1 **Characterization of *Aphanomyces euteiches* pathotypes infecting peas in Western Canada**

2 Nimllash T Sivachandra Kumar[1], Kiela B. Caudillo-Ruiz[1], Syama Chatterton[2], Sabine Banniza[1,†]

3 [1] University of Saskatchewan, Crop Development Centre/Department of Plant Sciences,

4 Saskatoon, S7N 5A8, Canada

5 [2] AAFC, Lethbridge Research and Development Centre, Lethbridge, AB T1J 4B1, Canada

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7

8 [†]Correspondence to: S. Banniza. sabine.banniza@usask.ca

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11

12 **Abstract**

13 Aphanomyces root rot, caused by the soil-borne oomycete *Aphanomyces euteiches* Drechs., has
14 developed into a serious disease in the pea and lentil-producing areas of the Great Plains of North
15 America. Based on six pea differentials previously used to differentiate 11 pathotypes in France,
16 pathotypes were identified among field isolates from Saskatchewan (14) and Alberta (18). Four
17 isolates from the USA and standard isolates for pathotypes I and III designated in the French study
18 were also included. Each isolate was tested twice in replicated experiments by inoculating French
19 pea differentials Baccara, Capella, MN 313, 902131, 552 and PI 80693, along with the Canadian
20 susceptible pea cultivar CDC Meadow and partially resistant USDA line PI 660736 under
21 controlled conditions. Pea plants grown in vermiculite were inoculated 10 days after seeding by
22 pipetting 5 mL of a suspension containing 1 x $10^3$ zoospores $mL^{-1}$ to the base of each plant. Root
23 discoloration was scored 10 days post-inoculation using a 0-5 scale. Testing revealed that 38 of
24 the isolates, including standard pathotype I isolate RB84 belonged to pathotype I, 4 isolates
25 including standard pathotype III isolate Ae109 were pathotype III, and USA isolate Ae16-01 was
26 a pathotype II isolate. An alfalfa isolate from Quebec was avirulent on all pea genotypes. These
27 findings indicate that pathotype type I is predominant on the Canadian prairies.

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30 **Keywords:** Pea differentials, breeding, virulence, oomycete

31 **Introduction**

32 Aphanomyces root rot (ARR) caused by the soil-borne oomycete *Aphanomyces euteiches*
33 Drechs. was recognized as a serious disease of pea as early as the 1920s in the USA (Jones and
34 Drechsler 1925), emerged as an economically important disease in France in the early 1990s
35 (Wicker et al 2001), and was confirmed on the Canadian prairies in 1997 (Mathur et al. 1998).
36 Management of ARR is hampered by the resilience of thick-walled oospores. Circumstantial
37 evidence indicates that oospores survive in field soils for 10 years or more (Papavizas and Ayers
38 1974). ARR management is further complicated by the pathogen's ability to infect at any time
39 during the cropping season. Traditionally, the avoidance of infested fields or long crop rotation
40 with non-host crops such as wheat, soybean, chickpea, and faba bean varieties with resistance to
41 ARR are the only tools available to producers (Moussart et al. 2013). In Canada, the seed
42 treatment Intego Solo® (Ethaboxam; Nufarm Agriculture Inc. Calgary) has been registered for
43 use as an *A. euteiches* suppressant. Application of the foliar fungicide Phostrol (mono- and
44 dibasic sodium, potassium and ammonium phosphites; Engage Agro Corporation, Guelph,
45 Ontario, Canada) was shown to control ARR infection to a certain extent, but control has not
46 been consistent (Gundersen et al. 2006; Porter and Coffman 2006, 2007; Conner et al. 2013).
47 Biological control agents have shown some effectiveness for controlling ARR infection under
48 controlled conditions, but no biological control is commercially available to date. Large-scale
49 screening programs in the USA have identified pea germplasm with partial resistance to this
50 pathogen (reviewed in Lavaud et al. 2015). Genetic control of resistance to *A. euteiches*,
51 considered the most desirable management tool, was shown to be complex and inherited
52 quantitatively, evident in the 52 loci that have been associated with resistance in pea based on a
53 genome wide association study (Desgroux et al. 2016).

54 The development of an effective breeding strategy for host resistance requires an
55 understanding of the pathogenic variability in populations prevalent in the growing region. Host
56 range studies in France and in the USA on isolates originating from pea, *Phaseolus* bean and
57 alfalfa revealed that isolates caused more disease on their host of origin than on other hosts,
58 including vetch and faba bean, but pea isolates also caused moderately high levels of disease on
59 vetch and alfalfa (Wicker et al. 2001, Malvick et al. 1998). For the characterisation of isolates
60 from pea, a set of six pea differentials was identified from among 33 diverse pea accessions
61 (Wicker et al. 2003), and was used to differentiate 11 virulence types (now referred to as
62 pathotypes) among 88 French, nine Scandinavian, six US American, three Canadian and three
63 New Zealand isolates (Wicker and Rouxel 2001). Most French isolates belonged to the highly
64 virulent pathotype I, whereas the three Canadian isolates grouped as pathotypes V, VI and X, and
65 six isolates from the USA were classified as pathotypes I and III. Pathotype III isolates appeared
66 to belong to the same group of isolates earlier described as a 'major group' in the USA (Malvick
67 and Percich 1998), which originally led to the assumption that pathotype III isolates may be
68 dominating the North-American population on pea. However, more recent assessments of
69 isolates from ARR field nurseries in Washington, Oregon and Minnesota indicated that
70 pathotype I was prevalent there as well (Hamon et al. 2011).

