield potential. Concurrently, our research team is actively engaged in identifying QTLs that confer resistance to F. avenaceum, and additional resistance sources for Aphanomyces and other Fusarium root rots. Looking forward, our breeding initiatives will focus on combining Aphanomyces and Fusarium root rot resistance QTLs in elite pea cultivars, thereby fortifying their resistance against multiple root rot pathogens to expand the acreage of pea in western Canada.



Figure 3. Aphanomyces root rot severity (growth chamber study) in an introgression line pyramided with major-effect QTLs.

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¹ 7090-F1-7-91 is an introgression line pyramided with Aphanomyces root rot major-effect QTLs *Ae-Ps7.6a* and *Ae-Ps7.6b* from PI 660733 and *Ae-Ps4.5* from 90-2079 in the genetic background of CDC Inca.

Searching for new sources of resistance to root rots in pea

by Sara RODRÍGUEZ-MENA, Diego RUBIALES and Mario GONZÁLEZ

Abstract: Root rot diseases are currently responsible for major losses in pea crops. Among the potential solutions to combat these diseases, plant breeding stands out as the most efficient and environmentally friendly control measure. Developing cultivars that exhibit both resistance and favourable agronomic characteristics is essential, underscoring the importance of identifying resistant accessions. Various germplasm collections of Pisum spp. from around the world are available. Screening these collections for resistance has led to the identification of some accessions with varying levels of resistance to root rots. Despite notable advances in recent years, there is still much work to be done to develop fully resistant varieties and improve phenotyping methods.

Keywords: phenotyping, Pisum, screening

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Introduction

Since its domestication 10,000 years ago, pea (*Pisum sativum*) has emerged as one of the main cultivated temperate legumes. The wide diversity in pea germplasm is shown by the ~98,000 accessions stored in ~25 major gene banks distributed around the world (Figure 1). Although wild genotypes typically exhibit a wide range of adaptations, which include resistance to biotic and abiotic stresses, they constitute only about 1% of the conserved germplasm [1].

Pea production can be constrained by aerial and root fungi, viruses, bacteria, nematodes, and parasite plants and pests, which reduce crop productivity and quality. Among them, root rot pathogens are responsible for the most devasting losses which is highlighted by their high prevalence in soil and worldwide distribution. Among the potential solutions to combat these diseases, plant breeding stands out as the most efficient and environmentally friendly control measure. Developing cultivars combining both resistance and favourable agronomic characteristics is essential. Screening collections for resistance has led to the identification of some accessions with varying levels of resistance to root rot pathogens, highlighting the significance of this method in discovering new sources of resistance. Despite notable advances in recent years, there is still much work to be done to develop fully resistant varieties [2].

Root rot diseases

Typically, multiple pathogens are involved in the disease collectively referred to as pea root rot complex which is one of the main concerns in pea production. It adversely affects seed ger-

mination, resulting in browning and softening of root tips, formation of root lesions, and yellowing and wilting of leaves. Furthermore, it hampers seed germination, slows down plant growth, and reduces both crop yield and quality. Depending on the causal agent, host susceptibility, and environmental conditions, crop losses can range from slight to complete crop failure. Pea root rot can be caused by fungi and oomycetes, including Aphanomyces euteiches, Fusarium oxysporum, F. solani, F. avenaceum, Rhizoctonia solani, Pythium spp., and Phytophthora spp. These pathogens commonly coexist, and the observed symptoms are a result of their collective action. Depending on the climate conditions, the effect of one prevails over the others. In humid and cold climates A. euteiches, R. solani and Phytophthora spp. are the predominant pathogens, while warm and dry conditions favour the effect



Figure 1. Diversity of pea seeds from Institute for Sustainable Agriculture (Córdoba, Spain), collection.

Pathogen	Resistant accession	Reference
Aphanomyces euteiches	PI180693, 552, E11, LISA, 90-2131, 90-2079	[4]
Pythium ultimum	PI140165, PI183910, PI194006, PI210587, PI223285	[5]
Fusarium solani	JI1794, PI140165, PI183910, PI194006, PI210587, PI223285, Carman	[5-7]
F. oxysporum f. sp. pisi	JI1412, JI1559, JI1559, P656	[8]
F.avenaceum	Carman	[9]

Table 1: Reported accessions with resistance to various root rot pathogens.

of Fusarium spp. [3].

