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Evaluation of Claviceps purpurea isolates on wheat reveals complex virulence and host susceptibility relationships

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4 **Evaluation of *Claviceps purpurea* isolates on wheat reveals complex virulence and host**
5 **susceptibility relationships.**

6

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23

24 **Abstract:** Ergot of cereals, caused by *Claviceps purpurea*, results in yield loss and downgrading
25 of infested grain because of toxic alkaloids in the sclerotia. Resistant wheat genotypes are
26 known, but their effectiveness against different *C. purpurea* isolates over geographic regions has
27 not been studied. The objective was to examine the pathogenic variability among isolates of *C.*
28 *purpurea* on wheat lines differing in resistance. Under controlled environmental conditions,
29 fourty one single spore isolates of *C. purpurea* were developed from Canadian and United
30 Kingdom collections and inoculated onto a set of wheat genotypes composed of durum wheat
31 lines 'Melita', 'Kyle', and 9260B-173A, and hexaploid spring wheat lines 'Cadillac', 'Vista',
32 'Kenya Farmer', 'Lee' and HY630. Honeydew production and weight of sclerotia produced per
33 spike were assessed. There were significant differences among the wheat genotypes for overall
34 reactions to the pathogen isolates, and among pathogen isolates for geographic origin and host
35 origin. Twenty virulence phenotypes were identified using the honeydew production data, and 23
36 virulence phenotypes identified using the sclerotial weight data from the 41 isolates. The
37 existence of different virulence phenotypes indicates that variability in virulence exists in
38 populations of *C. purpurea*, and knowledge of virulence phenotypes is necessary to effectively
39 breed for resistant commercial lines.

40

41 *Keywords : Claviceps purpurea*, *Triticum aestivum*, *T. aestivum* var. *durum*, wheat, virulence,
42 genetic variability

43 **Introduction**

44 Ergot of cereals, caused by *Claviceps purpurea* (Fr.) Tul., is a common disease in holoarctic
45 regions of the world (Linder, 1948; Gaudet et al., 2000). It's most conspicuous symptom is the
46 formation of dark purple to black ergot bodies or sclerotia that are visible on infected cereal
47 spikes, or in the harvested grain. This disease does not often result in large yield losses, but can
48 have a large impact on cereal production because of the downgrading of grain infested with
49 sclerotia (Canadian Grain Commission, 2012). The ergot bodies contain various alkaloids toxic
50 to humans and livestock, which can cause severe health problems if ingested. Ergot is often
51 considered a disease of the Middle Ages, as it was common during that period and resulted in
52 many thousands of people dying from consuming ergot infested grain. As more became known
53 about this disease, such as how to control ergot and avoid contaminated grain, it became less of a
54 problem. The last major outbreak of ergotism affecting people was recorded in 1951 in France
55 (Gabbai et al., 1951).

56 Ergot was not reported at significant levels throughout most of the 1980s and 1990s on
57 the Canadian Prairies. In 1999, surveys conducted by the Grain Research Laboratory as part of
58 the Harvest Survey Program of the Canadian Grain Commission (Canadian Grain Commission
59 2013) revealed that 12% of all samples of Canadian Western Red Spring (CWRS) wheat and 4%
60 of Canada Western Amber Durum (CWAD) contained ergot bodies. In Saskatchewan, 17% of
61 the CWRS wheat delivered to the elevators was infested with ergot. Ergot was a problem in
62 Manitoba in 2005, when 10% of CWRS wheat samples were infested with ergot sclerotia
63 (Menzies & Turkington, 2014). This was followed by ergot infestation levels of CWRS wheat
64 samples in Alberta, Saskatchewan and Manitoba of 12%, 15% and 13%, respectively in 2008.
65 These levels declined in 2009, but in 2011, ergot infestation levels rose to 29%, 19% and 15% in

66 Alberta, Saskatchewan and Manitoba, respectively. The 2012 levels of ergot infestation of
67 CWRS wheat declined by 50% or more from 2011, but in Alberta and Saskatchewan, they were
68 still three to five times greater than what was reported in 2002 to 2007. The ergot infestation of
69 grain on the Canadian prairies has resulted in decreased returns to farmers as ergot infested grain
70 must have the ergot bodies removed, or become downgraded or rejected at point of sale, and
71 increased risk to livestock as some of the ergot infested grain is used as feed.