71 In response to the increasing importance of *A. euteiches* in pea production in western Canada,
72 and with the initiation of an ARR resistance pea breeding program, experiments were conducted
73 to identify pathotypes of Saskatchewan and Albertan *A. euteiches* isolates. For this purpose,
74 established testing protocols (Sivachandra Kumar et al. 2020; Wicker and Rouxel, 2001) were
75 used to compare standard isolates of pathotype III (Ae109) and pathotype I (RB84) with
76 Canadian and US isolates.

77 **Materials and methods**

78 **Plant materials.** French pea differentials Baccara, Capella, MN 313, 90-2131, 552 and PI
79 80693 (Wicker and Rouxel 2001; Wicker et al. 2003) were used for the characterization of
80 virulence profiles of isolates. Susceptible Canadian pea cultivar CDC Meadow and USDA line
81 PI 660736 with partial resistance to ARR (McGee et al. 2012) were included as internal controls.
82 Pea genotypes were grown in a controlled environment chamber (Conviron Chamber, Model:
83 GR-48, Controlled Environments Inc., Winnipeg, Canada) with a 16 h photoperiod and a
84 temperature regime of 23/21°C day/night. Five to six seeds of each pea genotype were sown into
85 10 x 10 cm pots filled with vermiculite, which avoids confounding staining of roots grown in
86 regular potting mix (Sivachandra Kumar et al. 2020) . Four replicate pots were prepared for each
87 isolate – pea genotype combination, and an additional pot was used as a non-inoculated control.
88 Plants were watered as necessary (about every second day) and thinned to four plants per pot
89 prior to inoculation.

90 ***Aphanomyces euteiches* isolates.** Local isolates of *A. euteiches* included in this study
91 comprised 14 isolates collected in Saskatchewan (AE1, AE9, AE10, AE11, AE12, AE13, AE14,
92 AE15, AE17, AE18, AE19, AE20, AE33, AE34) and 18 isolates collected in Alberta (AE2, AE3,
93 AE4, AE5, AE6, AE7, AE21, AE22, AE23, AE24, AE25, AE26, AE27, AE28, AE29, AE30,
94 AE31, AE32). These were recovered from soil and pea root samples collected from across the
95 pea growing zone of both provinces. Additional isolates were Ae16-01 and Ae16-04 used for
96 germplasm screening in the USA (L. Porter, USDA Pullman, USA), isolates Ae3.1ND15 and
97 Ae2.1ND15 from North Dakota (J. Pasche, North Dakota State University, USA), and standard
98 isolates RB84 (Pathotype I, France) and Ae109 (Pathotype III, Wisconsin, USA) (Wicker and

99 Rouxel 2001) obtained from A. Moussard (INRA, France). Finally, alfalfa isolate DAOMC BR
100 694 from Quebec, Canada, was also included.

101 All 32 Canadian field pea isolates and the alfalfa isolate could not be tested simultaneously on
102 the eight pea genotypes due to space constraints and time limitations for ARR severity
103 assessments. Therefore, the experiment was divided into seven experimental sets. Isolates RB84,
104 Ae109, Canadian field isolate AE11, North Dakota field isolate Ae2.1ND15 and USDA isolate
105 Ae16-04 were included in every experimental set, in addition to four or five randomly selected
106 field isolates. To further confirm consistency across sets, an eighth set was tested, which
107 included one randomly selected isolate from each of the seven sets, plus the five isolates
108 included in every set. A ninth set was designed to accommodate late-sporulating isolate DAOMC
109 BR 694 and inconsistently reacting isolate AE24 (Set 6 *versus* Set 8). The data for AE24 from
110 Set 6 were ultimately omitted as disease reaction in Set 9 was consistent with that of Set 8. Each
111 isolate was tested in at least two independent experiments.