Cultural, physical, biological, and chemical control methods have been used as management strategies to control root rot. However, due to the broad range of hosts and the high prevalence of these pathogens in soil, these strategies remain incomplete solutions to date. Developing resistant varieties seems to be the key to manage root rot. Screening programs have enabled the identification of accessions with varying levels of resistance, particularly wild types, facilitating the exploration of their resistance mechanisms and their integration into breeding programs for the development of recombinant inbred lines (RILs) (Table 1).

Phenotyping

The traditional method to assess root rot symptoms in peas involves rinsing the roots at the end of the experiment and visually evaluating them based on a predefined scale (Figure 2). However, this method is laborious, destructive and provides only a single-point assessment. In addition, the evaluation is subjective and prone to human errors. Approaches such as high-throughput phenotyping using imaging techniques provide an objective and quantitative selection of resistant genotypes as well as reduce the time of evaluation.

Various sensing and imaging techniques have been studied for stress detection in both controlled and field conditions. As sensing imaging techniques, four types can be distinguished: digital Red-Green-Blue (RGB), florescent, mul-

ti/hyperspectral and thermal imaging. At present, RGB provides the best approach to study rot root. This technique also enables the characterisation of morphological traits and the analvsis of root architecture, which are associated with root rot resistance. Using RGB imaging, Divyanth et al. 2022 [10] developed a software called DeepArrNet that classifies images of pea roots as resistant, intermediate, or susceptible based on symptoms of A. euteiches. Similarly, Bari et al. 2023 [11] designed a high-throughput phenotyping platform that uses RGB imaging to assess resistance to Aphanomyces root rot based on aerial symptoms. Susceptible genotypes exhibit low chlorophyll concentration and show signs of senescence and necrosis in the foliage, which can be effectively detected with an RGB camera. This platform facilitates the rapid screening of numerous genotypes, allowing for the quick elimination of susceptible plants and tracking disease progression. However, a second evaluation of the roots is recommended after initial screening due to the possibility of the selection of tolerant genotypes which display rot necrosis but do not present visible symptoms in leaves. Despite this, the platform considerably reduces the time of screening. Additionally, open sources such as PlantCV are also available for high-throughput phenotyping analysis. PlantCV has been utilized in various studies, from characterizing germplasm in winter wheat to creating image masks in herbarium specimen data. Recently, this Phyton software library was employed in developing RootDS, a pipeline for

scoring Fusarium root rot in common beans [12].

Imaging techniques are not the only method for evaluating root rot. Both, biotic and abiotic stresses induce changes in plant physiology and metabolism (Figure 3). Detecting these changes through specific sensors has enabled the characterization of resistant and susceptible plants, as well as the elucidation of key factors in plant defence responses. One of the most studied factors in primary metabolism under these stresses is photosynthesis. Near-infrared (NIR) spectroscopy is one of the employed techniques to study changes in photosynthesis as response to *R. solani* in *Oryza sativa* [13].

Secondary metabolites, unlike primary metabolites, are considered non-essential for primary functions and are involved in plant immunity. These metabolites can serve as informative markers for resistance mechanisms or infection progress symptoms, as well as indicating the magnitude or quality of the plant immune response. Various techniques can be employed to detect these secondary metabolites. For instance, Raman spectroscopy has been utilized to create a spectroscopy fingerprint for the detection of Fusarium wilt in bananas [14].

Plant-pathogen interaction can lead to changes in plant tissue temperature. Measuring thermal energy dissipation provides another method of phenotyping [15]. However, a significant challenge with all these techniques is their cost and the high level of specialization required. More user-friendly devices have been developed in recent years. For the detection of volatile organic compounds, several e-nose models are commercially available for plant diagnosis purposes. While these devices yield promising results under controlled conditions, their effectiveness in field settings is limited [16]. Other devices, such as Multispeq, combine measurements of environmental conditions and photosynthetic parameters to characterize the plant response [17]. This device presents a user-friendly method of plant diagnosis with promising results in rot root. A strong correlation was observed between RGB, multispectral and hyperspectral trails with visual scores [18]. Integrating various phenotyping methods with evaluations of both foliage and roots would likely provide the most accurate assessment.

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Figure 2. Aphanomyces root rot scale of evaluation. Scale 0 to 4.

