Engineering 4 (2018) 542–551

Contents lists available at ScienceDirect

Engineering

journal homepage: www.elsevier.com/locate/eng

Research
Crop Genetics and Breeding—Review

Aphanomyces euteiches: A Threat to Canadian Field Pea Production

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ARTICLE INFO

Article history:
Received 2 February 2018
Revised 2 May 2018
Accepted 8 June 2018
Available online 17 July 2018

Keywords:
Field pea
Aphanomyces euteiches
Root rot
Pathogenicity variability
Quantitative trait loci

ABSTRACT

Field pea (*Pisum sativum* var. *arvense* L.) is an important legume crop around the world. It produces grains with high protein content and can improve the amount of available nitrogen in the soil. *Aphanomyces* root rot (ARR), caused by the soil-borne oomycete *Aphanomyces euteiches* Drechs. (*A. euteiches*), is a major threat to pea production in many pea-growing regions including Canada; it can cause severe root damage, wilting, and considerable yield losses under wet soil conditions. Traditional disease management strategies, such as crop rotations and seed treatments, cannot fully prevent ARR under conditions conducive for the disease, due to the longevity of the pathogen oospores, which can infect field pea plants at any growth stage. The development of pea cultivars with partial resistance or tolerance to ARR may be a promising approach to analyze the variability and physiologic specialization of *A. euteiches* in field pea and to improve the management of this disease. As such, the detection of quantitative trait loci (QTL) for resistance is essential to field pea-breeding programs. In this paper, the pathogenic characteristics of *A. euteiches* are reviewed along with various ARR management strategies and the QTL associated with partial resistance to ARR.

1. Introduction

Field pea (*Pisum sativum* var. *arvense* L.), along with common bean (*Phaseolus vulgaris* L.), faba bean (*Vicia faba* L.), soybean (*Glycine* max (L.) Merr.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* Medik.), belongs to the family Fabaceae. The interaction between pea and *Rhizobium* bacteria leads to the formation of root nodules, which enable pea roots to fix nitrogen directly from the atmosphere, thereby benefiting production of the pea and subsequent crops. Pea seeds have a high protein content, are rich in starch, dietary fiber, vitamins, minerals, and polyphenols, and provide a protein-rich food source for both humans and livestock [1]. Garden peas are processed for canning or freezing by the food industry, while field pea is one of the most widely cultivated crops for human consumption and livestock feed on the Canadian Prairies, with an export market value of 1.2 billion CAD in 2016 [2].

World grain pea production peaked in 1990 at 1.66×10^7 t; by 2014, it had decreased by 5.5×10^6 t due to a reduction in pea cultivation in Europe [3,4]. Since then, European pea cultivation has once again increased as a result of new Common Agricultural Policy (CAP) greening measures [5]. Pea cultivation was introduced to Canada more than a century ago [6], first in limited areas in Eastern Canada in the late 1800s. In 1985, there were only 8.05×10^4 hm^2 of field peas seeded in Canada. There was a significant increase in pea cultivation in North America (i.e., Canada and the United States) starting in the 1990s. Because of its adaptation to cool climates and its nutritional value for human and livestock consumption, field pea has become increasingly popular as a cash crop to meet demand for the export market in Canada. By 2014, Canada had become the largest field pea producer in the world, which now accounts for 21% of global production [4].

At present, *Aphanomyces* root rot (ARR) is one of the major limitations to pea production worldwide. This disease is caused by *Aphanomyces euteiches* Drechs. (*A. euteiches*), which is distinguished from most other soil-borne pathogens by the formation of thick-walled oospores [7]. It can cause severe root damage at all growth stages of its host. The longevity of *A. euteiches* oospores,

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https://doi.org/10.1016/j.eng.2018.07.006

combined with the absence of fully resistant pea genotypes, makes the management of ARR difficult. This review describes pathogenic variability in *A. euteiches*, and the application of traditional management strategies and partial resistance to control ARR in field pea.

Pea root rot complex (PRRC) has been reported to be a serious problem in field pea production in Canada [8] and worldwide [9]. When root rot is severe, yield reduction can be as high as 70% [10,11]. A number of soil-borne pathogens have been reported to be involved in PRRC, including *A. euteiches*, *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., and *Rhizoctonia solani* Kühn [12–15]. *Fusarium solani* (Mart.) Sacc. (*F. solani*) was the most common causal agent of pea root rot worldwide [16]. In addition to *Fusarium* spp., *A. euteiches* has been reported to occur in certain countries in North America and Europe, as well as in Japan, Australia, and New Zealand [17]. Yield losses due to infection by *Pythium ultimum* and *A. euteiches* were reported in the United States [18,19]. An estimated loss of 2.4×10^4 t of field pea caused by PRRC occurred in Southern Ontario in 1983 [10]. *F. avenaceum* (Corda ex. Fr.) Sacc. was reported to be the main cause of *Fusarium* root rot in pea crops in Alberta and Manitoba, accounting for as much as 80% of the isolates collected from field samples [20,21]. Hwang and Chang [22] reported that PRRC was prevalent in the Canadian province of Alberta. Tu [23] noted that the amount of damage to field pea caused by *Fusarium* spp. is influenced by soil compaction, temperature, and moisture levels, which may also impact the relative prevalence of *F. solani* [16] and *F. avenaceum* [20].

Infection by PRRC is associated with seed decay, damping-off, seedling blight, root rot, and wilt; however, the identity of the causal organisms cannot be determined solely by examining the symptoms [24]. This increases the difficulty of predicting and controlling pea root rot in western Canada and elsewhere. Direct invasion of the seeds by any of the fungi involved in the PRRC complex, but most often by *Pythium* spp., is usually the cause of seed decay [25,26], which results in a soft, mushy appearance of the seeds and in their rapid deterioration. Damping-off and seedling blight reduce seedling emergence and plant density, limit pea growth, delay canopy closure, and therefore increase weed competition. All of these factors may cause yield reductions [27]. Root rot also restricts the transport of water and nutrients in infected roots, and reduces canopy density and the uniformity of crop maturity [28]. Root rot may also destroy rhizobial nodules, leading to a reduction in nitrogen fixation in the roots [29].