72 The increased incidence and severity of this pathogen in fields of wheat in western
73 Canada has led to greater interest in the biology and control of this disease. The identification of
74 possible sources of resistance to *C. purpurea* in wheat, and the development of resistant
75 commercial lines are being explored. Studies examining the presence of ergot resistance in lines
76 of wheat have been conducted by Platford & Bernier (1970, 1976), Puranik & Mathre (1971),
77 Darlington & Mathre (1976), Watkins & Littlefield (1976), Coley-Smith & Watkinson (1987),
78 Pageau et al. (1994), Menzies (2004), and Bayles et al. (2009). Puranik & Mathre (1971)
79 inoculated male sterile lines of barley and wheat and observed no differences among the lines in
80 terms of percentage of florets infected. However, the other studies observed differences in
81 resistance among lines of wheat, although the most resistant lines were often not suitable for
82 commercial production. These results are significant in that they reveal that differences in
83 susceptibility among genotypes of wheat to *C. purpurea* do occur, even if some of the
84 differences are not great.

85 The identification of lines of wheat resistant to *C. purpurea* is a good step towards the
86 development of resistant commercial lines, but often only a few isolates of the pathogen have
87 been used in identifying resistant genotypes. The effectiveness of these resistant sources against
88 all or the majority of the strains in a local area, or from different geographic regions, has not

89 been addressed. Variability in pathogenicity of different strains of *C. purpurea* has not been
90 extensively studied so little is known about the amount of pathogenic variability that a resistant
91 host line may encounter in nature. Campbell (1957) studied the host specificity of 423 isolates of
92 this fungus on 38 different host species and observed no host specificity among the isolates. He
93 reported that each grass species became infected and concluded that indigenous and forage
94 grasses constituted a reservoir of ergot inoculum for cereal grain hosts. Darlington et al. (1977)
95 inoculated 48 isolates of the pathogen onto eight lines of barley and four lines of wheat to
96 determine if specific biotypes or races could be identified. They did not find good evidence of
97 the occurrence of specific races, but did observe differences in the percent infection of florets
98 among the isolates. Cagaš & Macháč (2002) inoculated seven lines of Kentucky bluegrass with
99 three European isolates and one isolate from the U.S.A. and found significant differences in the
100 number and weight of sclerotia between the European isolates and the isolate from the U.S.A.
101 The isolate from the U.S.A. produced more and heavier sclerotia than the European isolates. The
102 differences in percent infection of florets of isolates of *C. purpurea* led Darlington et al. (1977)
103 to recommend that plant breeders use a mixture of isolates in screening germplasm for resistance
104 to ergot.

105 The objective of this research was to determine if pathogenic variability exists among
106 different isolates of *C. purpurea* collected from Canada and the United Kingdom on lines of
107 wheat differing in their levels of susceptibility/resistance.

108

109 **Materials and methods**

110 *Pathogen isolates collection and maintenance*

111 Thirty seven samples of *C. purpurea* collected from Manitoba, Saskatchewan and Alberta,
112 Canada, and four cultures from the United Kingdom were used (Table 1). Twenty six of the 37
113 samples from Canada were obtained as sclerotia in grain samples from Randy Clear (Canadian
114 Grain Commission, Winnipeg, Canada) as part of the CGC's 2000 harvest survey program. Four
115 sclerotia were collected from field plots at the University of Manitoba, Winnipeg, MB, in 1996,
116 and 7 sclerotia were collected during annual barley, oat and wheat disease surveys in 1996, 1997
117 and 2005. The sclerotia were used to produce mycelial isolates. Each sclerotium was surface
118 sterilized by immersion and shaking in 95% ETOH for 30 seconds followed by igniting the
119 sclerotia with a flame. The surface sterilized sclerotia were then cut into small pieces using a
120 sterile scalpel. Pieces of the sclerotia were placed onto acidified malt extract agar [6 g dextrose
121 (Fisher Scientific), 3 g malt extract, 3 g yeast extract and 12 g bacto agar (Becton, Dickenson and
122 Company) in 1 L H_2O, acidified with 450 µL of 2.5% lactic acid after autoclaving] in Petri
123 dishes and incubated for up to one week or until mycelium started to grow on the agar at room
124 temperature (21°C) under ambient light. Small plugs of agar with growing mycelium were
125 transferred to new malt agar Petri dishes, allowed to grow for a few days at room temperature,
126 and then maintained at 5°C. The four cultures originating in the United Kingdom were supplied
127 as mycelial isolates and were also maintained on malt agar at 5°C.

