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Management Strategies and Distribution of Aphanomyces Root Rot of Alfalfa (Medicago sativa), a continuing threat to forage production in the United States

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PLANT PATHOGEN IMPACTS

Management strategies and distribution of Aphanomyces root rot of alfalfa (*Medicago sativa*), a continuing threat to forage production in the United States

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Abstract

Alfalfa (*Medicago sativa*) is one of several legumes that is affected by Aphanomyces root rot (ARR) caused by *Aphanomyces euteiches*. Symptoms of ARR on alfalfa seedlings include a yellow-grey discolouration of roots, rotting and loss of lateral roots, stunted growth, chlorotic foliage and reduction of nitrogen-producing nodules on roots. Infection can also occur on adult plants leading to loss of lateral roots and nodules. At the seedling stage, ARR decreases alfalfa stand establishment, and field longevity is reduced when adult plants are infected. *A. euteiches* is an oomycete pathogen that has motile zoospores and thick-walled oospores that can survive for many years in soil. Two races are currently recognized by pathogenicity on differential alfalfa check cultivars. Most alfalfa cultivars contain race 1 resistance, but there is an increasing development of cultivars with resistance to race 2. Management strategies include planting resistant cultivars, avoiding planting in fields with poor drainage and rotating crops with nonhost plants.

KEYWORDS
alfalfa, *Aphanomyces*, disease management, *Medicago*

1 | INTRODUCTION

Alfalfa (*Medicago sativa*), often called the "Queen of Forages," is grown worldwide as a forage crop for livestock due to its high biomass production and nutritional quality. It is the third most valuable crop in the United States and plays key roles in dairy and beef livestock feeds, protecting water and soil resources, enhancing soil fertility, breaking pest and pathogen cycles and sequestering soil carbon (Fernandez et al., 2019). Alfalfa is used as dry hay, for silage, and in grazing, as well as providing nectar for bees and habitat for wildlife. Early attempts to grow alfalfa in the United States were unsuccessful because of the differing climate from alfalfa's centre of origin in the Fertile Crescent, acidic soil and interactions with plant pathogens. The first successful alfalfa crops in the United States were produced in the warm, western part of the country. Alfalfa was not widely cultivated in the Midwestern United States until the late 1800s. The crop became successful in the Midwest by the development of varieties that displayed better survival in the region's harsh winters. Today, autumn dormancy and freezing tolerance are critical characteristics that effect the productivity and persistence of alfalfa in cold climates (Liu et al., 2019).

Early alfalfa breeding efforts focused on improving resistance to root rot and bacterial wilt diseases caused by *Phytophthora*

medicaginis and *Clavibacter insidiosus*, respectively. The development of cultivar Agate and additional *P. medicaginis*-resistant cultivars uncovered other root rot problems. Recurring issues with establishment of resistant cultivars were found to be caused by diseases incited by *Aphanomyces euteiches* and *Pythium* species (Munkvold & Carlton, 1995; Nygaard & Grau, 1989). Aphanomyces root rot (ARR) may be the most economically important alfalfa disease because it is widely distributed and affects alfalfa both at the seedling stage, causing poor stand establishment, and at the mature plant stage, causing yield losses and premature stand thinning (Figure 1). Primary symptoms on infected roots of seedlings are water-soaked grey lesions that become soft and honey-brown in appearance (Figure 2a). Seedlings may also be stunted with chlorotic, purple-tinted cotyledons and have reduced root volume and function. Severe infections lead to postemergence seedling death (Malvick & Grau, 2015). Seedlings with ARR often remain upright even after death and are referred to as standing corpses (Figure 1a), a symptom that distinguishes ARR from Phytophthora root rot (Holub & Grau, 1990). Adult plants with symptoms are stunted with yellow foliage, lack fibrous and lateral roots and have loss of root nodules (Figure 2b).

2 | LIFE CYCLE OF *A. EUTEICHES*

A. euteiches is a diploid, homothallic oomycete, which can produce both oospores via sexual reproduction and zoospores via asexual reproduction (Figure 3). Thick-walled oospores serve as the primary inoculum source (Grünwald & Hoheisel, 2006). Oospore germination is triggered by chemical signals released from the host. Germination occurs through the formation of a germ tube that branches to form hyphae that can infect plant roots or that form sporangia (Figure 3) (Hughes & Grau, 2013). Primary spores aggregate at the tip of sporangia, which produce biflagellate zoospores that emerge through a pore in the primary spore. The zoospores are motile and require water to move to host roots. Isoflavone legume root exudates, especially prunetin, chemically attract *A. euteiches* zoospores to pea (*Pisum sativum*) roots (Sekizaki & Yokosawa, 1988); however, the attractant from alfalfa roots has not been identified. Research conducted on pea demonstrated that zoospores are drawn to a region directly behind the root cap (Cannesan et al., 2011). Once in contact with roots, the zoospores encyst at the root surface (Gaulin et al., 2007). In alfalfa, zoospores encyst along the entire root and cyst germination is observed on root hairs and the root epidermis (Figure 4). Development of mycelium occurs throughout the root cortex, and the final stages of infection produce oogonia that are fertilized by antheridia to generate oospores (Cannesan et al., 2011), which range from 18 to 25 μm in diameter. Oospores can be visualized in susceptible root tissue under a light microscope (Figure 5). The thick-walled oospores provide a suitable resting state to survive harsh winter conditions (Billard et al., 2019). Oospores survive for years in tissues of infected plants or in soil. Water-saturated soils and temperatures of 24 to 28°C are optimal for infection and disease development, although symptoms are greatest if warm and dry soil conditions occur after infection. Under these conditions, symptoms of disease and production of oospores are usually observed 10 days after infection.

3 | RACE STRUCTURE

A. euteiches is categorized by races that are defined with respect to pathogenicity of *A. euteiches* isolates against a differential set of alfalfa cultivars (Hudelson & Grau, 1998) (Figure 6). Originally, there was thought to be only one race of the pathogen because alfalfa cultivars did not differ in their reactions to individual pathogen isolates (Delwiche et al., 1987). Resistance to *A. euteiches* race 1 was established in alfalfa