112 **Zoospore production.** Zoospores of the *A. euteiches* isolates were produced following a
113 standardized protocol (Sivachandra Kumar et al. 2020). Briefly, isolates were incubated for 4
114 days on autoclaved wheat leaf segments placed on corn meal-yeast extract-phosphate buffer
115 agar. Colonized wheat leaves were transferred into 100 mL distilled water in 250 mL flasks and
116 incubated at 100 rpm for 18 h at 24ºC to induce zoospore production. Isolates AE7 and DAOMC
117 BR 694 were slow growing, and production of zoospores through the standardized protocol was
118 challenging, so zoospores for these isolates were generated following the protocol by Moussart et
119 al. (2001) with modifications. Plugs from the advancing edge of the colony on 5% corn meal
120 agar mother plate were transferred to 100 ml of sucrose yeast peptone (1 g sucrose, 0.5 g yeast
121 extract, 1 g peptone, 500 mL of water). Cultures were grown in the dark for 4 days, after which

122 the mycelia were transferred into a new flask containing 100 mL of mineral salt solution (0.26 g
123 $CaCl_2 \cdot 2H_2O$, 0.07 g KCl, 0.49 g $MgSO_4 \cdot 7H_2O$ dissolved in 1 L of sterile distilled water)
124 (Windels 2000), soaked for 10 min, and transferred to 250 mL flasks containing 100 mL of
125 sterile distilled water. Flasks were incubated at 100 rpm for 18 h at 24ºC to induce zoospore
126 production. Zoospores of each isolate were adjusted to obtain the concentration of 1000
127 zoospores $mL^{-1}$.

128 **Plant inoculation and experimental design.** Pea plants were inoculated 10 days after
129 seeding by pipetting 5 mL of zoospore suspension at the base of each plant. The four replicate
130 pots were arranged in a randomized complete block design and were bagged and watered
131 carefully after inoculation to avoid splashing and cross-contamination. Non-inoculated control
132 plants of each genotype were kept separate from the inoculated plants and were used for
133 comparisons of root color during rating. Once uniform discoloration was confirmed in
134 susceptible genotypes (10-14 d after inoculation), plants were removed from pots, the
135 vermiculite was washed off and root discoloration of the root system of individual plants was
136 scored on a 0-5 scale where 0 indicates no root discoloration, 1 indicates 1-25% discoloration, 2
137 represents 26-50% discoloration, 3 indicates 51-75%, 4 indicates 76-100% discoloration, and 5
138 represents dead plants (Papavizas and Ayers 1974; Figure 1). No symptoms were observed on
139 non-inoculated control plants.

140 **Data analysis.** Individual root scores (0-5) were transformed into percentage data using 0%
141 for a score of 0, the mid-class values for scores of 1 to 4 (12%, 38%, 63%, 88%), and 100% for a
142 score of 5. The average of 4 root ratings per replicate pot was used for data analyses. All
143 analyses were conducted with SAS software Version 9.4 (SAS Institute Inc, Cary, NC). Data
144 from Repeats 1 and 2 of each set were pooled, and were analyzed with the mixed model

145 procedure of SAS. Experimental repeats, blocks nested within repeats, the interactions between
146 experimental repeats and pea genotypes, and the interaction between repeats and isolates were
147 considered random effects. Isolates, pea genotypes and their interaction were considered fixed
148 effects. Normality and homogeneity heterogeneous of variances of residuals were evaluated, and
149 heteroscedacity was modelled with the repeated statement in SAS when required. The successful
150 inoculation of each experiment was confirmed by comparing known reactions of susceptible
151 'CDC Meadow' with partially resistant 'PI 660736'after inoculation with isolate AE11, and
152 susceptible 'Baccara' with partially resistant MN 313 after inoculation with the pathotype III
153 standard Ae109 through simple linear contrast analyses. Variability among isolates was further
154 explored by comparing means based on Fisher's least significant differences.

155 Pathotypes were assigned based on the reaction of each isolates on the French pea
156 differentials Baccara, Capella, MN 313, 90-2131, 552 and PI 80693 as described by Wicker and
157 Rouxel (2001). Root rot discoloration of more than 25% (disease score > 1) was considered an
158 indication for a virulent reaction whereas root discolorations of 25% or less indicated an
159 avirulent reaction. Isolates of pathotype I are virulent on all differentials, whereas for other
160 pathotypes, isolates are avirulent on the following differentials: pathotype II on PI 80693;
161 pathotype III on MN 313; pathotype IV on MN 313 and 552; pathotype V on 90-2131 and PI
162 80693; pathotype VI on all differentials except Baccara; pathotype VII on 552 and PI 80693;
163 pathotype VIII on MN 313, 552 and PI 80693; pathotype IX on Capella, 552 and PI 80693;
164 pathotype X on 90-2131, 552 and PI 80693; and pathotype XI on 90-2131 and 552 (Wicker et al.
165 2003).

166

167 **Results**

168 *Aphanomyces euteiches* isolates were tested on the French pea differentials in nine sets, each
169 of which included isolates RB84 (pathotype I standard), Ae109 (pathotype III standard),
170 Canadian field isolate AE11, North Dakota field isolate Ae2.IND15 and USDA isolate Ae16-04
171 (Table 1). CDC Meadow (susceptible) had consistently more root discoloration than PI 660736
172 (partially resistant) upon inoculation with isolate AE11, and Baccara (susceptible) roots had
173 more disease symptoms than MN 313 after infection with Ae109 in all experiments ($P <$
174 $0.0001$)(Table 1). We also compared responses of isolates repeated in Set 8 with their reactions
175 in other sets and found a high level of consistency.