Conclusion

Despite the laborious process of screening collections for resistance, it remains one of the most effective ways to find new sources of resistance. Image programs and Deep Learning offer the potential to develop faster and more precise phenotyping methods, which could facilitate research. While accessions with varying levels of resistance to root rots have been identified, it is imperative to continue investigations in this field, understanding the genetic basis of this resistance and combining classical selection breeding with novel phenomic and genomic tools in the development of elite resistant cultivars to be released to farmers.

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Figure 3. Non-visual phenotyping changes during plant infection. Created with BioRender.com

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Faba beans: An option for sustainable Canadian crop production

by Ahmed ABDELMAGID

Abstract: Faba bean (*Vicia faba* L.) is gaining traction in the Canadian prairies due to its environmental benefits and economic value. Despite challenges like root rot and foliar diseases, faba bean offers promise for diversifying crop production and enhancing sustainability. Further research is needed to address disease management and optimize cultivation practices for successful faba bean production in western Canada.

Keywords: Canadian prairies, crop diversification, diseases, economic value, environmental benefits, Faba bean

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Faba bean (*Vicia faba* L.) are a crucial legume crop on a global scale [1]. It is widely grown as food, feed, and green manure worldwide [2] and is a rich source of protein, starch, dietary fiber, and vitamins [3]. Limitations on faba bean yield, once a major concern in the 1970s [4,5], no longer exist. Breeders strive to improve yield stability as well as seed quality [4]. Due to genetic resistance, both abiotic stresses [5] and biotic stresses [6] have been reduced, leading to an increase in productivity per unit.

Faba bean, while historically viewed as a less prominent crop in the Canadian prairies, is experiencing growing interest for cultivation owing to its positive environmental contributions and rising economic value [7]. Faba bean thrives in cooler temperatures and typically takes between 80 to 100 days to mature for harvesting [7]. Including faba bean in crop rotations enhances soil fertility by leveraging its strong symbiotic relationship with specific Rhizobium bacteria (i.e., Rhizobium leguminosarum). This partnership leads to biological nitrogen fixation, reducing the necessity for additional fertilizers in agricultural lands while boosting overall soil biological activity. Research conducted on pulses grown in western Canada revealed that faba bean was the most nitrogen-fixing pulse, acquiring 88% of its nitrogen directly from the atmosphere [3,8]. There is, however, a major obstacle to the adoption of faba beans: their utilization as a food source has been limited due to the presence of pyrimidine glycosides called vicine and convicine (v-c). Consumption of v-c can trigger a condition called favism in individuals with a hereditary deficiency in glucose-6-phosphate dehydrogenase (G6PD). V-C can reduce feeding efficiency in monogastric animals. Elimination of these glucosides is one of the main objectives of faba bean programs worldwide [9].

In western Canada (Manitoba, Saskatchewan, and Alberta), pulse crops have shown to benefit both agriculture and the environment [9]. Considering the growing population and the health benefits of pulses, western Canada will play an important role in meeting the global demand for pulses. Plant diseases such as Aphanomyces root rot and Anthracnose threaten field pea and lentil crops [8]. The Canadian prairies need alternative pulse crops, and faba bean is well suited to those areas due to its cool and moist climate. Currently, numerous root and foliar diseases are affecting faba bean in western Canada that are not well understood. Since the inception of faba bean in western Canada, the foremost hurdle affecting their yield has been chocolate spot, caused by Botrytis fabae Sard., representing a significant biotic stress factor [3]. Production of faba beans cultivars with low tannin seed on the Canadian prairies has the potential for use in human food, as a feedstock for livestock, and as a forage crop. Low tannin cultivars have a thin seed coat with low concentrations of saponins and alkaloids, which may increase susceptibility to seedling blight and root rot [10]. Therefore, root rot could be a major issue in the long-term production of faba bean in western Canada. Root rot disease in faba bean is caused by multiple pathogens, including Fusarium spp., Rhizoctonia spp., and Pythium spp. [11]. In China (the largest producer of faba beans), Fusarium spp. is reported to cause the most root rot in faba bean crops and to cause severe yield loss [11]. The yield loss ranges from 5% to 100% in