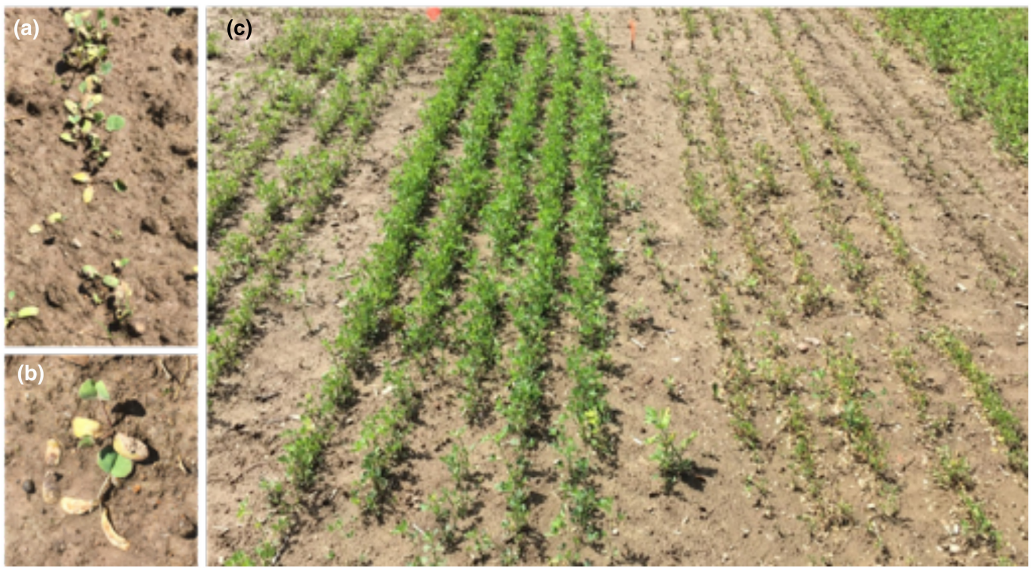

FIGURE 1 Field symptom of Aphanomyces root rot of alfalfa. (a) Infected seedlings show poor emergence and stunting. (b) Infected seedling with yellowed and reddened cotyledons. (c) Cultivar in centre has high levels of resistance compared to cultivars on the left and right, increasing stand density and plant growth

FIGURE 2 Root and shoot symptoms of Aphanomyces root rot of alfalfa. Alfalfa seeds were sown at the same time. (a) Infected seedlings (right) have stunted shoots with yellowed or reddened leaves and stunted yellowed roots compared to healthy seedlings (left). (b) Infected adult plants (right) have few lateral and fibrous roots and stunted herbage compared to healthy plants (left)

cultivars following recurrent selection (Holub & Grau, 1990). However, race 1-resistant alfalfa cultivars performed poorly in some fields infested with *A. euteiches*, which led to the discovery of the race 2 virulence phenotype (Grau et al., 1991). WAPH-1, an alfalfa cultivar with race 1 resistance, was developed as a differential to identify race 1 isolates (Grau, 1992), and WAPH-5 with resistance to both race 1 and race 2 isolates is used as the standard check cultivar to identify *A. euteiches* race 2 isolates (Hudelson & Grau, 1998; Figure 6). Both race 1 and race 2 isolates appear to occur throughout the United States (Malvick & Grau, 2001; Samac et al., 2017). Race 2 did not emerge in response to selective pressure from deploying race 1-resistant cultivars because race 2 isolates have been found in fields with no history of alfalfa cultivation (Malvick et al., 2009). Interestingly, races have not been identified in Europe, although ARR on pea is common in Europe and the *A. euteiches* isolates from pea can infect alfalfa (Gaulin et al., 2007). Genetic analyses to distinguish races have remained inconclusive. According to RAPD gel electrophoresis banding patterns, genetic variation was found to be as similar within race 1 and 2 isolates as it was between the two races (Malvick & Grau, 2001). Races were found not to cluster together in an AFLP analysis nor were there unique bands correlated with races (Malvick et al., 2009).

Currently, the only method of distinguishing races is by performing standardized bioassays on a differential set of alfalfa cultivars. Recently, alfalfa genetic suppliers have been marketing new alfalfa varieties with "race 3" resistance, although at this time there is no differential cultivar approved by the Association of Official Seed Certifying Agencies to identify race 3-resistant cultivars. Performing these bioassays on collected soils sometimes leads to misleading results due poor germination of all check cultivars, which can be partially explained by highly pathogenic *Pythium* species and *Fusarium* species causing seed rot and damping-off (Berg et al., 2017). Strains of *A. euteiches* were isolated from soil samples that inhibited WAPH-5 germination, and all strains were identified as either race 1 or race 2 (Samac et al., 2017). There is currently no way to determine if there are more than two races of *A. euteiches* that infect alfalfa because identifying a race 3 isolate using the standardized bioassay requires a differential for race 3, and there are currently only two differential cultivars of alfalfa, which identify race 1 and race 2 strains.

4 | DISTRIBUTION OF *A. EUTEICHES*

A. euteiches isolates that infect alfalfa have been reported from the United States, Canada, France, Australia and Sweden (Abbo & Irwin, 1990; Beghdadi et al., 1992; Levenfors et al., 2003; Malvick & Grau, 2001; McKeen & Traquair, 1980; Moussart et al., 2008; Tordsen et al., 2022). A recent and extensive study mapped *A. euteiches* infecting pea and lentil across all agricultural regions of Saskatchewan, Canada (Karppinen et al., 2020). Several field studies have mapped *A. euteiches* in alfalfa stands in the United States (Gibbs, 2009; Malvick et al., 2009; Munkvold et al., 2001). There seems to be no geographical boundaries for the pathogen. The alfalfa-infecting strains of *A. euteiches* may be endemic to North America because they have been detected in native rangeland and infect a wide host range of native prairie legumes (Karppinen et al., 2020; Malvick et al., 2009).

In these *A. euteiches* surveys, a susceptible alfalfa cultivar is used in bioassays to bait *A. euteiches* from field soil for isolation (Grau et al., 1991; Malvick & Grau, 2001). Selective media have been developed to inhibit the growth of other root rotting pathogens, such as *Pythium*