128 Single spore isolates of each culture were established by inoculating 125 mL of 2%
129 potato sucrose broth in a 500 mL Erlenmeyer flask with a small agar plug with growing
130 mycelium and incubating the culture for 14 days at room temperature under ambient light on a
131 rotary shaker at 150 rpm. The mature cultures were filtered through sterile cheesecloth, and the
132 spore suspensions diluted to 10^3 spores/mL with the aid of a haemocytometer. An aliquot of each
133 spore suspension was streaked on 2% water agar using an inoculation loop, and incubated at

134 room temperature under ambient light for 24 h. Single germinating spores were identified using a
135 dissecting microscope, and the single spores transferred into Petri dishes containing malt extract
136 agar. The single spore isolates were maintained on malt extract agar slants at 5°C.

137 The isolates were determined to be *C. purpurea* by comparing ITS region sequences with
138 those of Pažoutová et al. (2015) by K.A. Seifert and M. Liu (Ottawa Research and Development
139 Centre, AAFC-AAC, Ottawa, ON, Canada).

140 *Inoculum preparation*

141 Inoculum was produced following the procedure of Menzies (2004, stored for a maximum 2 mo
142 at 5°C until used (Platford & Bernier 1976). An aliquot of each sucrose spore suspension was
143 mixed in distilled water with one drop of Tween 20 (polyethylene glycol sorbitan monolaurate)
144 per L to make a final concentration of 10^4 spores per mL as determined using a haemocytometer,
145 prior to use as inoculum (Platford & Bernier 1970, 1976).

146 *Wheat differential set*

147 A differential set of spring wheat lines which varied in their response to infection by *C. purpurea*
148 was established based on the assessments by Platford & Bernier (1970, 1976) and Menzies
149 (2004). The differential set included the durum wheat (*Triticum aestivum* L. var. *durum*) lines
150 'Melita', 'Kyle', and 9260B-173A, and the hexaploid spring wheat (*T. aestivum* L.) lines
151 'Cadillac', 'Vista', 'Kenya Farmer' 'Lee' and HY630. 'Melita', 'Cadillac' and 'Vista' were
152 chosen as susceptible genotypes, and the others were selected because they had shown some
153 level of resistance in previous tests (Platford & Bernier 1970, 1976; Menzies 2004).

154 *Inoculation procedure*

155 Wheat lines were sown separately into 15 cm pots and grown in growth cabinets at a temperature
156 of 16ºC night/22ºC day with a 15 h/9 h light/dark period. Twelve seeds were sown per pot. Ten
157 to 12 spikes were allowed to develop per pot, and 9 spikes inoculated per pot. The spikes were
158 inoculated prior to anthesis (Platford & Bernier 1970), when the spikes had just completely
159 emerged from the boot. Twenty florets were inoculated per spike by selecting five healthy
160 spikelets on each side of the spike and injecting a spore suspension into the primary and
161 secondary florets on each spikelet using a syringe and hypodermic needle to fill the florets
162 (Campbell 1957; Platford & Bernier 1976). The spikelet below the group of inoculated spikelets
163 was removed as a marker for the inoculated spikelets (Menzies 2004). Three spikes per pot were
164 inoculated from four separate pots representing four replicates of three spikes each for each
165 fungal isolate on each wheat line.

166 Each spike was assessed for honey dew production approximately 14 days after
167 inoculation using a scale from 1 to 4 with 1 = none visible, 2 = honeydew confined within the
168 glumes, 3 = honeydew exuding from the florets in small drops, and 4 = large drops of honeydew
169 running down the spike (Menzies 2004). At maturity, each spike was harvested and threshed by
170 hand. Total weight in mg of sclerotia per spike was recorded.

171 *Statistical analysis*

172 Analysis of variance was performed on honeydew production and sclerotial weight data to
173 determine the relative contribution of isolate by differential interaction to the total variance
174 comprising of replicates, the simple effects and interaction. The model least square means were
175 calculated and used to tabulate the response of the differential wheat lines to the different
176 isolates.