severely infected fields [11]. In western Canada, only one disease survey was conducted on faba bean in Manitoba almost 50 years ago [6]. It indicated that Fusarium spp., Rhizoctonia solani Kuehn, and Sclerotinia sclerotiorum (Lib.) de Bary were associated with root rot of faba bean in Manitoba. Additionally, Saskatchewan and Alberta have not updated their root rot data since 1975 and 1981 respectivelly, two provinces that can potentially produce more faba beans in the future [12,13]. More research is crucial in comprehending how root rot diseases impact faba bean production in western Canada. The cultivation of faba bean presents an opportunity to diversify Canadian crop production. Its adaptability to particular climates and suitability for specific regions provides farmers with the chance to vary their crop selection, mitigating risks and bolstering resilience against environmental shifts. Moreover, there is a requirement for continued exploration into refining farming practices for faba bean in western Canada. This entails studying factors such as optimal plant density, fertilization techniques, irrigation methods, and effective strategies for managing weeds and diseases. These elements play a pivotal role in influencing both the quantity and quality of faba bean yields.

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Root parasitic plants threatening legume crops

by Diego RUBIALES*, Maria J. COBOS and Osman Z. WOHOR

Abstract: Root parasitic weeds are major threats for legume cultivation in large territories. The most important of these are the broomrapes (*Orobanche crenata*, *O. minor*, *O. foetida* and/ or *Phelipanche aegyptica*) threatening temperate legumes in the Mediterranean basin and Middle East; and *Striga gesnerioides* and *Alectra vogueli* threatening warm season legumes such as cowpea in Sub-Saharan Africa.

Genetic variation for resistance has been reported in most instances and is being used by breeders. Monogenic resistance has been reported in cowpea against S. generioides but not yet on any legume crop against Orobanche species, where resistance identified so far appears to be incomplete and complex in nature. Genetic studies have resulted in the identification of a number of quantitative trait loci (QTLs) in most crops, but progress in delivery of markers for use in marker assisted selection (MAS) has been slow. This, together with the insufficient understanding on the variability of the pathogen populations has complicated resistance breeding. Current achievements and prospects in breeding for resistance to broomrapes in major cool season food legumes will be discussed.

Keywords: biotechnology, breeding, crop management, legumes, Orobanche, resistance, Striga

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Several thousands of plant species have evolved to feed on other plants, most of them occurring only in the wild and not causing agricultural problems. However, a few have adapted to agricultural environments, becoming weedy and posing a serious economic threat mainly because they are at present almost uncontrollable [1]. They can be grouped in stem parasites (mainly Cuscuta and Viscum) and root parasites (mainly Striga, Orobanche and Phelipanche). The weedy root parasites are very problematic as they exert their greatest damage prior to their emergence, complicating diagnosis and control. A wide variety of approaches - physical, cultural, chemical, and biological - have been explored, but most of these are not effective enough [2].

The broomrapes (Orobanche and Phelipanche spp.) are widespread in Mediterranean areas in Asia and Southern and Eastern Europe, attacking dicotyledonous crops and depend entirely on their hosts for all their nutritional requirements. Orobanche crenata (Figure 1a, b) is the most widespread and damaging species, infecting grain and forage legumes, as well as some Apiaceous crops like carrot and celery. Phelipanche aegyptiaca (syn. O. aegyptiaca) is important on many crops including Fabaceae, Solanaceae, Cucurbitaceae, Brassicaceae and Asteraceae in Eastern Mediterranean countries, extending eastwards. Orobanche foetida is widespread in the Western Mediterranean infecting wild legumes only. However, a population has been described severely attacking legumes in areas of Tunisia. Orobanche minor has a wide host range among forage legumes in temperate climates. In addition to these, S. gesnerioides and A. vogelii can be of importance in Sub-Saharan

Africa in legume crops, especially cowpea, but soybean, bambara groundnut, common bean, mung bean and many legume fodder crops are also parasitized [1,2].

Management

Rather than being under control, the parasitic weed problem is increasing both in intensity and in acreage. With climate change, these invasive broomrapes are spreading further north in Europe, and further south in Africa, with new areas at risk of invasion if care is not immediately taken to limit the introduction of parasitic weed seeds and to educate farmers and others to be on alert for new infestations. So far, the effectiveness of conventional control methods is limited, due to numerous factors particularly the complex nature of the parasites, which reproduce by tiny and long-living seeds, and are difficult to diagnose until they irreversibly damage the crop. Chemical control with glyphosate, imidazolinones or sulfonylurea herbicides has been developed for a small number of crops, but these can not always be applied due to economic and environmental considerations.