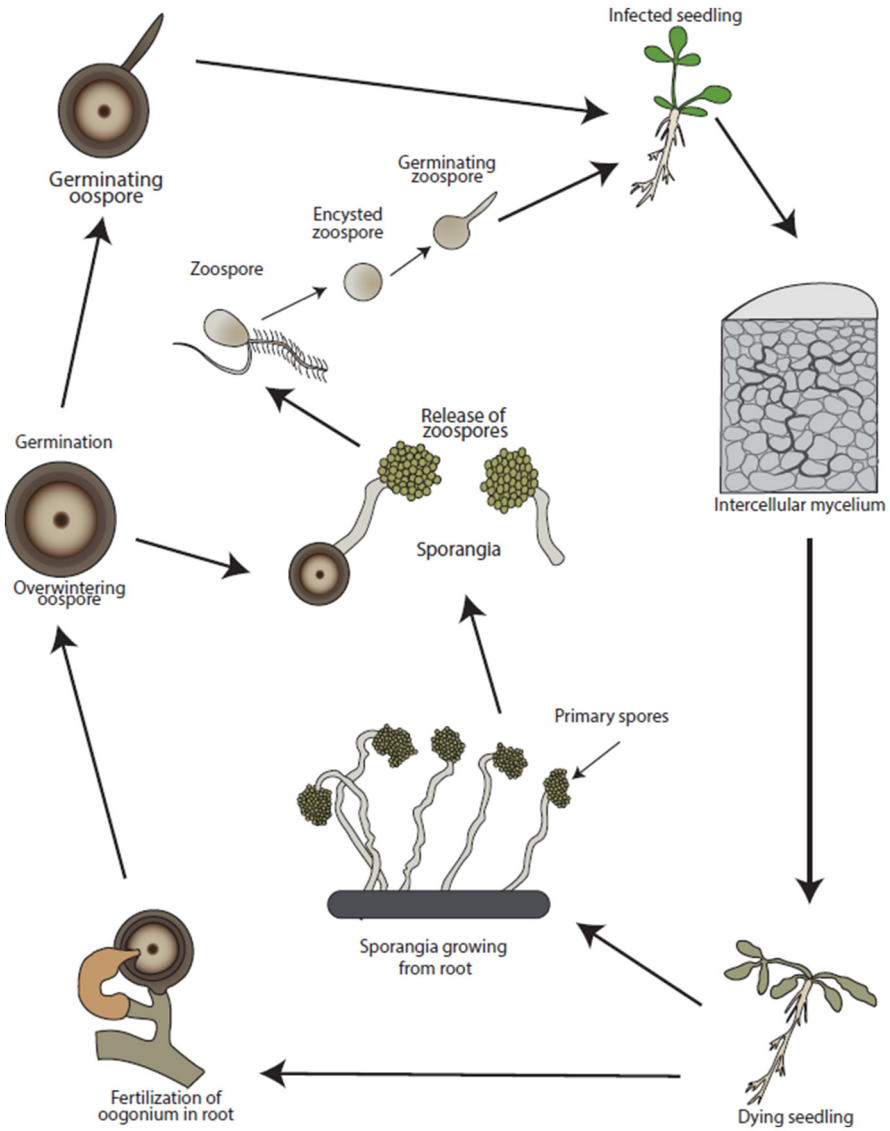

FIGURE 3 Life cycle of *Aphanomyces euteiches* on alfalfa

spp., which commonly outcompete *A. euteiches* in culture (Pfender et al., 1984). *A. euteiches* isolates from alfalfa have large diameter granular hyphae, short side branching hyphae, and main hyphae branch in a Y-shaped junction. Maximal growth occurs at 28°C (Delwiche et al., 1987). Once isolated in pure culture, DNA can be extracted, and specific PCR primers are used to validate *A. euteiches* to the species level but cannot distinguish between races of the pathogen (Vandemark et al., 2002). Also, a polyclonal antiserum highly specific for *A. euteiches* has been developed for a root diagnostic ELISA (Kraft & Boge, 1994).

5 | MANAGEMENT

5.1 | Genetic resistance

Planting disease-resistant cultivars of alfalfa is currently the best approach to reduce damage from ARR. Through recurrent phenotypic selection, alfalfa cultivars with high levels of resistance, greater than 50% of plants in a population being resistant, have been developed for race 1 and race 2 of *A. euteiches*. Heritability of resistance to ARR is high, which suggests that it is generated by a small number of genes (Samac et al., 2021). ARR-resistant cultivars have demonstrated improved seedling health, increased forage yields, and persistence under field conditions. Stands planted with resistant alfalfa varieties have increased dry matter yields up to 0.87 Mg/ha and increased ground cover up to 32% (Vincelli et al., 2000). Most commercial cultivars have resistance to race 1, which was previously thought to be the prevalent race of *A. euteiches* (Malvick & Grau, 2001). Race 2 of *A. euteiches* in Iowa and Wisconsin soils limited the yield benefits of race 1-resistant alfalfa cultivars (Munkvold et al., 2001). Resistance of alfalfa to the two races of *A. euteiches* appears to be controlled by different genes because race 1-resistant cultivars are susceptible to race 2 (Vandemark et al., 2004). In response to *A. euteiches* inoculation, a rapid hypersensitive response (HR) occurs in both race 1- and race 2-resistant plants in which the epidermal cell dies soon after penetration (Figure 7). This resistance response indicates resistance is mediated by a resistance (*R*) gene, most probably separate

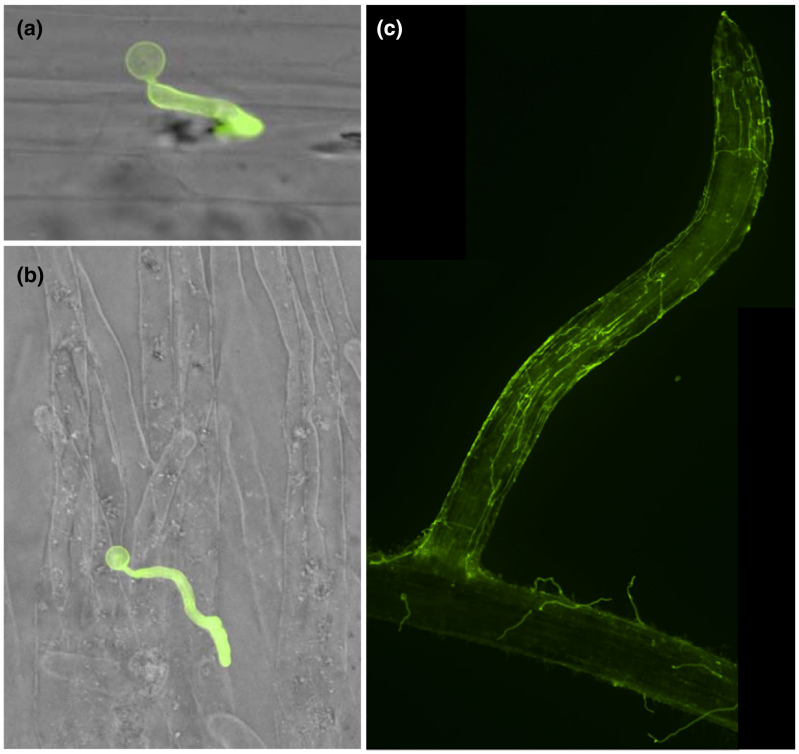

FIGURE 4 Germination and growth of encysted zoospores of *Aphanomyces euteiches* on alfalfa roots. Roots were stained with wheat germ agglutinin-fluorescein isothiocyanate. (a) Germination on the root surface. (b) Germination on root hairs. (c) Germ tube growth on the root surface and internal mycelial growth