2. ARR caused by *A. euteiches*

A. euteiches belongs to the class Oomycota (oomycetes), which comprises a large group of eukaryotes that includes the most diverse, important, and earliest-known water molds [30]. Oomycetes resemble fungi in morphology (i.e., mycelial growth) and many are parasitic. Unlike true fungi, oomycetes produce motile, biflagellate zoospores [30,31]. Cytological and biochemical studies indicate additional differences that distinguish oomycetes from fungi [32–34]. At the vegetative stage, the mycelia of oomycetes consist of a coenocytic thallus that remains diploid [33] (Fig. 1 (a)). The formation of haploid nuclei only occurs through meiosis for gamete formation. At this stage, fungal thalli produce septate cells, each of which carry one haploid nucleus. In addition, in contrast to fungal cell walls, which are composed mainly of chitin (acetylglucosamine polymers), along with glucans, polysaccharides, mucopolysaccharides, waxes, and pigments, the cell walls of oomycetes contain cellulose, β-glucans and hydroxyproline, but no chitin [35].

The genus *Aphanomyces* includes a number of water molds that are saprophytes or parasites of fish, crayfish, and plants [36]. There are about 40 described species of *Aphanomyces* [37]. Most have a wide range of hosts belonging to different families, although there are a few exceptions such as *A. cochlioides* Drechs., which only affects sugar beet (*Beta vulgaris* L.) [37] and *A. iridis* Ichitani et Tak. Kodama, which only affects iris (*Iris* spp.) [36]. Although *A. euteiches* has a broad host range within the family Fabaceae, it causes the greatest economic damage to pea and lentil crops [38–40]. This parasite has been isolated from pea, alfalfa (*Medicago sativa* L.), snap and red kidney bean (*Proteus vulgaris* L.), faba bean, red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), lentil, and several weed species [38,41]. Nevertheless, its occurrence and degree of pathogenicity may differ from one host to another. Pea-infecting strains and alfalfa-infecting strains of *A. euteiches* from the United States and France have been identified, and some strains can infect both pea and alfalfa [39,42,43]. Papavizas and Ayers [38] reported that infection by *A. euteiches* caused large economic losses in pea and alfalfa crops in North America and Europe. The wide host range of *A. euteiches*, combined with its long-lived oospores, makes the management of ARR with crop rotation difficult.

Since it was first described by Jones and Drechsler [44] and extensively reviewed by Papavizas and Ayers [38], *A. euteiches* has been considered to be one of the most damaging soil-borne pathogens of legumes. At present, *A. euteiches* has been reported in all of the main pea cultivation regions of the world [17]. In France, it affects pea crops in the northern regions of the country [41]. In North America, it causes severe yield losses in the Great Lakes region in both Canada and the United States, as well as in the Northeastern [25] and the Pacific Northwest [45] regions of the United States. A high incidence and severity of pea root rot caused by *A. euteiches* was recently reported in Alberta [46]. Yield losses caused by this parasite can be as high as 86% in heavily infested pea fields [47].

2.1. Favorable conditions

Symptoms of ARR can develop within 7–14 days after first infection, depending on soil moisture, temperature, and the concentration of oospores [38,47]. High inoculum densities of *A. euteiches* increase the incidence and severity of ARR. Chan and Close [7] observed a positive correlation between the number of oospores per 100 g of soil and root rot severity. Oospores can form germ tubes, which directly penetrate the cortex of the pea roots. Soil moisture levels influence the formation of sporangia and the release of zoospores, and allow the flagellated zoospores to travel to the plant roots in the moisture films surrounding soil particles [48,49]. Zoospore infection also facilitates the leakage of metabolites from pea roots [50], which stimulates the germination of oospores and attracts more zoospores [9]. High rainfall favors ARR outbreaks, and only a short period is required for the completion of the infection process by *A. euteiches* [25]. The minimum level of soil moisture needed for the initiation of ARR is about 30% of the water-holding capacity of the soil [51,52].

ARR may occur over the same wide soil-temperature range that is conducive for pea growth [25]; however, the optimal temperatures for infection are about 16 °C, and 20–28 °C for disease development [53,54]. High temperatures may accelerate pea root decay following infection by *A. euteiches*, since severe infection further limits water and nutrient movement within field peas [55].

Gaulin et al. [56] reported that *A. euteiches* can infect legume hosts at any growth stage, while others have suggested that infection occurs most commonly at the seedling stage [57,58].