Therefore, the main current means for controlling parasitic weeds should be focusing on (a) reducing the soil seedbank, (b) preventing seed set and (c) inhibiting seed movement from infested to non-infested areas, *i.e.* sanitation. Seedbank demise can be efficiently achieved by fumigation or solarization, however, this is hardly economically feasible in low input legume crops. There is promise in a number of strategies such as rotations with trap or catch crops; intercropping; biological control with insects (*Phytomiza orobanchia* and *Smicronyx* spp.) and fun-



Figure 1. a) Pea crop heavily infected by Orobanche crenata. b) Details of an uprooted pea plant showing O. crenata plants attached to pea roots.

gal species (particularly some *Fusarium* spp.); mychorrization; induction of suicidal germination by the application of synthetic strigolactones to the soil; inhibition by fungal metabolites, natural amino acids, or plant or algae extracts; activation of systemic acquired resistance; and others [2,3]. However, all these suggested methods are currently still under development, and need further verification in the field before registration.

Resistance breeding

Target site herbicide resistance might be a promising solution for controlling broomrape that is being explored in some crops, particularly with non-transgenic imidazolinone target-site resistant cultivars. Still, the most suitable control option is the development of resistance to the parasite, avoiding the use of herbicides.

Breeding for broomrape resistance is a difficult task considering the scarce and complex nature of resistance in most crops. Monogenic resistance has been identified in cowpea against S. gesnerioides but not in any legume crops against any broomrape species, with existing reports suggesting complex inheritance. Still, breeders have succeeded in developing varieties with certain levels of resistance in most legume crops, including pea (Figure 2a, b). Adoption of genomic tools, rapidly developing in both the legumes and in the parasite, will facilitate breeding. For instance, reference genomes of most legume crops are becoming increasingly available as well as those of some Orobanche species, although not yet for O. crenata. Meanwhile, it has been possible to empirically identify genotype resistance in the field. This might be due to a battery of escape and resistance mechanisms, acting at different stages of the infection process, starting from escape due to growth cycle or root morphology, to reduced stimulation of broomrape seed germination, to later barriers preventing or delaying the infection, each of them with putatively different genetic control. The genetic basis of this resistance has not been studied yet, but there are indications that it might be monogenic. Identifying resistance remains a challenge, but the successful combination of multiple resistance mechanisms might be the key to provide durable resistance [4,5].

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Figure 1. a) Example of *Pisum* accessions susceptible (left) vs resistant (right) to Orobanche crenata; b) Resistant pea variety (right) resulting from the IAS-CSIC breeding program, surrounded by susceptible lines.

Plant-parasitic nematodes in low-input legume crops

by María CÓRDOBA-SÁNCHEZ, Diego RUBIALES and Juan Emilio PALOMARES-RIUS

Abstract: Low-input legume crops can be affected by plant-parasitic nematodes, a homogenous group of multicellular eukaryotic organisms that are present in practically all habitats and ecosystems of the biosphere. They cause unspecific symptoms, such as poor growth, vellowing, root damage and early senescence, which can be easily confused with other stresses and hampers proper diagnoses. For this reason, qualitative and quantitative nematological analysis on soil and/or plant roots are needed in order to design efficient field management strategies. In low-input legume crops a few tools are available to reduce nematode populations, including rotation with non-host, modifying sowing dates and plant resistance or tolerance. We will focus on the following genera of nematodes affecting low-input legume crops: rootknot nematodes (Meloidogyne spp.), root-lesion nematodes (Pratylenchus spp.), cyst-forming nematodes (Heterodera spp.), and stem and bulb nematodes (Ditylenchus spp.).

Keywords: *Ditylenchus, Heterodera,* legumes, *Meloidogyne,* nematodes, *Pratylenchus*

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Introduction

Phytoparasitic nematodes are included in the Phylum Nemata, a homogeneous group of multicellular eukaryotic organisms. They are invertebrate animals, vermiform and non-segmented, present in practically all habitats and ecosystems (soil, plant-tissue, animals...) of the biosphere. They are obligate pathogens that can cause plant diseases directly by infecting plants, or indirectly by acting as virus vectors or interacting with other soil pathogens. Their damage is usually associated with their abundance in the soil. However, other factors, such as climatic stress, cultivar susceptibility and size/age of the plant could increase the damage in the field. Parasiticplant nematodes damage usually appears in an aggregate pattern in the field or following cultivation lines (Figure 1). They cause rather unspecific symptoms, such as poor growth, yellowing, root damage and early senescence, which can be easily confused with other stresses and hampers proper diagnoses. For this reason, qualitative and quantitative nematological analysis on soil and/or plant roots are needed to design efficient field management strategies.