176 Root rot discoloration on pea genotypes ranged from 1 to 6% after inoculation with alfalfa
177 isolate DAOMC BR 694. For pea isolates the lowest root rot discoloration at 5% was observed
178 for US isolate Ae16-01 on PI 180693 and PI 660736, whereas the highest at 89% was recorded
179 for Albertan isolate AE32 on Capella (Table 2). On average, root discoloration was highest on
180 Baccara, Capella and CDC Meadow, intermediate on 90-2131 and 552, and lowest on MN 313,
181 PI 180693 and PI 660736. Even on the three most resistant pea genotypes, most isolates caused
182 more than 20% root discoloration, and for half of isolates root discoloration was 50% and higher.

183 Pea genotypes, isolates and their interaction had significant effects on root discoloration
184 (Table 1). Most isolates induced root discolorations similar to, or significantly lower than the
185 pathotype I standard isolate RB84 from France (Supplementary Table 1). A few isolates (AE7,
186 AE13, AE17, AE33) induced significantly more root discoloration compared to RB84 on PI
187 180693, and AE9 and AE13 caused more root rot than RB84 on MN 313. Isolates from the
188 Canadian provinces of Saskatchewan and Alberta could not be distinguished based on their
189 virulence on individual pea genotypes or based on their virulence profiles on all. Comparisons
190 with US isolates were not feasible because of unequal sample sizes.

191 Based on the French pathotyping system described by Wicker and Rouxel (2001), 29
192 Canadian field isolates with more than 25% root discoloration (disease score > 1) were classified
193 as pathotype I based on their virulent interaction with all differentials similar to the pathotype I
194 standard isolate RB84 (Table 2). US isolates Ae16-04, Ae2.IND15 and Ae3.IND15 also revealed
195 a virulence profile consistent with pathotype I. Canadian field isolates AE3, AE19 and AE33
196 were avirulent on MN 313, but virulent on all other pea differentials, similar to the pathotype III
197 standard isolate Ae109. US isolate Ae16-01 was avirulent on PI 180693 and PI 660736, and
198 virulent on all other pea differentials, thus had a virulence profile consistent with that of
199 pathotype II.

200

201 **Discussion**

202 *Aphanomyces euteiches* poses a serious threat to several pulse crop species including pea and
203 lentil due to the severity of root rot it causes and the persistence of oospores in infested field soils
204 in the absence of host plants. While advances have been made in the identification and utilization
205 of partial resistance in pea and lentil (e.g. Desgroux et al. 2016, Ma et al., 2020), an
206 understanding of the population dynamics and virulence mechanisms of *A. euteiches* is only
207 slowly emerging. The first step in this direction was the differentiation of pathotypes, based
208 largely on French isolates with the inclusion of only a few North-American isolates (Wicker and
209 Rouxel 2001). The majority of French isolates were identified as pathotype I whereas nine
210 North-American isolates belonged to pathotypes I, III, V, VI and X. Following the same
211 methodology here, we demonstrated that 29 out of 33 Canadian isolates and four out of five US
212 isolates belong to pathotype I, three Canadian isolates belong to pathotype III and one US isolate
213 belongs to pathotype II.

214 The original assumption had been that pathotype III, considered identical with a 'major
215 group' described by Malvick and Percich (1998), dominated the *A. euteiches* population in the
216 USA. Those isolates were mostly from Minnesota, in addition to a few from Wisconsin and
217 Oregon. However, Hamon et al. (2011) mentioned, but did not describe in detail, a prevalence of
218 pathotype I in field nurseries in Washington, Oregon and Minnesota, indicating that the US
219 population of *A. euteiches* may have experienced a major shift from pathotype III to pathotype I
220 in the decade following the late 1990s. If so, the evolutionary potential of *A. euteiches*
221 populations is high, which highlights the needs for regular monitoring to ensure that *A. euteiches*
222 resistance breeding programs screen with representative isolates to ensure that new varieties have
223 effective field resistance. In Canada, most peas are produced in the Prairie provinces and *A.*
224 *euteiches* was identified in Manitoba in 1997 (Mathur et al. 1998), in Saskatchewan in 2012
225 (Banniza et al. 2013) and in Alberta in 2013 (Chatterton et al. 2015). Isolates characterized in
226 this study were isolated between 2013 and 2016, so further monitoring for changes in virulence
227 patterns in the Canadian population may be prudent in the near future.