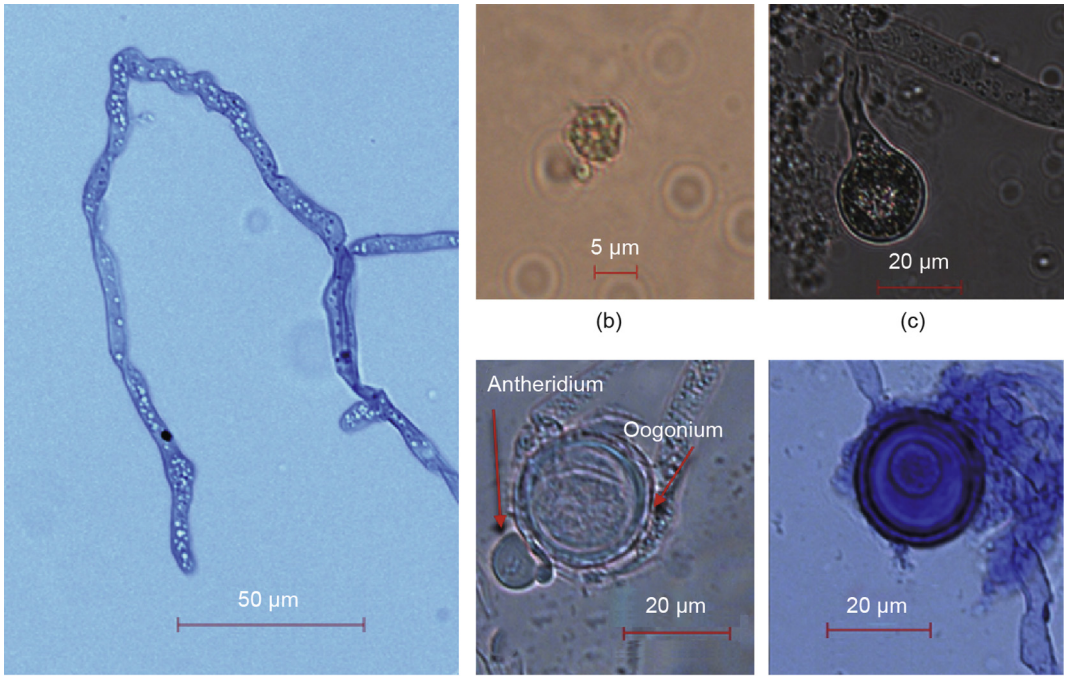

Fig. 1. Structures of *A. euteiches*. (a) Coenocytic hyphae with no septa; (b) encysted zoospore losing both flagella; (c) oogonium of *A. euteiches*; (d) antheridium and oogonium of *A. euteiches* during the sexual stage; (e) thick-walled oospore for survival in unfavorable conditions.

2.2. *Life-cycle of* A. euteiches

The life-cycle of *A. euteiches* includes both asexual and sexual stages, which allow for its efficient dissemination via zoospores and its survival as oospores during harsh winter conditions [41]. The oospores are 18–25 μm in diameter, have a thick protective wall, and contain energy reserves in the form of a large oil globule [9,38]. They can survive in the soil for over ten years [47] and may be spread over long distances by the transportation of infested soil and/or infected plant residue [38].

When adjacent to pea roots, the oospores germinate under conducive temperature and moisture conditions, and form either a mycelium or a zoosporangium. The zoosporangium, which forms as long tubes on the oospores, may release a large number of zoospores [59]. The biflagellate motile zoospores are attracted to a suitable host by chemical signals in the root exudates [60], and encyst within minutes on the rhizoplane (Fig. 1(a)). The resulting cysts germinate and penetrate the host cortical cells within hours [38]. Once an infection site has been established, coenocytic hyphae develop rapidly in the intercellular spaces of the host root tissue and the pathogen spreads from the roots to the stem (hypocotyls and epicotyls), eventually colonizing the entire root system. The infected roots become soft and water-soaked, and take on a honey-brown or blackish-brown coloration, which turns orange-brown or blackish-brown during the later stages of disease development (Fig. 2(b) and (c)).

Within a few days of infection, *A. euteiches* may enter its sexual stage with the formation and fusion of haploid antheridia and oogonia [59] (Fig. 1(a) and (d)). Subsequently, thick-walled oospores are formed, which ensure the long-term survival of the pathogen and serve as the primary source of inoculum for new infections in subsequent years [61] (Fig. 1(e)). The parasite may progress from first infection of the roots to formation of oospores in as few as 10–14 days [62].

The translocation of water and nutrients within infected plants can be restricted by severe root rot [63] (Fig. 2(a) and (f)). Infected plants may become stunted during the early growth stages and then start to wilt, resulting in premature death [64] (Fig. 2(d)). Moreover, ARR may severely delay pea maturity, reduce pod size and seed number, and decrease seed quality [64] (Fig. 2(e) and (g)).

2.3. *Variability and physiological specialization in* A. euteiches

Information on pathogenic variability and physiologic specialization in *A. euteiches* is limited. Given the absence of completely resistant or immune pea genotypes, it is difficult to create a differential set to distinguish races, and the races identified by the limited differential genotypes may exhibit atypism [38]. Nonetheless, differences among isolates have been detected based on zoospore and oospore size, the time required for sporulation and the ability to produce zoospores, growth rate on culture media, and the production of pectinolytic and cellulolytic enzymes [38].

Physiological specialization in *A. euteiches* was first examined by King and Bissonnette [65], who indicated that isolates of the parasite differed in their virulence patterns on various pea cultivars in Minnesota. Carlson [55] tested ten isolates of *A. euteiches*, which were isolated from infested soil from Minnesota, New York, and Wisconsin, by inoculating the root tips of tolerant and susceptible pea cultivars, and reported considerable differences in the ability of the isolates to infect plants and produce oospores. Variable virulence and growth characteristics on culture media were also observed among seven single-zoospore isolates obtained from germinated oospores [48]. Beute and Lockwood [66] inoculated six differential cultivars with 15 *A. euteiches* single-zoospore isolates, and identified two races based on their virulence on those pea cultivars (Table 1) [66–70]. The two races displayed a different disease reaction pattern on the six pea cultivars, based on disease severity. Employing the same differentials as Beute and Lockwood [66], Sundheim and Wiggen [67] confirmed the existence of four physiological races of *A. euteiches* in a collection of 14 isolates from four counties in Norway. Sundheim and Wiggen [67] evaluated resistance by counting the number of dead plants ten days after