177 Correspondence analysis (CA; Savary et al. 1994) was performed to determine the
178 association between the factors (differential lines, isolate sampling location and the isolate host
179 source) and the variables honeydew production and sclerotial weight because honeydew
180 production values were categorical and ordinal in nature. The median honeydew production and
181 mean total sclerotial weights were determined for each pot. Medians and means were converted
182 into classes. The classes of the honeydew production were determined by the class ranges of 1,
183 1.5-2.5, 3 – 3.5 and 4 for the classes H1, H2, H3 and H4 respectively. Mean sclerotial weights
184 were also converted to classes by assigning the data to classes Sc1, Sc2, Sc3 and Sc4 with the
185 end points of 0-35mg, >35-130 mg, >130-350 and >350. For both categorical variable H and Sc,
186 contingency tables were constructed with isolate sampling location (AB, SK, MB and UK),
187 isolate host source (Wht for hexaploid spring wheat, Dur for durum wheat, Tri for triticale, Bar
188 for barley, BG for blackgrass, Oat for oat and Rye for rye), and the wheat differential lines. Tests
189 of independence for each contingency table were performed. In addition, a test of independence
190 was calculated for the categorical variables for honeydew production and sclerotial weight.

191 Correspondence analysis is a multivariate statistical technique for creating an ordination
192 similar to principal component analysis (PCA). In contrast to PCA which is based on Euclidian
193 distances, CA uses the chi-square distances between rows. As in PCA, eigenvalues and
194 eigenvectors were determined, and coordinates determined by weighing the rows of the
195 contingency tables by the first and second eigenvectors. The calculations of the analysis were
196 performed using the package "ca" in R (R Core Team 2016). To determine the significance of
197 the first eigenvalue, a Monte Carlo simulation was performed on 1000 permutations of the
198 dependent variable and the eigenanalysis performed on each resulting contingency table with the
199 p-value of the test statistic compared to the given percentile of the simulations.

200

201 **Results**

202 The different isolates of *C. purpurea* caused a wide range of disease symptoms on the different
203 wheat genotypes. Honeydew production ratings ranged from 1 to 4 (Table 2), and the weight of
204 sclerotia produced on a spike ranged from 0 mg to more than 1000 mg (Table 3). A test of
205 independence for a contingency of honeydew production versus sclerotial weight per spike
206 indicated a significant positive association ($X^2 = 0.864$, $P<0.0001$).

207 ANOVA indicated that the effects of the wheat differential lines, pathogen isolates and
208 the wheat differential line by pathogen isolate interactions were significant ($P<0.0001$) for both
209 honeydew production and sclerotial weights. Differential lines, isolates and their interaction
210 accounted for 32%, 39% and 29% of the total treatment sum of squares for honeydew
211 production, respectively. For log transformed sclerotial weights, the sum of squares for
212 differential lines accounted for 40% of the total, 33% for the isolates, and 27% for the
213 interaction.

214 The CA involving wheat differential line, isolate sampling location and isolate host
215 source for honeydew production is presented in the plot in Figure 1. The graph is derived from
216 the eigenanalysis of these contingency tables. The first eigenvalue accounted for 73.2% of the
217 variation inherent in the contingency tables and the second eigenvalue accounted for 23.2% of
218 the variation. The H1 to H4 points on the honeydew biplot (Figure 1) are based on column scores
219 derived from the eigenanalysis of the counts of the contingency tables of the three factors. The
220 path from H1 to H4 indicates increasing honeydew production with means of 1.0, 2.0, 3.0 and
221 4.0 for H1, H2, H3 and H4, respectively. Contingency table analysis and Pearson's chi square

222 analysis indicated significant differences (P<0.0001) among the wheat differential lines, the
223 isolate sampling locations, and the isolate host sources. 9260B-173A had significantly lower
224 honeydew production than the other lines, followed by 'Kenya Farmer'. This was followed by a
225 group of lines consisting of HY630, 'Kyle', 'Lee', 'Vista' and 'Cadillac' which had lower
226 honeydew production than 'Melita'. Isolates from the UK had higher honeydew production than
227 those from Alberta, which were higher than those from Saskatchewan and Manitoba, which did
228 not differ. Isolates sourced from rye had greater honeydew production than the isolates sourced
229 from other hosts, with durum wheat isolates having higher honeydew production than the
230 remaining hosts isolates. The Blackgrass, triticale and oat isolates were combined for analysis
231 because of low isolate numbers, and this combination of isolates had higher honeydew
232 production than the wheat and barley sourced isolates. The wheat isolates had greater honeydew
233 production than the barley isolates. The first eigenvalue (0.1541) was significant (P<0.001) as
234 determined by Monte Carlo at the 99.9 percentile of 0.0131.