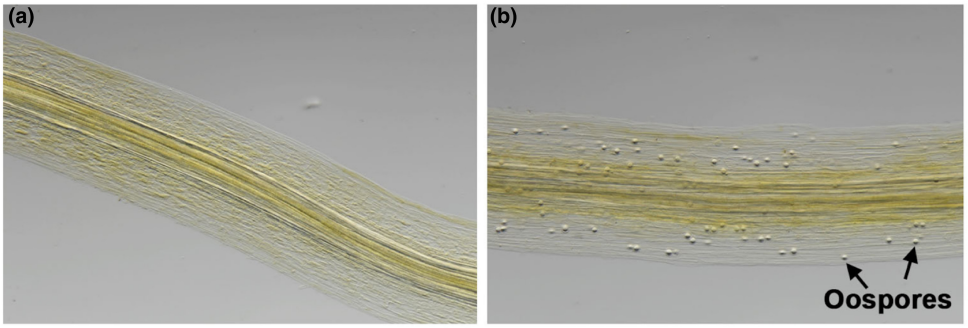

FIGURE 5 Oospores of *Aphanomyces euteiches* within alfalfa roots at 14 days after inoculation. (a) Root of a resistant plant lacks oospores. (b) Root of a susceptible plant with numerous oospores

R genes for each race (Samac et al., 2021), although no *R* gene from alfalfa has been cloned to confirm activity. In contrast, in susceptible plants, the pathogen rapidly colonizes the cortical cells, grows in the intercellular spaces and causes massive cellular degradation. Resistance is also associated with browning of the infected cell and a few neighbouring cells, which fluoresce under UV light, indicating the presence of phenolic compounds. Transcript profiling found strong up-regulation of genes in the phenylpropanoid pathway, jasmonic acid synthesis, receptor kinase, transcription factor and defence response genes such as those encoding chitinase, glucanase and peroxidases (Samac et al., 2021). Improved understanding of the *R* gene–race interaction is needed so alfalfa breeders can rationally design crosses to maximize resistance loci and increase the frequency of resistant plants. To protect and enhance the *A. euteiches* resistance found in alfalfa, it is important to characterize the strains of *A. euteiches* that can overcome this resistance through frequent disease surveys and selecting alfalfa plants with resistance to highly aggressive strains.

A few DNA markers associated with ARR resistance have been identified in alfalfa. Marker-assisted recurrent selection has made little improvement at increasing alfalfa broad-spectrum resistance to *A. euteiches* (Audy et al., 2017). A genotyping-by-sequencing analysis was performed on 373 alfalfa plants, and highly significant single-nucleotide polymorphism (SNP) markers, possibly for race 2 resistance, were identified at the top of chromosome 2 (Samac et al., 2017). For race 1 resistance, SNP markers were identified at the top of chromosome 1, around 38 kb away from a cluster of *R* genes in the Cultivated Alfalfa at the Diploid Level (CADL) genome sequence (Samac et al., 2017). To our knowledge, marker-assisted selection for

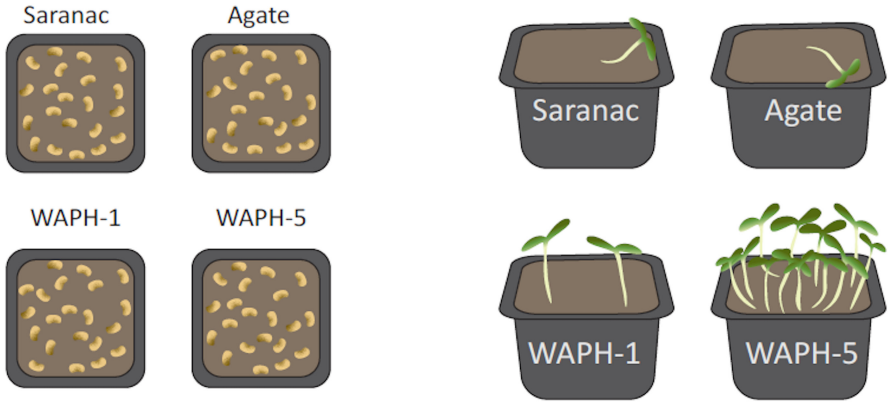

FIGURE 6 Aphanomyces root rot bioassay in alfalfa. A differential set of standardized alfalfa check cultivars are planted in field soil. After seeds germinate, the soil is flooded for 5 days. The water is released, and symptoms are scored at 21 days after planting to identify the races of *Aphanomyces euteiches* present in the soil sample

Cultivar	Phytophthora Root Rot	Aphanomyces Race 1	Aphanomyces Race 2
Saranac	Susceptible	Susceptible	Susceptible
Agate	Resistant	Susceptible	Susceptible
WAPH-1	Resistant	Resistant	Susceptible
WAPH-5	Resistant	Resistant	Resistant

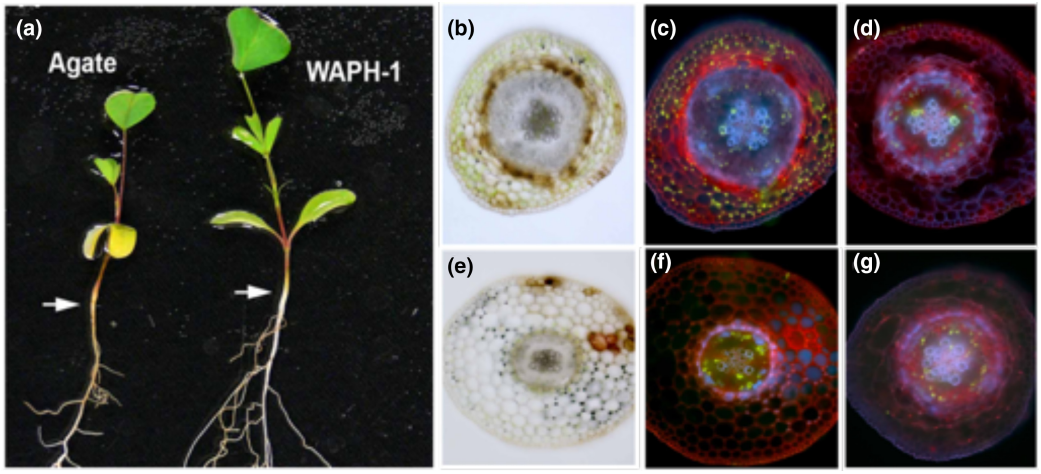

FIGURE 7 Resistance to Aphanomyces root rot of alfalfa is mediated by a hypersensitive reaction. (a) Plant symptoms at 7 days after inoculation with a race 1 strain. Cross sections were made at the top of the roots indicated by the arrows. Plants from cultivar Agate are susceptible, and WAPH-1 is resistant to race 1 strains. (b) Cross section of Agate root with necrotic brown cortical cells by light microscopy. (c) Same cross section as in (b) stained with wheat germ agglutinin-fluorescein isothiocyanate under UV illumination showing growth of *A. euteiches* (green fluorescence) between cortical cells and invasion into the stele. Red fluorescence indicates phenolic compounds. (d) Control noninoculated plant showing fluorescence in some vascular cells. (e) Cross section of WAHP-1 root by light microscopy. (f) Same cross section as in (e) stained with wheat germ agglutinin-fluorescein isothiocyanate showing penetration of a single epidermal cell by *A. euteiches* (green fluorescence). Red fluorescence indicates phenolic compounds. (g) Control noninoculated plant showing fluorescence in some vascular cells

increased resistance to any alfalfa disease has not been used in any commercial alfalfa breeding programmes.