228 Pathotype I is more virulent than pathotype III (Wicker and Rouxel, 2001) and the prevalence
229 of pathotype I rather than pathotype III in North-America has implications for resistance
230 breeding as it affects the selection of the most effective sources of resistance. Developing and
231 testing near-isogenic lines with zero to three QTLs associated with aphanomyces resistance,
232 Lavaud et al (2015) could show that major QTL *Ae-Ps4.5* was more effective against pathotype
233 III standard isolate Ae109 than pathotype I standard isolate RB84. This was also evident here on
234 MN 313, the pea differential that carries major QTL *Ae-Ps4.5* (described as *Aph1* in Pilet-Nayel
235 et al. 2002). In contrast, QTL *Ae-Ps7.6* is considered the most effective resistance QTL for
236 pathotype I. Resistance breeding to Aphanomyces root rot in pea was initiated at the Crop

237 Development Centre (CDC) of the University of Saskatchewan in 2014 using a marker-assisted
238 backcrossing program that relied on two sources of resistance, breeding line 90-2079 (Kraft
239 1992) carrying, among others, major effect QTL *Ae-Ps4.5*, and recombinant inbred lines PI
240 660729, PI 660733 and PI 660736 derived from a cross with breeding line 90-2131 (McGee et al.
241 2012) carrying major effect QTL *Ae-Ps7.6*, also in addition to minor QTLs. PI 660736 was
242 included here to evaluate virulence of field isolates on this genotype in comparison to the
243 pathotype I standard RB84. Among the 33 field isolates, one isolate, AE25, from Alberta caused
244 significantly more disease (42%) on PI 660736 compared to RB84 (Supplementary Table S1).

245 Only a relatively small number of isolates were evaluated here, primarily because the
246 maintenance of *A. euteiches* cultures is labor-intensive requiring sub-culturing every 4 to 6
247 months without the option of cryo-preservation, and the occurrence of one highly virulent isolate
248 among 33 is concerning. Pea varieties with improved Aphanomyces root rot resistance derived
249 from PI 660736, once released, could select for such isolates in the pathogen population by
250 giving them a significant competitive advantage over less virulent isolates. Their increase in the
251 population could render newly developed, partially resistant varieties ineffective in a relatively
252 short period of time. This was also suggested by Quillévéré-Hamard et al. (2021) who tested 43
253 French pathotype I isolates of *A. euteiches* on near-isogenic lines (NILs) with one to three QTLs
254 and identified a group of isolates with higher virulence on those carrying QTL *Ae-Ps7.6*. One of
255 their resistant control lines, AeD990SW45-8-D, which is assumed to carry resistance alleles at
256 all seven main resistance QTLs, however, maintained high levels of resistance against all
257 isolates, indicating that the pyramiding of resistance QTLs will be essential for effective
258 Aphanomyces root rot resistance breeding. As in France, this strategy has also been implemented
259 at the CDC.

260 French and US isolates were included in the current study to allow for comparison of data
261 across country boundaries. This is particularly important when germplasms are shared for
262 resistance breeding, or when commercialization of varieties is considered in locations other than
263 the region where they were developed and screened. For example, US isolates Ae16-01 and
264 Ae16-04 have both been used for this purpose and were shown here to belong to pathotypes II
265 and I, respectively. On most differentials, US isolate Ae16-04 reacted similarly to AE11, a
266 Saskatchewan field isolate used routinely for phenotyping of pea lines at the CDC, but it was
267 significantly less virulent on PI 180693 and PI 660736. Isolate AE11 itself is moderately virulent
268 on these two pea genotypes compared to other field isolates tested here, as were the other two US
269 isolates from North Dakota, so resistant germplasms identified with Ae16-04 may require re-
270 evaluation with local isolates prior to utilization in resistance breeding in or for locations such as
271 Saskatchewan and North Dakota.

272 We also included one isolate from alfalfa, which proved to be avirulent on all pea accessions.
273 Alfalfa is a common rotational crop in North-America, and Aphanomyces root rot has been
274 reported as a serious disease in the USA (Vandemark et al. 2002, Malvick and Grau 2001).
275 Previous testing of *A. euteiches* isolated from different hosts indicated that isolates from alfalfa
276 were almost exclusively pathogenic on alfalfa, whereas isolates from pea caused root rot on
277 alfalfa, although to a more moderate degree than on pea (Malvick et al. 1998). This indicates that
278 only alfalfa varieties with resistance to pea isolates can be safely grown in rotations that also
279 include pea.

280 In conclusion, pathotyping of Canadian field isolates confirmed that the Canadian population
281 is dominated by the more virulent pathotype I. The identification of one isolate with significantly
282 higher virulence on one of the major sources of resistance, PI 660736, indicates that ongoing

283 monitoring of the pathogen population and the search for new sources of resistance will be

284 necessary for the development of durable resistance in pea.