Some species of plant-parasitic nematodes are more adapted to subtropical and tropical environments and others to cooler areas. Here we cover nematodes parasitizing temperate season legumes in low-input systems. Low-input systems are characterized by restricted farmers' margins in profitability which reduce many of the tools to control plant-parasitic nematodes (available nematicides, solarization...) and options for crop rotations. Given these limitations, quarantine and preventive measures to limit nematode introduction into new fields are crucial. Once the nematode is introduced and established in the field, eradication is very difficult. In low-input legume crops a few tools are available to reduce nematode populations to under threshold levels. These include: i) Rotations with non-host or poor host crops in order to reduce nematode population levels; accurate species identification is critical in order to choose the proper rotations; ii) Modifying sowing dates might help for some species; and iii) Plant resistance or tolerance. Plant resistance is an essential tool in these low-input systems that should be managed carefully in order not to create nematode resistant (virulent) population selection to a specific resistance gene.

We will focus on the following genera of nematodes affecting low-input legume crops: *Meloidogyne* spp., *Pratylenchus* spp., *Heterodera* spp., and *Ditylenchus* spp.

Root-knot nematodes (Meloidogyne spp.)

The genus *Meloidogyne* is composed by endoparasitic species and is the most damaging group



Figure 1. Patches of reduced growth in chickpea plants infected by *Meloidogyne artiellia*.



Figure 2. A) Chickpea root without inoculation with *Meloidogyne artiellia*; B) Root inoculated with *M. artiellia* showing the giant cells (CG); C) Nodules on roots of chickpea infected with *M. artiellia*, the black line shows the section where the feeding site with the giant cells is located. H = female, M = gelatinous mass with eggs inside.



Figure 3. Differences in *Meloidogyne* nodulation in chickpea plants: A) Control without inoculation; B) Chickpea roots infected with *M. incognita* showing strong nodulations; C) Chickpea roots infected with *M. artiellia* showing tiny nodulations.



Figure 4. A) Healthy roots of pea; B) Heavily infected roots with *Pratylenchus thornei* showing necrotic roots; C) *P. thornei* female; D) Anterior part of *P. thornei*; E) Posterior part of *P. thornei* showing an egg. (Scale bars: C, $E = 50 \mu m$, D =15 μm). V = vulva.

of nematodes in agriculture. Infection is characterized by the formation of nodules (galls) on roots (Figure 2, 3). Nematodes belonging to this genus cause direct damage through parasitism on the plant, forming a feeding site composed of several giant cells (Figure 2), and indirect damage by the distortion on root physiology caused by the nodule and feeding site, thereby interfering with efficient absorption and translocation of water and mineral salts. Juveniles (second stage juveniles) hatch from eggs and actively seek in the soil for viable roots. Upon root penetration, the nematode selects root parenchyma cells, starts to feed and became sedentary, inducing the development of giant cells and the gall in the root. When the nematodes become sedentary, the females deposit eggs in a gelatinous matrix (Figure 2).

Meloidogyne artiellia (cereal and legume rootknot nematode) affects cereals, legumes, and some cruciferous plants during winter. This nematode forms minute nodules on the roots compared to others species of this genus (Figure 3). Other species, such as *M. hapla* or *M. chitwoodi* could affect legumes in temperate climates. The infection of *M. artiellia* can break the resistance to *Fusarium oxysporum* f. sp. *ciceris* in chickpea [1]. Controlling this nematode is challenging due to the current lack of resistant cultivars, the wide host range, and the common practice of wheat-legume rotation, which promotes nematode population growth, due to its ability to reproduce in both crops. For this reason, management involves preventing the introduction of the nematode into the farm, rotations with nonhosts such as sunflower [2], or early sowing. Infections are more severe in spring sowings as the activity for these nematodes increases with temperature and tolerance to damage increase with plant age [3].

Root-lesion nematodes (Pratylenchus spp.)