The model legume *Medicago truncatula* has emerged as the genetic system to study *A. euteiches* resistance in legumes. The broad host range of *A. euteiches* and high levels of synteny between *M. truncatula* and alfalfa suggest that resistance may be conserved between these two species (Choi et al., 2004). However, ARR resistance is a complex trait. *A. euteiches* race-specific resistance that has been identified in alfalfa has not been detected in *M. truncatula* (Djebali et al., 2009; Pilet-Mayel et al., 2009). *M. truncatula* usually displays quantitative disease resistance (QDR) where a few quantitative trait loci (QTLs) with large effects and numerous QTLs with small-to-intermediate effects interact to form the resistance phenotype (Bonhomme et al., 2019). Bulk segregant analysis (BSA) on *M. truncatula* inoculated with a pea isolate of *A. euteiches* was used to identify and map *AER1*, a major dominant

ARR resistance gene, to a nucleotide-binding site leucine-rich repeat (NBS-LRR)-rich region of chromosome 3, and most classical *R* genes encode proteins that contain a central NBS domain and a carboxy-terminal LRR domain (Pilet-Nayel et al., 2009). The same locus was identified when *M. truncatula* was inoculated with both race 1 and race 2 alfalfa isolates (Hamon et al., 2010). Identified *M. truncatula* resistance mechanisms include increased lignin deposition, frequent pericycle cell divisions to protect the central root cylinder and accumulation of soluble phenolic compounds (Djebali et al., 2009, 2011).

5.2 | Fungicides

Alfalfa seeds are frequently treated with mefenoxam (Apron XL; Syngenta), which inhibits the growth of some alfalfa root rotting pathogens (*Pythium* spp. and *P. medicaginis*) but fails to control *A. euteiches*. Pyraclostrobin (Stamina; BASF) is labelled for use as a seed treatment against *A. euteiches* and also inhibits the growth of *Rhizoctonia solani*, *P. medicaginis* and *Fusarium* species. Pyraclostrobin prevents fungal respiration, depriving the pathogen of energy for growth and development (Venancio et al., 2003). It was suggested that race 2 isolates of *A. euteiches* were more sensitive to Stamina treatments than the race 1 isolates but increasing concentrations of Stamina inhibited growth of *A. euteiches* in both race 1 and 2 strains (Smith & Watson, 2014). This implies that certain *A. euteiches* strains may be more resistant to some fungicides than others.

Ethaboxam (INTEGO Solo) is used as a legume seed coating to suppress early season root and seed rots. Ethaboxam treatments have activity against *Phytophthora* spp., *Pythium* spp. and *A. euteiches* (Wu et al., 2019). Three fungicide treatments, INTEGO Solo, BAS 516F (pyraclostrobin, boscalid) and BAS 720F (metalaxyl, pyraclostrobin, fluxapyroxad), increased pea plant health and reduced disease severity significantly, compared with the inoculated control under greenhouse conditions (Wu et al., 2019). However, no fungicide was found to limit ARR severity in pea field tests. Evaluating these fungicides as alfalfa seed treatments may lead to improved ARR management.

5.3 | Biological control

With no current fungicides providing successful ARR management in field trials, using biological control agents as seed treatments may be useful in reducing ARR severity. Biological control agents are often discovered in naturally occurring disease-suppressive soils. From suppressive soils for ARR of pea in New Zealand, four bacterial isolates were found to inhibit *A. euteiches* in both mycelial growth and zoospore germination (Wakelin et al., 1998). In western Canada, bacteria from 18 different genera isolated in soil samples from pea fields also demonstrated inhibition of *A. euteiches* mycelial growth and zoospore germination (Gobedo et al., 2020).

Antagonistic effects against ARR in pea plants have been demonstrated by multiple bacterial species including *Streptomyces* spp. (Brahim et al., 2018), *Lysobacter capsici* K-Hf-H2 (Gobedo et al., 2020), *Bacillus mycoides* MW27 (Wakelin et al., 2002) and *Bacillus velezensis* UCMB5113 (Lagerlöf et al., 2020). Isolates OB21 and BA15 of *Streptomyces* spp. applied as a seed treatment reduced disease by 33% and 47%, respectively, compared to untreated control pea seeds (Brahim et al., 2018). In a field study, seed treatments containing *Bacillus mycoides* MW27 increased pea plot stands by approximately 9% in an *A. euteiches*-infested field (Wakelin et al., 2002). These promising biocontrol agents have yet to be tested against alfalfa-infecting isolates of *A. euteiches*.

Combining biocontrol treatments with other management strategies may provide effective disease control. In greenhouse experiments, the association of *B. velezensis* UCMB5513 seed treatments with earthworms (*Lumbricus terrestris*) resulted in taller, healthier pea plants in *A. euteiches*-inoculated soils (Lagerlöf et al., 2020). A limited set of plant defensins, plant antimicrobial peptides, failed to inhibit *A. euteiches* growth even though they displayed activity against other alfalfa pathogens (Sathoff et al., 2019). Further surveys of plant defensins for activity against *A. euteiches* are warranted because these peptides have widespread antimicrobial activity and could potentially be developed into a novel seed treatment.

5.4 | Cultural control

A. euteiches is a persistent pathogen due to its thick-walled oospores and longevity; at high inoculum levels it can remain a threat to susceptible species for up to 10 years (Gaulin et al., 2007). A common strategy used for disease management is cropping system diversification. Soil- or residue-borne pathogens can be avoided by selecting and rotating crops with nonhost plants. The cultivated legume hosts of *A. euteiches* include pea, alfalfa, snap bean (*Phaseolus vulgaris*), red kidney bean (*P. vulgaris*), fava bean (*Vicia faba*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) and lentil (*Lens culinaris*) (Wu et al., 2019). By planting legume species with high resistance to *A. euteiches*, the inoculum potential of the soil was reduced in greenhouse conditions (Moussart et al., 2013). But, in order to eliminate inoculum potential in pea fields, the recommended length of rotation between host and nonhost crops is more than 6 years (Hossain et al., 2012). Growers may find this amount of time unsustainable for their practices.