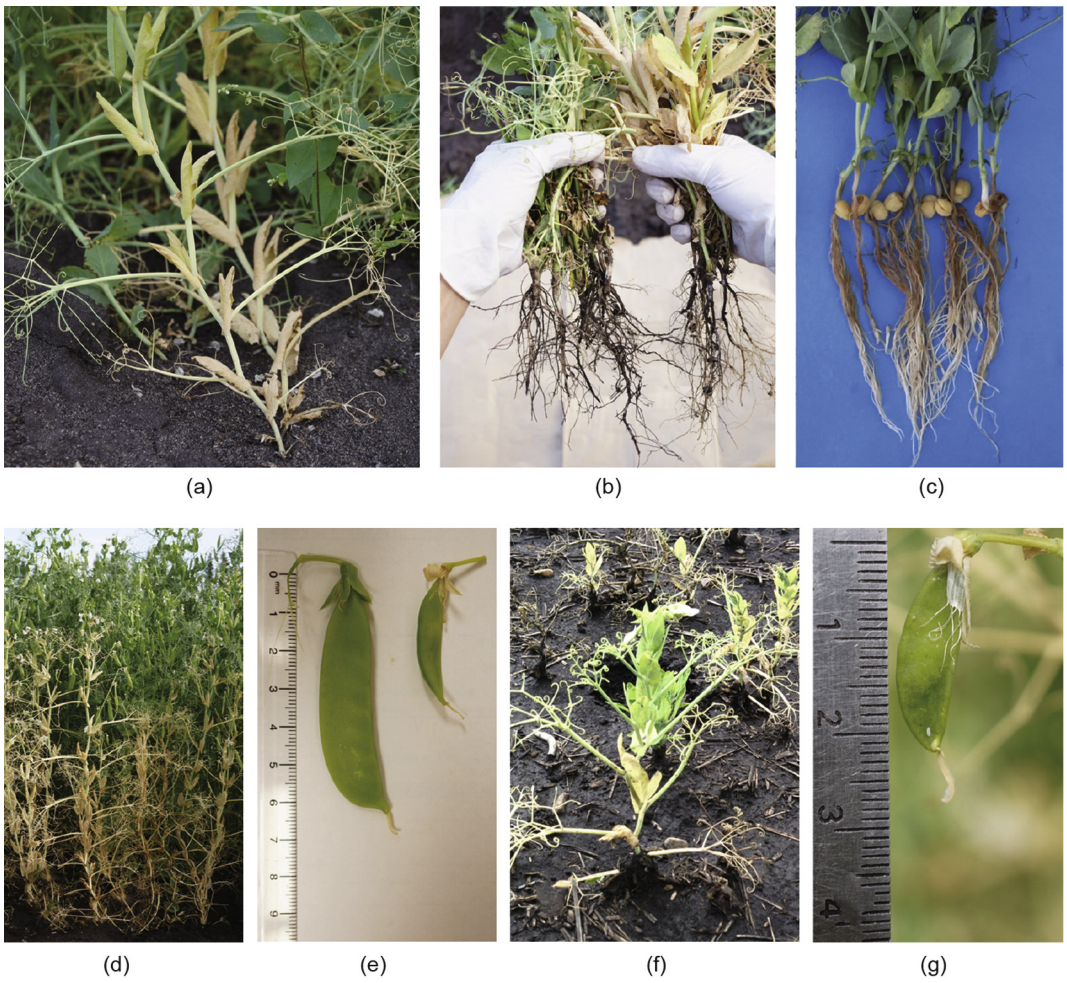

Fig. 2. Symptoms of pea ARR caused by *A. euteiches*. (a) Yellowing and stunting of pea stems in the field; (b) comparison of healthy (left) and diseased (right) plants; (c) discoloration and water-soaking of diseased pea rootlets; (d) wilted pea plants in the field near harvest; (e) comparison of healthy (left) and diseased (right) pods; (f) Seedling blight in a low area of a field after heavy rainfall; (g) bleaching of leaflets and premature ripening of the pod.

Table 1
Studies on pathogenic variability and physiological specialization in *A. euteiches* isolates from pea using various sets of differential pea genotypes.

Method	Differential genotypes	Identified race/ virulence type	Isolate region	Ref.
Race identification	Miragreen; Early Perfection; PI 175232; PI 169604; PI 180693; PI 166159	Races 1 and 2	United States	[66]
Race identification	Miragreen; Early Perfection; PI 175232; PI 169604; PI 180693; PI 166159	Races 1–4	Norway	[67]
Race identification	Miragreen; Early Perfection; PI 175232; PI 169604; PI 180693; PI 166159	Race 5	New Zealand	[68]
Pathogenic variability	MN313; MN314; 90-2079; WI-8904; Little Marvel; Saranac; Early Gallatin	Virulence groups I–IV	United States	[69]
Pathogenic variability	Baccara; Capella; 90-2131; MN313; 552; PI 180693	Virulence types I–XI	North America, Europe, and Oceania	[70]

inoculation. The method of race identification described by Sundheim and Wiggen [67] was questioned by Manning and Menzies [68], who suggested that the irreversibly wilted plants 10 d after inoculation could not fully reflect the virulence spectrum of *A. euteiches*. The inconsistencies between these studies underscore the difficulties associated with race identification in *A. euteiches*.

Malvick and Percich [69] developed a new differential set (consisting of the pea genotypes MN313, MN314, 90-2079, WI-8904, Little Marvel, Saranac, and Early Gallatin) to evaluate pathogenic diversity among 114 *A. euteiches* isolates from the United States (Table 1), and also examined genetic variation via random amplified polymorphic DNA (RAPD) analysis. All isolates were pathogenic on one or more pea cultivars, and 18% and 14% were pathogenic on alfalfa (Saranac) and bean (Early Gallatin), respectively. Malvick and Percich [71] concluded that *A. euteiches* populations were genotypically (based on the RAPD analysis) and phenotypically variable in the central and western United States. In a subsequent study, four virulence groups were identified, in which a disease severity of greater than 3.0 (i.e., > 90% of the roots brown or yellow, but no symptoms present on the epicotyl or hypocotyl) was used as the threshold for a clear pathogenic interaction [72].