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288

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368 **Table 1.** Effects of pea genotypes, isolates and their interaction on aphanomyces root rot
369 measured as root discoloration. *Aphanomyces euteiches* isolates were tested in nine sets, each of
370 which included Saskatchewan isolate AE11, Washington isolate Ae16-04, North Dakota isolate
371 Ae2.IND15, Wisconsin isolate Ae109 and French pathotype I standard isolate RB84. Simple
372 linear contrasts of CDC Meadow (susceptible) and PI 660736 (partially resistant) after
373 inoculation of AE11, and of Baccara (susceptible) and MN 313 (partially resistant) after
374 inoculation with Ae109 confirmed successful inoculations.

| Set | Isolates | Effect | Num DF | Den DF | F Value | Pr > F |
|---|---|---|---|---|---|---|
| 1 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84, Ae-16-01, Ae1, AE2, AE4 | pea genotypes | 7 | 7 | 21.57 | 0.0003 |
| | | isolates | 8 | 8 | 6.56 | 0.0077 |
| | | pea genotypes*isolates | 56 | 482 | 6.3 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 482 | 28.69 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 482 | 87.02 | <.0001 |
| 2 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84, AE20, AE23, AE25, AE34 | pea genotypes | 7 | 7 | 22.72 | 0.0003 |
| | | isolates | 8 | 8 | 16.39 | 0.0003 |
| | | pea genotypes*isolates | 56 | 482 | 7.58 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 482 | 23.9 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 482 | 69.11 | <.0001 |
| 3 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84,AE9, AE12, AE13, AE15, AE30 | pea genotypes | 7 | 7 | 4.89 | 0.0265 |
| | | isolates | 9 | 9 | 3.55 | 0.0363 |
| | | pea genotypes*isolates | 63 | 535 | 8.14 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 535 | 9.61 | 0.002 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 535 | 54.01 | <.0001 |
| 4 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84,Ae3.IND15, AE5, AE10, AE17, AE27 | pea genotypes | 7 | 7 | 82.83 | <.0001 |
| | | isolates | 9 | 9 | 11.79 | 0.0005 |
| | | pea genotypes*isolates | 63 | 537 | 8.92 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 537 | 54.22 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 537 | 125.31 | <.0001 |
| 5 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84,AE14, AE21, AE22, AE28, AE29 | pea genotypes | 7 | 7 | 13.97 | 0.0013 |
| | | isolates | 9 | 9 | 3.44 | 0.0398 |
| | | pea genotypes*isolates | 63 | 535 | 7.6 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 535 | 33.98 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 535 | 35.45 | <.0001 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 6 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84, AE6, AE24, AE18, AE31, AE32 | pea genotypes | 7 | 7 | 25.19 | 0.0002 |
| | | isolates | 9 | 9 | 48.18 | <.0001 |
| | | pea genotypes*isolates | 63 | 537 | 12.61 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 537 | 16.97 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 537 | 122.06 | <.0001 |
| 7 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84,AE3, AE7, AE19, AE26, AE33 | pea genotypes | 7 | 7 | 215.17 | <.0001 |
| | | isolates | 9 | 9 | 8.01 | 0.0024 |
| | | pea genotypes*isolates | 63 | 537 | 11.41 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 537 | 24.3 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 537 | 465.17 | <.0001 |
| 8 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84, Ae16-01, AE12, AE17, AE24, AE 25, AE29, AE33 | pea genotypes | 7 | 7 | 64.91 | <.0001 |
| | | isolates | 11 | 11 | 17.95 | <.0001 |
| | | pea genotypes*isolates | 77 | 647 | 10.84 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 647 | 58.22 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 647 | 163.6 | <.0001 |
| 9 | AE11, Ae16-04, Ae2.IND15, Ae109, RB84, DAOMC BR 694, AE24 | pea genotypes | 7 | 7 | 41.06 | <.0001 |
| | | isolates | 6 | 6 | 160.94 | <.0001 |
| | | pea genotypes*isolates | 42 | 372 | 25.97 | <.0001 |
| | | AE11 on CDC Meadow *vs* PI 660736 | 1 | 372 | 102.53 | <.0001 |
| | | Ae109 on Baccara *vs* MN 313 | 1 | 372 | 125.3 | <.0001 |

375

377 **Table 2.** Mean Aphanomyces root rot severity and standard error of the mean caused by isolates
378 of *Aphanomyces euteiches* from France, Saskatchewan (SK), Alberta (AB), Washington (WA),
379 and Wisconsin (WI) based on a 0 – 5 scale where 0 indicates no root discoloration, 1 indicates 1-
380 25% discoloration, 2 represents 26-50% discoloration, 3 indicates 51-75%, 4 indicates 76-100%
381 discoloration, and 5 represents dead plants (Papavizas and Ayers 1974). Gray shading reflects
382 scores of the rating scale. Means were calculated after transforming individual root scores (4
383 subsamples, 4 replications, 2 experiments) into percentage using 0% for a score of 0, the mid-
384 class values of 12%, 38%, 63%, 88% for scores of 1 to 4, and 100% for a score of 5. score > 1:
385 (virulent) and rating of ≤ 1 (avirulent) this is based on the mean of two experiments from each
386 sets. NA-Not aggressive. Root rot discoloration of more than 25% (disease score > 1) was
387 considered an indication for a virulent reaction whereas root discolorations of 25% or less
388 indicated an avirulent reaction, and pathotypes were assigned following the method by Wicker
389 and Rouxel (2001).