Although *A. euteiches* infects a wide range of plants, a relationship has been found between the host from which a strain was isolated and virulence. Approximately 20% of *A. euteiches* isolates obtained from alfalfa soil showed high virulence on both pea and alfalfa, whereas 80%–100% of isolates obtained from pea/alfalfa soil were found to be highly virulent to pea and alfalfa (Holub et al., 1991). An increase in inoculum concentration is also suggested to occur due to the ability of *A. euteiches* to infect other plants, releasing a new batch of viable oospores into the soil (Papavizas & Ayers, 1974). Alfalfa cultivars are synthetic mixtures

of heterozygous plants. Even when using an ARR-resistant cultivar of alfalfa, there is still a proportion of susceptible plants, so there is always the potential for disease and an increase in pathogen inoculum when growing a resistant cultivar.

In addition to crop rotations and diversification strategies, other possible methods to reduce *A. euteiches* soil population densities include ensuring proper drainage using subsurface drainage tiles, avoiding clay-heavy soils and using conservation tillage methods to reduce waterborne inoculum spread (Sturz et al., 1997). Alfalfa growth is restricted in poorly drained, wet soils by pathogens that cause root disease. These poorly drained soils are favourable for pathogen growth and frequently have problems with alfalfa stand establishment. Soil moisture, total and organic carbon and total nitrogen contents were found to be positively correlated with *A. euteiches* abundance in lentil and pea fields (Karppinen et al., 2020). *A. euteiches* was found not to be limited to any specific soil or land use type (annual cropland, roadside ditches or rangeland; Karppinen et al., 2020). Overall, it is unlikely that there is a single *A. euteiches* management strategy to entirely control ARR. A collective approach integrating genetic host resistance with seed treatments and crop diversification will probably be necessary to control ARR in alfalfa.

6 | FUTURE PERSPECTIVES

With the goal of improving genetic resistance, several genome-wide association studies (GWAS) have been performed in *M. truncatula*, which identified causative SNPs for loci that impart quantitative resistance to ARR (Bonhomme et al., 2014). These markers may be used to identify race-nonspecific resistance in alfalfa. Quantitative resistance is controlled by multiple genes that impart partial resistance and is predicted to be more durable than *R* gene-mediated resistance (Palloix et al., 2009). SNPs highly associated with variation in resistance to *A. euteiches* in *M. truncatula* were identified in the promoter and coding region of an F-box protein encoding gene located inside the 440 kb *AER1* genomic region (Bonhomme et al., 2014). Alleles corresponding to a nonfunctional F-box were associated with resistance, indicating that the protein acts as a negative regulator of disease resistance. A local score approach, which takes advantage of cumulative association signals of small effect, was applied to a GWAS study and used to uncover many minor QTLs for *A. euteiches* resistance in *M. truncatula* (Bonhomme et al., 2019). Potentially, these genes from *M. truncatula* could be transferred to alfalfa, which may confer race-nonspecific resistance.

With improved pathogen detection methods, resistant alfalfa cultivars can be selectively deployed in areas with *A. euteiches*-infested soil, which should reduce the selective pressure on the pathogen. Previous surveys have relied on the inefficient process of baiting pathogens from collected soil samples using susceptible seedlings to determine the inoculum potential. Also, soil sampling strategies may lead to false negatives because *A. euteiches* of pea appears in clusters of disease foci (Moussart et al., 2009). A quantitative PCR assay can detect *A. euteiches* at concentrations as low as 10 oospores per gram of soil (Gangneux et al., 2014). Frequent *A. euteiches* soil surveys will provide growers with the essential pathogen distribution data so current integrative management strategies to combat ARR can be used only when necessary.

The genome of a pea-infecting isolate of *A. euteiches* was recently sequenced, annotated and deposited in AphanoDB (Gaulin et al., 2018). AphanoDB is a genomic database used for the study of *A. euteiches* containing a collection of gene sequences and annotations from *Aphanomyces* species along with tools for comparative genomic approaches (Madoui et al., 2007). The sequenced pea isolate of *A. euteiches* possesses a large and diverse suite of small secreted protein (SSP)-encoding effector genes, including cell wall-degrading enzymes, that are strongly expressed upon *M. truncatula* inoculation (Gaulin et al., 2018). Draft genome sequences of a race 1 and race 2 strain of alfalfa isolates of *A. euteiches* have been assembled and are available in AphanoDB. New long read DNA sequencing methods and bioinformatics tools should facilitate the development of more complete genome sequences for *A. euteiches*. The addition of new sequences will facilitate gene prediction, which can be used as a tool in understanding genes involved in pathogenicity and race specificity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no data sets were generated or analysed in this study.

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REFERENCES

Abbo, E.O. & Irwin, J.A.G. (1990) *Aphanomyces euteiches*, a possible cause of poor lucerne establishment in the Lockyer Valley, Queensland. *Australian Journal of Experimental Agriculture*, *30*, 361–364.

Audy, P., Rocher, S. & Claessens, A. (2017) Aphanomyces and Phytophthora root rot tolerance in alfalfa: recurrent selection and marker development under stringent disease conditions. *Canadian Journal of Plant Pathology*, *39*, 544.

Beghdadi, A., Richard, C. & Dostaler, D. (1992) *Aphanomyces euteiches* of lucerne in Quebec: isolation, morphology, variability of growth, and pathogenicity. *Canadian Journal of Botany*, *70*, 1903–1911.

Berg, L.E., Miller, S.S., Dornbusch, M.R. & Samac, D.A. (2017) Seed rot and damping-off of alfalfa in Minnesota caused by *Pythium* and *Fusarium* species. *Plant Disease*, *101*, 1860–1867.

Billard, E., Quillévéré-Hammard, A., Lavaud, C., Pilet-Nayel, M.L. & Le May, C. (2019) Testing of life history traits of a soilborne pathogen in vitro: do characteristics of oospores change according to the strains of *Aphanomyces euteiches* and the host plant infected by the pathogen? *Journal of Phytopathology*, *167*, 313–320.

Bonhomme, M., André, O., Badis, Y., Ronfort, J., Burgarella, C., Chantret, N. et al. (2014) High-density genome-wide association mapping implicates an F-box encoding gene in *Medicago truncatula* resistance to *Aphanomyces euteiches*. *New Phytologist*, *201*, 1328–1342.

Bonhomme, M., Fariello, M.I., Navier, H., Hajri, A., Badis, Y., Miteul, H. et al. (2019) A local score approach improves GWAS resolution and detects minor QTL: application to *Medicago truncatula* quantitative disease resistance to multiple *Aphanomyces euteiches* isolates. *Heredity*, *123*, 517–531.

Brahim, O., Nafis, A., Baz, M. & Mauch, F. (2018) The potential of antagonistic Moroccan *Streptomyces* isolates for the biological control of damping-off disease of pea (*Pisum sativum* L.) caused by *Aphanomyces euteiches*. *Journal of Phytopathology*, *167*, 82–90.

Cannesan, M.A., Gangneux, C., Lanoue, A., Giron, D., Laval, K., Hawes, M. et al. (2011) Association between border cell responses and localized root infection by pathogenic *Aphanomyces euteiches*. *Annals of Botany*, *108*, 459–469.