Later, Wicker and Rouxel [70] examined 109 isolates of *A. euteiches* from France, Denmark, Sweden, Norway, the United States, Canada, and New Zealand on another differential set (Baccara, Capella, 90-2131, MN313, 552, and PI 180693) and identified 11 virulence types (Table 1). In that study, the isolates belonging to virulence type I, which caused severe ARR symptoms on all of the differentials, were predominant and the most aggressive. Wicker and Rouxel [70] also calculated a disease severity index (DSI) based on the mean of the individual disease severity ratings (0–5), and regarded a DSI < 1 as indicative of resistance.

In a later study, Wicker et al. [17] indicated that the differential pea genotypes used by Malvick and Percich [69] were inadequate to distinguish French strains of *A. euteiches*. To more accurately evaluate the virulence of the pathogen from different countries, Wicker et al. [17] evaluated 33 pea lines and the five differentials originally described by Wicker and Rouxel [70]. The resistance detected in the differential pea genotypes in these studies has been used in the development of commercial pea cultivars with ARR resistance [17]. Wu [73] conducted greenhouse screening of eight *A. euteiches* isolates from Alberta and Manitoba on the same differential set as Wicker and Rouxel [70]. Most strains were classified as virulence type I, although one strain was identified as virulence type III. Further testing of additional isolates from other Canadian regions with more differential breeding lines is still needed in order to better understand physiological specialization in this pathogen.

2.4. Isolation of A. euteiches

The isolation of *A. euteiches* strains is difficult. Pea root and rootlet samples easily slough off infected tissue into the soil [74]. Numerous fungi also interfere with the isolation of *A. euteiches* [75]. Manning and Menzies [75] successfully isolated *A. euteiches* on potato dextrose agar (PDA) plates using soil baiting. To increase the isolation success rate, metalaxyl-benomyl-vancomycin (MBV) [25] medium has been widely used to isolate *A. euteiches*, since it suppresses the growth of *Pythium* spp., *Phytophthora* spp., and most bacteria.

Wu [73] used both direct isolation from infected root samples and soil baiting. For direct isolation, root and soil samples were collected at 2–3 weeks after seeding, when roots were not yet completely infected by PRRC. The soil samples were later used for pathogen baiting with susceptible pea cultivars [68]. The tips were cut from water-soaked pea roots and examined under a microscope for the presence of oospores. The root tips were surface-sterilized in 1% NaClO for 30 s, rinsed in sterilized water, and plated on MBV medium. However, *A. euteiches* was detected in < 0.1% of the samples, based on the results of a real-time polymerase chain reaction (PCR) assay described by Vandemark et al. [76].

2.5. Inoculation methods

Zoospores are the most common form of *A. euteiches* inoculum employed in greenhouse experiments [17,43,70,77–83], while oospore-based inoculum has also been used in both greenhouse and field trials [38,73]. The zoospore-based inoculum has been used widely for the detection of partial resistance to ARR in field pea [77–83]. Zoospore inoculum is usually produced in a broth made from corn kernels, maltose-peptone, and oat (*Avena sativa* L.), or from pea seeds suspended in water, which are inoculated with *A. euteiches* and incubated for 5–7 d in the dark at room temperature [84]. The resulting mycelial mats are placed in a mineral salt solution and aerated overnight to produce a zoospore suspension of 3×10^5–8×10^5 zoospores per milliliter. The zoospores are usually used to precisely inoculate seven-day-old pea seedlings, before the seedlings are transplanted into pots in a greenhouse, with a determined zoospore concentration, thus eliminating the undesirable effect of nutrient substances in the media.

Oospore inocula have been produced on autoclaved rolled oats with sand, cornmeal, and water. This substrate is inoculated with *A. euteiches* and incubated in the dark for 30 days at room temperature [38]. Wu [73] modified this method by replacing cornmeal with oat grain. The grain-sand inocula were often used in field trials, as well as in greenhouse experiments, which need intensely infected disease conditions. Thygesen et al. [85] incubated *A. euteiches* in an oatmeal broth at 20 °C in the dark for 4–8 weeks; the broth was homogenized in a blender and then filtered and washed with a mineral salt solution. The suspension was mixed with sterilized sand, dried at room temperature, and stored at 4 °C. The oospore suspension also provides a precise inoculation for both the pea seedlings and pea seeds, which could continuously release zoospores in a greenhouse experiment.

3. Traditional disease management

ARR has been recognized as one of the most damaging root diseases of field pea for almost a century [86]. The options for management of this disease, however, are limited. Pea cultivars completely resistant to ARR are not available [25,87] and only partial resistance and/or tolerance has been reported in several studies [80,81,88]. Some studies have focused on the efficacy of fungicidal seed treatments at the seedling stage, which have been shown to improve plant health [89,90]. At present, the most widely recommended method to manage ARR is avoidance via crop rotation and evaluation of infestation levels in the field prior to seeding [91]. Biological control, including seed and soil treatments, has also shown promise at the experimental stage [9,92].