235 Correspondence analysis provides a graphical representation of the trends in a
236 contingency table, unlike analysis of variance. The eigenanalysis resulted in a number of axes
237 that account foremost of the inertia (variance) present in the contingency table. Only the first two
238 axes were used. Scores for each of the rows and columns are determined for each axis. These
239 scores are used in the biplot. For a given factor, the distance of a point from the origin gives a
240 measure of the strength of this point. In addition, a way to interpret the graphs is to draw lines
241 from the origin to a disease point (e.g. H1) and determine the angle from this line to that formed
242 by two independent sources [e.g. sources (wheat and barley)]. If the angle is acute, then the
243 profiles for the two variables are similar and becomes more dissimilar as the angle increases.
244 Alternatovely, the relative frequencies of the honeydew categories in the source sample may be

245 determined by drawing a line through the origin to the source sample (e.g. barley) and looking at
246 the point on this line by projecting a perpendicular to this line to the disease category points.
247 Frequencies of points on the line formed by the perpendicular projections from the point of a
248 disease category on the same side from the origin as the corresponding source point occur more
249 often than average profile with distance from the origin, and less often on the opposite side. For
250 example, using the latter method using rye and barley, the frequency of honeydew level H4
251 occurred more often than average using isolates derived from rye and H1 less often than average,
252 whereas H1 occurred more often than average using isolates from barley and less often than
253 average for H4.

254 The CA involving wheat differential line, isolate sampling location and isolate host
255 source for sclerotial weight is presented in the plot in Figure 2. The graph is derived from the
256 eigenanalysis of these contingency tables. The first eigenvalue accounted for 80.9% of the
257 variation inherent in the contingency tables and the second eigenvalue accounted for 16.3% of
258 the variation. The Sc1 to Sc4 points on the biplot (Figure 2) are based on column scores of the
259 contingency tables of the three factors. The biplot is derived from the eigenanalysis of these
260 contingency tables. The path from Sc1 to Sc4 indicates increasing sclerotial weight production
261 with means of 11.2 mg for Sc1, 80.1 for Sc2, 226.9 for Sc3 and 610.5 mg for Sc4. Contingency
262 table analysis and Pearson's chi square analysis indicated significant differences ($P<0.0001$)
263 among the different wheat lines, the isolate sampling locations, and the isolate host sources. The
264 wheat lines were all different from each other in terms of sclerotial weight, with 9260B-173A
265 having the lowest sclerotial weight and 'Cadillac' having the greatest weight based on the
266 distribution in the contingency table. The isolate sampling locations also differed in sclerotial
267 weight with the UK having the highest sclerotial weights and Manitoba having the lowest.

268 Isolates sourced from rye had greater sclerotial weight production than the isolates sourced from
269 other hosts, with durum wheat isolates having higher sclerotial weight production than the
270 remaining host isolates. The wheat isolates had greater sclerotial weight production than the
271 barley isolates. The first eigenvalue (0.1504) of this analysis was also significant ($P<0.001$) as
272 determined by Monte Carlo at the 99.9 percentile of 0.0126.

273 The phenotypic data for the 41 individual isolates of *C. purpurea* on the eight wheat lines
274 for honeydew production are presented in Table 2. The susceptible reaction for honeydew
275 production was set at a rating of 2.1 or greater because this is just above the H2 point in the CA.
276 The number of pathogen isolates that caused a susceptible or compatible reaction on the different
277 wheat lines was as follows: 8 isolates (20%) on 9260B-173A, 30 isolates (73%) on 'Kyle', 40
278 (98%) on 'Melita', 22 (54%) on HY630, 17 (41%) on 'Kenya Farmer', 28 (68%) on 'Lee', 30
279 (73%) on 'Vista', and 30 (73%) on 'Cadillac'. There were 20 different virulence phenotypes
280 identified as separated by resistant and susceptible reactions using the honeydew production
281 data. The most common virulence phenotype possessed by 12 pathogen isolates (isolates 44-2,
282 89-1, 90, 93-2, 95, 101 148, 155, 169-6, 179-1, 179-2 and UK 03-20-1) had virulence to all the
283 wheat lines except 9260B-173A (Table 2). The five isolates 7-1, 7-2, 125, 128 and 166-1 had
284 virulence to 'Kyle', 'Melita', 'Lee', 'Vista' and 'Cadillac'. The UK isolates UK 03-43-1, UK
285 04-02-1 and UK 04-97-1 and isolate 169-1 possessed virulence to all the wheat lines. There were
286 three groups of two isolates each, isolates 22 and 38, isolates 40 and 100 and isolates 172 and
287 192-1, which had the same virulence phenotype, The other 14 isolates in this study had unique
288 virulence phenotypes.