Nematodes belonging to the Pratylenchus genus are migratory endoparasites. They rank third in global economic damage across crops due to their wide range of host plants. Severely infected root systems are characterized by necrotic lesions in shades of brown or black distributed throughout the root system (Figure 4). Nematodes puncture cells for feeding and intracellular migration, and cells and tissues become necrotic in the root cortex. These necrotic tissues could affect wide root areas providing for opportunistic soil pathogens to invade the root, resulting in even more extensive damage. Pratylenchus spp. complete several life cycles within the crop and all stages are infective, from the second-stage juvenile to the adults. There are several species more associated with legume and cereal crops in low-input systems, such as P. thornei (Figure 4) and P. neglectus in drier conditions. Other species such as P. penetrans are predominant in more humid and temperate conditions. In addition to these, other Pratylenchus species could also infect legumes as they have a wide host range. Nematode control is challenging, because all stages of its life cycle are infective, and crop damage can be further exacerbated by other factors, such as drought or coinfection with other pathogens. Currently, there are no resistant cultivars commercially available.

Cyst-forming nematodes (Heterodera spp.)

These nematodes are sedentary endoparasites characterized by the transformation of the mature female body into a dry cyst containing numerous eggs, serving as a survival and dispersal structure (Figure 5). These nematodes usually parasitize a limited and specific range of botanically related species. Similar to root-knot nematodes, the infective stage is the second-stage juvenile, which infects the roots inducing a syncytium, but these nematodes do not induce a gall. The extent of damage sustained by the crop is closely tied to the synchrony between nematode emergence and planting time. Furthermore, it may also be correlated with soil texture, being more pronounced in light, sandy-textured soils compared to other soil types.

Heterodera spread is facilitated by cyst passive dispersal by machinery, livestock, footwear, rainwater, irrigation, or wind. Among the best control practices are long fallowing because of the long survival of these nematodes in the field, rotation with non-host plant crops (because of limited range of host plants, usually related to plant family or genus), or the use of resistant varieties (soybean). Among the species of *Heterodera* that have the greatest impact on extensive legume crops are *H. goettingiana* (pea cyst nematode) and *H. glycines* (soybean cyst nematode).



Figure 5. Root infection and light microscopy micrographs of *Heterodera* goettingiana parasitizing garden pea and black medick in southern Italy. A, B) Root segments of garden pea and black medick with cysts protruding from the root surface; C, D) Lip region and tail of secondstage juvenile. (Scale bars: A, $B = 500 \mu m_{\odot}$ C, D =20 µm). N = nematode.

Stem and bulb nematodes (Ditylenchus gigas and Ditylenchus dipsaci)

More than 60 species are presently recognized in the genus *Ditylenchus*, but only a few are parasites of higher plants, whilst the majority of species are mycophagous. *Ditylenchus dipsaci* is listed as an A2 quarantine organism in many countries [4]. The stem and bulb nematode is an obligate endoparasite of higher plants that feeds in parenchymatous tissue in the stem and bulbs of about 500 plant species [5]. Ditylenchus dipsaci consists of a number of biological races and populations differing in host preferences and occurring at different stages of speciation and reproductive isolation, which might represent different species status [5]. The formerly "giant race" of D. dipsaci is now regarded as a new species (D. gigas) [6]. Ditylenchus gigas seems to have a limited host range and causes important damage in faba bean (Figure 6). This nematode can be spread through dry seeds and planting material of host plants. All developmental stages outside the egg are capable of infecting plants. In the field, the fourth-stage juvenile can withstand desiccation for many years, and although soil densities seem to decrease rapidly, the nematode can survive for years without a host plant. Cool moist conditions during the winter growing season in the Mediterranean region favour nematode infection and disease development.



Figure 6. Symptoms of damage caused by Ditylenchus gigas in faba beans. A) Stems at the apex and leaves necrotic and deformed in severe attacks; B) Range of necrotic areas in stems; C) Longitudinal sections of healthy (left) and damaged (right) stems showing internal necrosis; D, E) Deformed and smaller pods; F) Deformed (centre and bottom) and healthy seeds (top).

Conclusions

As we have seen in each of the nematode genera, the control of plant-parasitic nematodes is not easy and requires a previous nematological survey to identify and quantify the species present. Integration of different management strategies in a concerted manner is needed. Prevention of introduction is crucial. Once introduced and stablished in a field, the nematode population should be kept under damaging threshold levels. Crop rotations and early sowings can help in some instances, but development of resistant cultivars remains as the most promising measure. Unfortunately, none or few resistant cultivars, or promising sources of resistance are available at present, calling for the urgent need to intensify efforts on resistance screenings and resistance breeding.

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