3.1. Cultural practices

Crop rotation is one of the oldest and most fundamental methods to manage diseases caused by soil-borne pathogens, although its effectiveness directly coincides with the length of rotation [93]. A positive relationship exists between the frequency of pea crops and root rot severity [86]. Rotation with non-host crops can therefore reduce the density of *A. euteiches* in the soil and thereby reduce the severity of ARR. Long-term crop rotations can reduce *A. euteiches* inoculum density in the soil, but they are not always effective in eradicating the disease [94]. Nonetheless, the practicality and effectiveness of crop rotation as a method to manage ARR is questionable, because the oospores can survive for 10–15 years in the absence of a host [95]. Furthermore, many alternative hosts, including chickpea, lentil, alfalfa, and weedy species, can sustain inoculum levels in the absence of pea [38]. Hossain et al. [96] recommended a crop rotation interval of 6–8 years. Williams-Woodward et al. [97] examined the effect of oats as a rotation crop with pea, and observed that oat residues improved ARR suppression. Therefore, increased crop diversity may represent a good long-term strategy for disease management [98].

Soil conditions can be suppressive or conducive to ARR [99]. Heyman et al. [100] observed a strong negative correlation between calcium concentration and disease development, which indicated that free calcium was a major variable in the degree of soil suppression of *A. euteiches*. This finding led to the suggestion that calcium might play a role in the inhibition of zoospore production from the oospores [100].

Residues from two plant families, the Brassicaceae—such as cabbage (*Brassica oleracea* L.), mustard (*Brassica nigra* L.), turnip

(*Brassica rapa* L.), and rapeseed (*Brassica napus*) [7,8,63,101–103]—and the Poaceae—such as oats, rye (*Secale cereale* L.), and maize (*Zea mays* L.) [8,97,104–108]—can reduce the severity of ARR.

Soil compaction can exacerbate the development of ARR, causing pea yield losses as high as 63% [107]. In contrast, the yield of pea plots covered with oat shoots and residues increased by 48% relative to plots planted without residues, suggesting that oat residues provide a promising method for the cultural control of pea ARR. Allmaras et al. [87] confirmed the effect of oats as a pre-crop in the suppression of ARR, and pointed out that excessive compaction related to tillage and traffic management may impair internal soil drainage and thus reduce the effectiveness of oat residues in controlling the disease.

Field indexing by sampling soils to determine the *A. euteiches* inoculum potential can be an effective method to manage ARR of field pea prior to seeding. Studies have identified and distinguished heavily infested fields from non-infested or lightly infested fields under greenhouse conditions [109,110], and this method of prior land selection can be an economical and dependable practice for avoiding ARR [111]. Real-time PCR analysis has also been used to measure populations of *A. euteiches* in field soil. Vandemark et al. [112] and Armstrong-Cho et al. [113] demonstrated that a positive relationship existed between ARR severity and the DNA concentration of several isolates of *A. euteiches* in pea roots.

3.2. Disease prediction and molecular detection of A. euteiches

Molecular markers are useful tools for the identification of fungal and oomycete plant pathogens. The testing of soil or plant samples for the presence of *A. euteiches* DNA by PCR analysis with species-specific primers has been widely used [76]. Chatterton et al. [46] and Armstrong-Cho et al. [113] detected *A. euteiches* in pea fields in Alberta and Saskatchewan, respectively, based on a PCR assay. A number of commercial kits have also been used to identify *A. euteiches* efficiently [46,76,112]. Nonetheless, information on the use of molecular markers for the identification of specific races or pathotypes of *A. euteiches* is still limited and preliminary.

Malvick and Percich [69] conducted RAPD analyses to evaluate genotypic diversity among strains of *A. euteiches* in the United States, but none of the 76 polymorphic RAPD markers were associated with pathogenic variation. In another study, the same researchers successfully distinguished one major group and two closely related minor groups in a collection of 114 isolates from four locations in the United States, based on a pathogenicity test of five pea genotypes and RAPD analysis [69]. Sauvage et al. [111] used two sets of markers, 136F/136R and 11F/280R, to amplify different-sized PCR products from 105 isolates of *A. euteiches*. They demonstrated a close relationship between the quantity of soil inoculum and ARR severity.

3.3. Seed and soil treatments

Certain soil fungicides for ARR control are prohibited in some regions, including much of Europe [96]. In addition, the cost and adverse environmental effects of treating the soil with chemicals makes this approach impractical and undesirable across the broad area over which pea crops are grown [114,115]. Seed-coating treatments such as hymexazol can effectively improve seedling emergence [116]. However, Tu [106] pointed out the limitations in the control of pea root rot using Captan (*N*-trichloromethylthio-4-cyclohexene-1,2-dicarboximide). Furthermore, *A. euteiches* is resistant to some of the fungicides that are registered for the control of other oomycetes. For example, metalaxyl is active against most oomycetes, but not against *Aphanomyces*. It is the main ingredient of the selective medium used to isolate *A. euteiches* [25]. Neither the systemic acylalanine-type of oomycete fungicides, such as metalaxyl, nor the ethyl phosphonates, such as fosetyl-Al or cymoxanil, effectively control ARR [117]. Some chemicals effectively suppress *A. euteiches* under controlled conditions, but have limited beneficial effects in field trials [89,90]. Tachigaren (hydroxyisoxazole or hymexazol) was reported to reduce root rot severity and increase yield under experimental field conditions [116]; this compound is available commercially in Japan for the control of the *Pythium* and *Aphanomyces* diseases of sugar beets [117]. The effectiveness of Tachigaren for the control of ARR, however, was variable in other studies [118–120]. A recent study determined that Intego Solo (ethaboxam) (Valent, Guelph, ON, Canada), BAS 516F, and BAS 720F reduced disease severity under greenhouse conditions, but not under field conditions [73]. At present, ethaboxam is the only fungicide registered for *Pythium* root rot control and the suppression of seed rot caused by *Phytophthora* spp. and *Aphanomyces* spp. in legumes in Canada.