289 The phenotypic data for weight of sclerotia for the 41 individual isolates of *C. purpurea*
290 on the eight wheat lines is presented in Table 3. A wheat line was considered susceptible to an

291 individual isolate if 81 mg or more of sclerotia were produced per spike because this is just
292 above the Sc2 point in the CA. The number of isolates causing a susceptible or compatible
293 reaction on the different wheat lines were as follows: 5 isolates (12%) on 9260B-173A, 12
294 isolates (29%) on 'Kyle', 33 (80%) on 'Melita', 23 (56%) on HY630, 18 (44%) on 'Kenya
295 Farmer', 30 (73%) on 'Lee', 37 (90%) on 'Vista', and 32 (78%) on 'Cadillac'. There were 23
296 different virulence phenotypes identified using the sclerotial weight data. The most common
297 virulence phenotype possessed by 6 isolates (isolates 90, 93-2, 101, 148, 179-1 and 179-2) had
298 virulence to all the wheat lines except 9260B-173A (Table 3). There were 2 sets of five isolates
299 which possessed virulence to all the wheat lines except 9260B-173A and 'Kyle' (isolates 44-2,
300 89-1, 95, 155 and 169-6), or virulence to 'Melita', 'Lee', 'Vista' and 'Cadillac' (isolates 7-2, 29-
301 2, 33-2, 85 and 128). There were five groups of two isolates each, isolates 7-1 and 115, isolates
302 40 and 77, isolates 169-1 and UK 03-43-1, isolates 7-3 and 138, and isolates 125 and 166-1,
303 which had the same virulence phenotype. Fifteen of the 23 virulence phenotypes were
304 represented by only one isolate including isolate UK 04-02-1, which was the only isolate to have
305 virulence to all the wheat differential lines.

306 Nine of the 41 isolates had the same virulence phenotype for both honeydew production
307 and sclerotial weight (Tables 2 and 3). Isolates 90, 93-2, 101, 148, 179-1, and 179-2 were
308 virulent to all differential wheat lines except 9260B-173A, isolate 150 was virulent to 'Melita'
309 and 'Cadillac', isolate 192-4 was virulent to 'Melita', HY630, 'Lee' and 'Vista', and isolate
310 UK04-02-1 was virulent to all wheat differential lines.

311

312 **Discussion**

313 Variability among the isolates of *C. purpurea* was evident for honeydew production and
314 sclerotial weight per spike, which is in agreement with previous studies (Platford & Bernier,
315 1976. Pathogenic variability was observed on each wheat line used in these experiments,
316 indicating it was because of genetic variation among isolates. These two disease symptoms
317 represent two different aspects of the disease cycle of *C. purpurea*. The honeydew is important
318 in secondary spread of the pathogen (Gaudet *et al.*, 2000) and a reduction in honeydew would
319 likely lead to reduced spread. The weight of sclerotia per spike provides an indication of the
320 success of the pathogen in reproducing on the host. The weight of sclerotia per spike reflects the
321 success of the pathogen in diverting plant resources, and considers the role of larger, heavier
322 sclerotia in pathogen reproduction. Cooke & Mitchell (1966) and Rapilly (1968) observed
323 positive linear relationships between the size of sclerotia and the number of clavae produced by
324 the sclerotium. The number of clavae produced would influence the number of ascospores
325 produced and released from a sclerotium. Therefore, lighter sclerotia would result in fewer
326 ascospores being produced to start infections in the next season.