Choi, H.-K., Mun, J.-H., Kim, D.-J., Zhu, H., Baek, J.-M., Mudge, J. et al. (2004) Estimating genome conservation between crop and model legume species. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 15289–15294.

Delwiche, P.A., Grau, C.R., Holub, E.B. & Perry, J.B. (1987) Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. *Plant Disease*, *71*, 155–161.

Djebali, N., Jauneau, A., Ameline-Torregrosa, C., Chardon, F., Jaulneau, V., Mathe, C. et al. (2009) Partial resistance of *Medicago truncatula* to *Aphanomyces euteiches* is associated with protection of the root stele and is controlled by a major QTL rich in proteasome-related genes. *Molecular Plant-Microbe Interactions*, *22*, 1043–1055.

Djébali, N., Mhadhbi, H., Lafitte, C., Dumas, B., Esquerré-Tugayé, M.-T., Aouani, M.E. et al. (2011) Hydrogen peroxide scavenging mechanisms are components of *Medicago truncatula* partial resistance to *Aphanomyces euteiches*. *European Journal of Plant Pathology*, *131*, 559–571.

Fernandez, A.L., Sheaffer, C.C., Tautges, N.E., Putnam, D.H. & Hunter, M.C. (2019) *Alfalfa, wildlife, and the environment* (2nd edition). 2, St Paul, MN: National Alfalfa and Forage Alliance.

Gangneux, C., Cannesan, M., Bressan, M., Castel, L., Moussart, A., Vicre-Gibouin, M. et al. (2014) A sensitive assay for rapid detection and quantification of *Aphanomyces euteiches* in soil. *Phytopathology*, *104*, 1138–1147.

Gaulin, E., Jacquet, C., Bottin, A. & Dumas, B. (2007) Root rot disease of legumes caused by *Aphanomyces euteiches*. *Molecular Plant Pathology*, *8*, 539–548.

Gaulin, E., Pel, M.J.C., Camborde, L., San-Clemente, H., Courbier, S., Dupouy, M.-A. et al. (2018) Genomic analysis of *Aphanomyces* spp. identifies a new class of oomycete effectors associated with host adaptation. *BMC Biology*, *16*, 43.

Gibbs, A.J. (2009) *The prevalence and geospatial distribution of Aphanomyces euteiches (race 1 and race 2) and Phytophthora medicaginis in Wisconsin and southeast Minnesota alfalfa fields*. MS thesis, University of Wisconsin-Madison.

Godebo, A.T., Germida, J.J. & Walley, F.L. (2020) Isolation, identification, and assessment of soil bacteria as biocontrol agents of pea root rot caused by *Aphanomyces euteiches*. *Soil Science*, *100*, 206–216.

Grau, C.R. (1992) Registration of WAPH-1 alfalfa germplasm with resistance to Aphanomyces root rot. *Crop Science*, *32*, 287–288.

Grau, C.R., Muehlchen, A.M., Tofte, J.E. & Smith, R.R. (1991) Variability in virulence of *Aphanomyces euteiches*. *Plant Disease*, *75*, 1153–1156.

Grünwald, N.J. & Hoheisel, G.A. (2006) Hierarchical analysis of diversity, selfing, and genetic differentiation in populations of the oomycete *Aphanomyces euteiches*. *Phytopathology*, *96*, 1134–1141.

Hamon, C., Baranger, A., Miteul, H., Lecointe, R., Le Goff, I., Deniot, G. et al. (2010) A complex genetic network involving a broad-spectrum locus and strain-specific loci controls resistance to different pathotypes of *Aphanomyces euteiches* in *Medicago truncatula*. *Theoretical and Applied Genetics*, *120*, 955–970.

Holub, E.B. & Grau, C.R. (1990) Ability of *Aphanomyces euteiches* to cause disease of seedling alfalfa compared with *Phytophthora megasperma* f. sp. *medicaginis*. *Phytopathology*, *80*, 331–335.

Holub, E.B., Grau, C.R. & Parke, J.L. (1991) Evaluation of the forma specialis concept in *Aphanomyces euteiches*. *Mycological Research*, *95*, 147–157.

Hossain, S., Bergkvist, G., Berglund, K., Mårtensson, A. & Persson, P. (2012) Aphanomyces pea root rot disease and control with special reference to impact of Brassicaceae cover crops. *Acta Agriculturae Scandinavica, Section B - Soil & Plant Science*, *62*, 477–487.

Hudelson, B.D. & Grau, C.R. (1998) Development of a standardized test for evaluating alfalfa for resistance to *Aphanomyces euteiches* race 2. In: *Proceedings from the 36th North American Alfalfa Improvement Conference*. St Paul, MN: NAAIC, p. 33

Hughes, T.J. & Grau, C.R. (2013) Aphanomyces root rot (common root rot) of legumes. *The Plant Instructor*. https://doi.org/10.1094/PHI-I-2007-0418-01

Karppinen, E.M., Payment, J., Chatterton, S., Bainard, J.D., Hubbard, M., Gan, Y. et al. (2020) Distribution and abundance of *Aphanomyces euteiches* in agricultural soils: effect of land use type, soil properties, and crop management practices. *Applied Soil Ecology*, *150*, 103470.

Kraft, J.M. & Boge, W.L. (1994) Development of an antiserum to quantify *Aphanomyces euteiches* in resistant pea lines. *Plant Disease*, *78*, 179–183.

Lagerlöf, J., Ayuke, F., Heyman, F. & Meijer, J. (2020) Effects of biocontrol bacteria and earthworms on *Aphanomyces euteiches* root-rot and growth of peas (*Pisum sativum*) studied in a pot experiment. *Soil & Plant Science*, *5*, 427–436.

Levenfors, J.P., Wikstrom, M., Persson, L. & Gerhardson, B. (2003) Pathogenicity of *Aphanomyces* spp. from different leguminous crops in Sweden. *European Journal of Plant Pathology*, *109*, 535–543.

Liu, Z.Y., Baoyin, T., Li, X.L. & Wang, L.Z. (2019) How fall dormancy benefits alfalfa winter-survival? Physiological and transcriptomic analyses of the dormancy process. *BMC Plant Biology*, *19*, 205.

Madoui, M.A., Gaulin, E., Mathe, C., Clemente, H.S., Couloux, A., Wincker, P. et al. (2007) AphanoDB: A genomic resource of *Aphanomyces* pathogens. *BMC Genomics*, *8*, 471.

Malvick, D.K. & Grau, C.R. (2001) Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the midwestern United States. *Plant Disease*, *85*, 740–744.

Malvick, D.K. & Grau, C.R. (2015) Aphanomyces root rot. In: Samac, D.A., Rhodes, L.H. & Lamp, W.O. (Eds.) *Compendium of alfalfa diseases and pests* (3rd edition, pp. 6–7). 3, St Paul, MN: APS Press.