3.4. Biological control

Antagonistic microorganisms applied to the seeds or soil may help to protect pea plants from infection by fungal and oomycete pathogens. The spores of arbuscular mycorrhizal (AM) fungi and some spore-forming bacteria, which were applied as seed coatings to control ARR in pea fields, significantly suppressed the development of ARR in a field trial [121]. The application of isothiocyanate, a compound produced by members of the Brassicaceae in shoot tissues, has also been shown to have potential for the management of ARR due to its toxic effects on *A. euteiches* under controlled conditions [96].

Biocontrol and fungicide treatments are often integrated into seed treatments. Recent studies have demonstrated that some fungal and bacterial strains, such as *Gliocladium roseum* (*Clonostachys rosea* (Link) Schroers), *Pseudomonas fluorescens* (Flügge) Migula, and species involved in the *Burkholderia cepacia* (Palleroni and Holmes) Yabuuchi et al. complex, which are formulated for seed coat application in combination with a fungicide, improved seedling emergence in fields infested with *A. euteiches* to a greater extent than treatments in which only a fungicide was applied [17,18,90]. Xue [90] evaluated a seed treatment consisting of the fungal strain ACM941 (*Clonostachys rosea*) and a fungicide (Thiram 75 WP (thiram) or Apron FL (metalaxyl)), and found that a seed coating with ACM941 + fungicide improved pea seed germination in an *A. euteiches*-infested field. AM fungi have also been proven to increase the seedling emergence of peas when inoculated with *A. euteiches* in greenhouse experiments, but they were not always effective in the field [85,122]. Several studies indicated that solarization was effective for the control of pea root rot in temperate regions, when used in combination with green manure crops, lower dosages of chemicals, or biological control organisms [123,124].

4. Genetic resistance to *A. euteiches*

4.1. Partial resistance to ARR

Genetic resistance to ARR in field pea could be the most economical and effective strategy for managing this disease. A number of pea-breeding lines with partial resistance or tolerance to ARR have been developed, and are used to prevent yield losses in some pea-producing regions [78,79,88,125]. Some differential pea genotypes, such as Capella, MN144, MN313, MN314, 90-2131, 90-2079, 552, and PI 180693, have been reported to be partially resistant to certain races of *A. euteiches* [17,72,125]. The differentials PI 180693 and 552 have drawn considerable attention due to their high level of stable partial resistance to ARR [17,126].

Conner et al. [88] reported a high level of tolerance in pea line 00-2067, resulting in good plant vigor and yield despite high disease severity in an ARR disease nursery in Manitoba, Canada. Similar findings were reported by Wu [73]. Therefore, line 00-2067 may be a candidate for the transfer of ARR tolerance into agronomically desirable pea genotypes. Some sources of resistance, however, have been linked to undesirable traits for node length and for flower and hilum colors, which increases the difficulties in transferring resistance or tolerance to agriculturally acceptable pea-breeding lines [127]. Traditional phenotypic breeding for partial resistance to ARR has been hampered by the polygenic nature of the inheritance of resistance in field pea [79]. Therefore, the identification and mapping of genes for partial resistance is essential for the breeding of resistant pea lines in order to effectively pyramid resistance genes.

4.2. Evaluation of resistance to ARR in field pea

Papavizas and Ayers [38] described the most common method of evaluating ARR severity in detail. Plants were uprooted from the soil at 3–4 weeks after seeding and washed under tap water. The roots of each plant were rated on a 0–4 disease severity scale, where: 0 = healthy roots with no visible symptoms of root rot; 1 = slight water-soaking of the primary or secondary roots; 2 = moderate water-soaking of the primary or secondary roots or epicotyls with light-brown areas and more extensive discoloration; 3 = extensive infected areas, soft, but the entire root is not collapsed, and the epicotyl is not markedly shriveled; and 4 = extensive discoloration of the roots with tissue collapse and disintegration, or the plant is completely dead (Fig. 3(a)). Rao et al. [128] developed a 1–5 scale to assess disease severity based on the extent of symptoms on both the roots and the epicotyl. Xue [129] developed a 0–9 scale, which not only assessed the percentage of root infection, but also considered the degree of necrosis (Fig. 3(b)).

4.3. Partial resistance to ARR

Partial resistance is controlled by many quantitative trait loci (QTL) expressing minor to major effects on disease-symptom suppression [130,131]. Several QTL associated with partial resistance to *A. euteiches* have been identified using linkage mapping populations derived from crosses between various combinations of parental genotypes [77–83]. Three stable QTL—namely, *Aph1*, *Aph2*, and *Aph3*—were identified in a recombinant inbred line (RIL) population derived from Puget × 90-2079, located on linkage group (LG) IVb, V, and Ia, respectively [77]. Furthermore, *Aph1* and *Aph3* were associated with partial resistance to both American and French strains of *A. euteiches*, while *Aph2* was resistant only to the French strain [78].

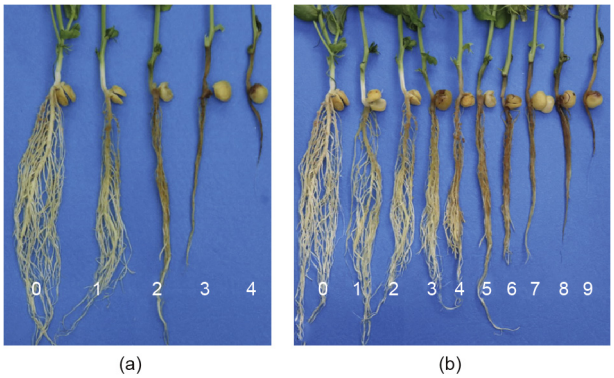

Fig. 3. Comparison of ARR disease ratings (a) on a 0–4 scale [38] and (b) on a 0–9 scale [129].