| Isolates | Origin | Pea differentials | | | | | | | | | | | | Internal controls | | | | Pathotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Baccara | | Capella | | 90-2131 | | 552 | | MN 313 | | PI180693 | | CDC Meadow | | PI 660736 | | |
| RB84 | France | 87 | ±5 | 87 | ±5 | 81 | ±6 | 79 | ±6 | 69 | ±5 | 49 | ±6 | 84 | ±5 | 60 | ±7 | I |
| AE13 | SK | 87 | ±8 | 86 | ±8 | 61 | ±9 | 66 | ±9 | 58 | ±8 | 73 | ±9 | 88 | ±8 | 73 | ±9 | I |
| AE22 | AB | 86 | ±5 | 88 | ±4 | 54 | ±5 | 61 | ±5 | 69 | ±6 | 29 | ±5 | 79 | ±5 | 26 | ±6 | I |
| AE6 | AB | 84 | ±5 | 86 | ±5 | 82 | ±5 | 74 | ±5 | 51 | ±5 | 69 | ±5 | 82 | ±5 | 71 | ±5 | I |
| AE28 | AB | 84 | ±6 | 65 | ±7 | 61 | ±6 | 72 | ±7 | 68 | ±8 | 48 | ±5 | 63 | ±6 | 59 | ±5 | I |
| AE34 | AB | 83 | ±5 | 86 | ±5 | 79 | ±7 | 72 | ±7 | 23 | ±6 | 62 | ±7 | 82 | ±6 | 55 | ±7 | I |
| AE24 | AB | 82 | ±5 | 87 | ±5 | 75 | ±5 | 59 | ±5 | 60 | ±5 | 32 | ±5 | 85 | ±5 | 26 | ±5 | I |
| AE2 | AB | 81 | ±8 | 86 | ±7 | 67 | ±8 | 54 | ±8 | 46 | ±8 | 52 | ±8 | 82 | ±8 | 33 | ±8 | I |
| AE12 | SK | 81 | ±6 | 86 | ±6 | 72 | ±7 | 50 | ±7 | 47 | ±7 | 50 | ±7 | 81 | ±6 | 52 | ±7 | I |
| AE14 | SK | 80 | ±6 | 75 | ±6 | 65 | ±6 | 70 | ±6 | 43 | ±5 | 63 | ±8 | 61 | ±8 | 73 | ±6 | I |
| AE4 | AB | 80 | ±8 | 83 | ±7 | 77 | ±8 | 59 | ±8 | 31 | ±8 | 69 | ±8 | 83 | ±8 | 31 | ±8 | I |
| AE32 | AB | 80 | ±5 | 89 | ±5 | 76 | ±5 | 77 | ±5 | 56 | ±5 | 45 | ±5 | 84 | ±5 | 64 | ±5 | I |
| AE11 | SK | 79 | ±6 | 80 | ±6 | 65 | ±6 | 54 | ±6 | 37 | ±6 | 49 | ±6 | 83 | ±5 | 43 | ±6 | I |
| AE23 | AB | 79 | ±5 | 81 | ±5 | 36 | ±7 | 31 | ±7 | 51 | ±6 | 38 | ±7 | 77 | ±6 | 16 | ±7 | I |
| AE7 | AB | 79 | ±5 | 84 | ±4 | 76 | ±6 | 76 | ±6 | 35 | ±5 | 73 | ±6 | 80 | ±5 | 68 | ±8 | I |
| AE17 | SK | 79 | ±4 | 77 | ±5 | 59 | ±5 | 56 | ±4 | 37 | ±5 | 54 | ±4 | 78 | ±4 | 56 | ±4 | I |
| AE9 | SK | 79 | ±8 | 77 | ±8 | 45 | ±9 | 56 | ±9 | 81 | ±8 | 29 | ±9 | 72 | ±8 | 38 | ±9 | I |
| AE25 | AB | 79 | ±5 | 86 | ±5 | 76 | ±6 | 52 | ±6 | 24 | ±6 | 52 | ±6 | 83 | ±5 | 72 | ±6 | I |
| AE26 | AB | 79 | ±5 | 80 | ±6 | 67 | ±6 | 53 | ±5 | 39 | ±8 | 28 | ±11 | 72 | ±6 | 29 | ±10 | I |
| AE20 | SK | 78 | ±5 | 87 | ±5 | 65 | ±7 | 45 | ±7 | 22 | ±6 | 66 | ±7 | 77 | ±6 | 51 | ±7 | I |
| AE27 | AB | 77 | ±4 | 74 | ±4 | 35 | ±4 | 31 | ±5 | 40 | ±5 | 32 | ±6 | 79 | ±5 | 65 | ±5 | I |
| Ae16-04 | WA | 76 | ±6 | 77 | ±6 | 57 | ±6 | 46 | ±6 | 45 | ±6 | 24 | ±6 | 72 | ±6 | 22 | ±6 | I |