Malvick, D.K., Grünwald, N.J. & Dyer, A.T. (2009) Population structure, races, and host range of *Aphanomyces euteiches* from alfalfa production fields in the central USA. *European Journal of Plant Pathology*, *123*, 171–182.

McKeen, W.E. & Traquair, J.A. (1980) *Aphanomyces* sp., an alfalfa pathogen in Ontario. *Canadian Journal of Plant Pathology*, *2*, 42–44.

Moussart, A., Even, M.N., Lesné, A. & Tivoli, B. (2013) Successive legumes tested in a greenhouse crop rotation experiment modify the inoculum potential of soils naturally infested by *Aphanomyces euteiches*. *Plant Pathology*, *62*, 545–551.

Moussart, A., Even, M.N. & Tivoli, B. (2008) Reaction of genotypes from several species of grain and forage legumes to infection with a French pea isolate of the oomycete *Aphanomyces euteiches*. *European Journal of Plant Pathology*, *122*, 321–333.

Moussart, A., Wicker, E., Le Delliou, B., Abelard, J.-M., Esnault, R., Lemarchand, E. et al. (2009) Spatial distribution of *Aphanomyces euteiches* inoculum in a naturally infested pea field. *European Journal of Plant Pathology*, *123*, 153–158.

Munkvold, G.P. & Carlton, W.M. (1995) Prevalence and distribution of *Aphanomyces euteiches* and *Phytophthora medicaginis* in Iowa alfalfa fields. *Plant Disease*, *79*, 1251–1253.

Munkvold, G.P., Carlton, W.M., Brummer, E.C., Meyer, J.R., Undersander, D.J. & Grau, C.R. (2001) Virulence of *Aphanomyces euteiches* isolates from Iowa and Wisconsin and benefits of resistance to *A. euteiches* in alfalfa cultivars. *Plant Disease*, *85*, 328–333.

Nygaard, S.L. & Grau, C.R. (1989) *Phytophthora megasperma* virulence to alfalfa measured using single-isolate zoospore suspensions. *Canadian Journal of Plant Pathology*, *11*, 101–108.

Palloix, A., Ayme, V. & Moury, B. (2009) Durability of plant major resistance genes to pathogens depends on the genetic background, experimental evidence and consequences for breeding strategies. *New Phytologist*, *183*, 190–193.

Papavizas, G.C. & Ayers, W.A. (1974) Aphanomyces species and their root diseases in pea and sugarbeet: a review. *United States Department of Agriculture*, *Agricultural Research Service Technical Bulletin*, *1485*, 154.

Pfender, W.F., Dewliche, P.A., Grau, C.R. & Hagedorn, D.J. (1984) A medium to enhance recovery of *Aphanomyces* from infected plant tissue. *Plant Disease*, *68*, 845–847.

Pilet-Nayel, M.L., Prospéri, J.M., Hamon, C., Lesné, A., Lecointe, R., Le Goff, I. et al. (2009) *AER1*, a major gene conferring resistance to *Aphanomyces euteiches* in *Medicago truncatula*. *Phytopathology*, *99*, 203–208.

Samac, D.A., Bucciarelli, B., Dornbusch, M.R., Miller, S.S. & Yu, L.-X. (2017) Identification of markers associated with race-specific resistance to Aphanomyces root rot in alfalfa. *Phytopathology*, *107*(S5), 1.

Samac, D.A., Yu, L. & Missaoui, A.M. (2021) Identification and characterization of disease resistance genes in alfalfa and *Medicago truncatula* for breeding improved cultivars. In: Yu, L. & Kole, C. (Eds.) *The alfalfa genome*. Cham: Springer, pp. 213–233.

Sathoff, A.E., Velivelli, S., Shah, D.M. & Samac, D.A. (2019) Plant defensin peptides have antifungal and antibacterial activity against human and plant pathogens. *Phytopathology*, *109*, 402–408.

Sekizaki, H. & Yokosawa, R. (1988) Studies on zoospore-attracting activity. I. Synthesis of isoflavones and their attracting activity to *Aphanomyces euteiches* zoospore. *Chemical and Pharmaceutical Bulletin*, *36*, 4876–4880.

Smith, D.L. & Watson, Q. (2014) Aphanomyces root rot management in alfalfa. In: *Proceedings of the 2014 Wisconsin Crop Management Conference*, 53, 86–92. Available at: https://soilsextension.webhosting.cals.wisc.edu/wp-content/uploads/sites/68/2014/12/2014_wcmc_proc.pdf [Accessed 18th March 2022]

Sturz, A.V., Christie, B.R., Matheson, B.G. & Nowak, J. (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biology and Fertility of Soils*, *25*, 13–19.

Tordsen, C.L., Giles, J.M. & Sathoff, A.E. (2022) First report of *Aphanomyces euteiches* race 1 and race 2 causing Aphanomyces root rot on alfalfa (*Medicago sativa*) in South Dakota. *Plant Disease*, *106*, 771.

Vandemark, G.J., Barker, B.M. & Gritsenko, M.A. (2002) Quantifying *Aphanomyces euteiches* in alfalfa with a fluorescent polymerase chain reaction assay. *Phytopathology*, *92*, 265–272.

Vandemark, G.J., Barker, B.M. & Hughes, T.J. (2004) Heritability of resistance to *Aphanomyces euteiches* races 1 and 2 in alfalfa. *Euphytica*, *136*, 45–50.

Venancio, W.S., Rodrigues, M.A.T., Begliomini, E. & Souza, N.L. (2003) Physiological effects of strobilurin fungicides on plants. *Agricultural Science Engineering*, *9*, 59–68.

Vincelli, P., Henning, J., Hendrick, T., Brown, J., Osborne, L.J., Prewitt, B. et al. (2000) Improved seedling health, yield, and stand persistence with alfalfa resistant to Aphanomyces root rot. *Agronomy Journal*, *92*, 1071–1076.

Wakelin, S.A., McCarthy, T., Stewart, A. & Walter, M. (1998) In vitro testing for biological control of *Aphanomyces euteiches*. In: *Proceedings of the 51st New Zealand Plant Protection Conference*, 1988. Auckland: NZPPC, pp. 90–95.

Wakelin, S.A., Walter, M., Jasper, M. & Stewart, A. (2002) Biological control of *Aphanomyces euteiches* root-rot of pea with spore-forming bacteria. *Australasian Plant Pathology*, *31*, 401–407.

Wu, L., Chang, K.-F., Hwang, S.-F., Conner, R., Fredua-Agyeman, R., Feindel, D. et al. (2019) Evaluation of host resistance and fungicide application as tools for the management of root rot of field pea caused by *Aphanomyces euteiches*. *Crop Journal*, *7*, 38–48.

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