Hamon et al. [79] reported 135 additive-effect QTL corresponding to 23 genomic regions and 13 significant epistatic interactions associated with partial resistance to *A. euteiches* in two RIL populations from the crosses Baccara × PI 180693 and Baccara × 552. Five consistent genomic regions (*Ae-Ps1.2*, *Ae-Ps2.2*, *Ae-Ps3.1*, *Ae-Ps4.1*, and *Ae-Ps7.6*) affecting a root rot index (RRI) and an aerial decline index (ADI) in two RIL populations were identified on LG I, II, III, IV, and VII [79], and *Ae-Ps1.2* was co-localized to *Aph3*, as identified by Pilet-Nayel et al. [77]. A QTL meta-analysis was also conducted to examine three previously described RIL populations derived from Puget × 90-2079 [77], Baccara × PI 180693, and Baccara × 552 [79], along with a fourth new population derived from DSP × 90-2131 [80]. A total of 27 meta-QTL were identified for ARR resistance; these were well distributed over seven linkage groups, with 11 of the meta-QTL being located on seven genomic regions.

Lavaud et al. [81] also validated the two major QTL, *Ae-Ps4.5* and *Ae-Ps7.6*, and some minor QTL in near-isogenic lines (NILs) from crosses with the resistant parental genotypes, 90-2131, PI 180693, and 552. In a subsequent study, Lavaud et al. [82] examined the functions of *Ae-Ps4.5*, *Ae-Ps7.6*, and some other minor QTL in field pea and found a significant effect of these QTL on ARR symptom expression and root colonization by *A. euteiches*.

Simple sequence repeat (SSR) markers developed by Loridon et al. [132] were widely utilized in the abovementioned studies to screen for reference markers. Various other molecular markers have also been used in the study of ARR resistance, including amplified fragment length polymorphism (AFLP), RAPD, inter simple sequence repeats (ISSRs), and sequence-tagged sites (STSs) markers. Due to rapid developments in molecular marker technology and the decreasing cost of genotyping in recent years, the genome-wide association study (GWAS) method has become widely used as a standard approach to detect the natural variation underlying complex traits, especially polygenic resistance to major diseases in legumes [133,134]. Compared with linkage mapping analysis between resistant and susceptible genotypes, GWAS enables the analysis of wider genetic diversity along with higher recombination rates due to the evolutionary history of the species; thus, it substantially refines the location of the genomic regions associated with trait variations.

Desgroux et al. [83] conducted GWAS mapping with 13 204 single-nucleotide polymorphism (SNP) markers in order to narrow down the confidence intervals of the QTL associated with ARR severity. That analysis resulted in the identification of 52 QTL at short intervals, which may be extremely valuable for use in pea breeding as a means of increasing levels of partial resistance to *A. euteiches* [83].

Root rot severity is the most commonly evaluated trait for assessing ARR development in field pea in QTL detection studies [77–83]. Other measurements to assess the adverse effects of ARR include the ADI [79,80], as well as root weight, foliar weight, and plant vigor in the field [88]. Wu [73] correlated ARR severity with vigor, height, root dry weight, and foliar dry weight.

5. Concluding remarks

Field pea is an important legume crop that provides a valuable protein source for human and livestock consumption. Pea production is limited by ARR, a severe soil-borne disease caused by *A. euteiches*. The longevity of the oospores, lack of high levels of host resistance, and severe economic losses associated with ARR make this disease particularly problematic. Compared with other pathogens involved in the root rot complex, *A. euteiches* can be highly destructive, causing yield losses in excess of 80% in highly infested

fields [47]. Although the importance of *A. euteiches* in pea production has been recognized for nearly a century [44], species-specific surveys on the incidence and severity of ARR based on a mixture of molecular and morphology identification methods have only begun in recent years. Studies on pathogenic variability in *A. euteiches* have not been carried out consistently. Race names and differential sets often differed between studies, making direct comparisons of the results difficult. To analyze the genetic diversity within species of *A. euteiches*, genotyping-by-sequencing (GBS) could be applied by leveraging next-generation sequencing (NGS).

The successful management of ARR continues to be a challenge worldwide. Traditional cultural practices such as crop rotation have limited effects on the control of ARR due to the long-term survival of thick-walled oospores in the soil. Seed treatments do not persist long enough to suppress ARR through the whole life of the field pea plant, and few commercial products are available at present. The application of seed coatings consisting of combinations of fungicides and biological controls should be investigated further as a means of suppressing ARR. Partial resistance and/or tolerance may be the most promising way to reduce the seed yield and quality losses caused by ARR. Many major-effect QTL have been identified via various molecular techniques; these techniques may provide valuable resources for gene pyramiding in pea-breeding programs. The use of genome-wide, transcriptome-based pea SNP marker platforms using NGS technology could further refine the precision and utility of the QTL that have been detected.

Additional efforts are required to better understand the traits and phenotypes that contribute both tolerance and resistance to *A. euteiches*. Mechanisms such as effector-triggered immunity have been well studied for other soil-borne oomycetes, such as the Phytophthora root rot of soybean caused by *Phytophthora sojae*, and should be investigated in the *A. euteiches*-pea interaction. Further research is also needed on the mechanisms involved in pathogenesis, such as the breakdown, acquisition, ingestion, and metabolism of the affected host tissue, and the chemical signals that may trigger oospore formation. This information should facilitate the development of new chemistries that inhibit the growth of the pathogen, prevent spore formation, or curtail infection.

Acknowledgements

We are grateful for funding support received from Agriculture and Agri-Food Canada, the Saskatchewan Pulse Growers Association, the Manitoba Pulse and Soybean Growers through the Growing Forward 2, and the Pest Management and Surveillance Implementation Program.

Compliance with ethics guidelines

Longfei Wu, Kan-Fa Chang, R.L. Conner, Stephen Strelkov, Rudolph Fredua-Agyeman, Sheau-Fang Hwang, and David Feindel declare that they have no conflict of interest or financial conflicts to disclose.

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