| AE15 | SK | 76 | ±8 | 83 | ±8 | 73 | ±9 | 68 | ±9 | 55 | ±8 | 65 | ±9 | 86 | ±8 | 63 | ±9 | I |
| AE18 | SK | 75 | ±7 | 75 | ±7 | 74 | ±7 | 35 | ±7 | 20 | ±7 | 45 | ±7 | 66 | ±7 | 52 | ±7 | I |
| AE29 | AB | 74 | ±6 | 65 | ±5 | 47 | ±6 | 35 | ±5 | 42 | ±6 | 33 | ±5 | 68 | ±5 | 26 | ±5 | I |
| AE21 | AB | 73 | ±7 | 69 | ±6 | 43 | ±6 | 50 | ±7 | 54 | ±6 | 37 | ±4 | 68 | ±7 | 24 | ±7 | I |
| AE10 | SK | 73 | ±5 | 66 | ±4 | 60 | ±6 | 63 | ±5 | 27 | ±7 | 35 | ±4 | 61 | ±8 | 60 | ±6 | I |
| AE1 | SK | 73 | ±8 | 44 | ±7 | 39 | ±8 | 28 | ±8 | 34 | ±8 | 24 | ±8 | 60 | ±8 | 5 | ±8 | I |
| Ae2.IND15 | ND | 71 | ±6 | 72 | ±5 | 60 | ±6 | 41 | ±6 | 20 | ±6 | 42 | ±6 | 70 | ±6 | 46 | ±6 | I |
| AE31 | AB | 69 | ±6 | 60 | ±6 | 59 | ±6 | 47 | ±6 | 15 | ±6 | 43 | ±6 | 67 | ±6 | 54 | ±6 | I |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Ae3.IND15 | ND | 68 ±8 | 68 ±5 | 65 ±6 | 45 ±3 | 22 ±7 | 49 ±5 | 72 ±4 | 55 ±7 | I |
| AE5 | AB | 67 ±6 | 68 ±4 | 58 ±4 | 58 ±7 | 61 ±4 | 43 ±3 | 66 ±7 | 68 ±5 | I |
| AE30 | AB | 65 ±8 | 62 ±8 | 54 ±9 | 57 ±9 | 48 ±8 | 42 ±9 | 76 ±8 | 45 ±9 | I |
| Ae16-01 | WA | 77 ±6 | 65 ±6 | 46 ±7 | 32 ±7 | 33 ±6 | 5 ±6 | 64 ±6 | 5 ±6 | II |
| AE33 | SK | 84 ±4 | 81 ±5 | 70 ±6 | 40 ±9 | 10 ±7 | 47 ±6 | 78 ±5 | 61 ±7 | III |
| AE109 | WIS | 81 ±6 | 75 ±6 | 63 ±7 | 51 ±6 | 14 ±6 | 46 ±6 | 79 ±6 | 49 ±6 | III |
| AE19 | SK | 75 ±6 | 66 ±6 | 53 ±8 | 20 ±6 | 2 ±5 | 31 ±7 | 54 ±5 | 37 ±10 | III |
| AE3 | AB | 72 ±5 | 85 ±5 | 48 ±5 | 39 ±7 | 14 ±5 | 43 ±4 | 65 ±7 | 65 ±6 | III |
| DAOMC BR 694 | SK | 2 ±3 | 6 ±3 | 5 ±3 | 9 ±3 | 1 ±3 | 5 ±3 | 4 ±3 | 1 ±3 | unknown |

391 Fig. 1. Root discoloration of pea seedlings 10 to 14 days after inoculation with *Aphanomyces euteiches*
392 representing the six categories of the 0-5 scale developed by Papavizas and Ayers (1974) where
393 (from left to right) 0 = no root discoloration, 1 = 1-25% discoloration, 2 = 26-50% discoloration,
394 3 = 51-75%, 4 = 76-100% discoloration, and 5 = dead plants.

Fig. 1. Root discoloration of pea seedlings 10 to 14 days after inoculation with *Aphanomyces euteiches* representing the six categories of the 0-5 scale developed by Papavizas and Ayers (1974) where (from left to right) 0 = no root discoloration, 1 = 1-25% discoloration, 2 = 26-50% discoloration, 3 = 51-75%, 4 = 76-100% discoloration, and 5 = dead plants.

220x146mm (300 x 300 DPI)