327 Isolates from the United Kingdom had higher honeydew ratings and greater sclerotial
328 weights per spike than isolates from Alberta, which in turn, were more severe than isolates from
329 Saskatchewan and Manitoba. This suggests that the U.K. isolates of *C. purpurea* have greater
330 potential to cause disease than the western Canadian isolates and the Alberta isolates had a
331 greater potential than the Saskatchewan and Manitoba isolates. These results suggest the
332 severity of disease caused by isolates of *C. purpurea* may vary with geographic origin of the
333 isolate.

334 *Claviceps purpurea* isolates originating from different hosts varied in the severity of
335 disease they caused on the 8 wheat differential lines. Isolates from rye had significantly greater

336 honeydew production and weight of sclerotia per spike than the other isolates, while those from
337 barley had significantly lower honeydew production and weight of sclerotia per spike. These
338 results suggest that the amount of disease caused by an isolate of *C. purpurea* may be influenced
339 by the host of origin. The hosts of origin in this study are known to vary in their susceptibility to
340 *C. purpurea*. Platford & Bernier (1976) listed the susceptibility of the different crop species in
341 the following order of decreasing susceptibility: rye, triticale, wheat, barley and oats. Possibly,
342 host species which are more susceptible or prone to infection allow the more rapid spread of
343 more virulent isolates, i.e. those with greater honeydew production, as compared to less virulent
344 isolates of *C. purpurea*, once crop plants become infected. This more rapid spread of infection by
345 the more virulent isolates would result in their sclerotia becoming predominant in infested grain
346 samples from the field. The positive association between honeydew production and sclerotial
347 weight per spike would lead to greater weights of sclerotia being produced by the more virulent
348 isolates on these more susceptible host crops. The nature of our sampling technique, in which
349 one sclerotium is sampled from a grain sample, likely results in the dominant or common
350 genotypes, or in our case the more virulent genotypes, being collected more often than the less
351 common or less virulent isolates. Popovic & Menzies (2006), working with *Ustilago tritici*,
352 suggested that if one genotype per field is sampled, a dominant genotype would be more
353 commonly collected than other genotypes. Other hosts which are not as susceptible or prone to
354 infection by *C. purpurea* as rye may not be so conducive to the rapid secondary spread of more
355 virulent isolates, and the sclerotia collected in infested grain of these crops may be more
356 reflective of the pathogen isolates that originally invaded the field from sporulating sclerotia or
357 neighbouring grasses.

358 The development of a differential host series for a pathogen is dependent on identifying
359 lines with different responses to the pathogen. The inoculation of the eight wheat lines with 41
360 isolates confirmed that the lines do differ in their reactions to *C. purpurea* as previously reported
361 by Menzies (2004) and Platford & Bernier (1970, 1976). There are other wheat genotypes that
362 have been reported to show resistance to *C. purpurea*, which could be considered for inclusion in
363 a more comprehensive differential set. For instance, the winter wheat line 'Robigus' has recently
364 been shown to have lower average sclerotium weight and sclerotial weight per ear than other winter
365 wheat lines (Gordon et al. 2015). It is also important to note that the resistance in our differential
366 wheat lines has not been characterized, and some of these lines may possess more than one gene
367 for resistance, or some of the same genes for resistance. The wheat lines used in this study lack the
368 sophistication and refinement of a near-isogenic set of lines, but nevertheless, provide useful
369 information.

370 The significant and large differential wheat line by pathogen isolate interaction suggests a
371 vertical resistance interaction (i.e. a gene for gene mechanism) is operating in this pathosystem
372 (Kranz 2003) and honeydew production and/or sclerotial weight may be used to determine
373 virulence phenotypes of *C. purpurea*. The division of pathogen isolate by host genotype
374 interactions into avirulent (resistant) or virulent (susceptible) reactions was set at a honeydew
375 production rating of 2.1 or higher and a sclerotial weight per spike of 81 mg or higher being
376 virulent reactions. The honeydew production data separated the 41 isolates into 20 different
377 virulence phenotypes and the sclerotial weight data separated the isolates into 23 virulence
378 phenotypes. The most common virulence phenotype for both honeydew production and sclerotial
379 weight per spike possessed virulence to all wheat differential lines except 9260B-173A. Four
380 isolates possessed virulence to all the wheat differential lines for honeydew production and one

381 isolate for sclerotial weight per spike. Some of the virulence phenotypes displayed a very good
382 demarcation between virulence and avirulence such as isolate 90 with a honeydew production
383 rating of 1 on 9260B-173A and 3.7 on 'Cadillac' and sclerotial weight per spike of 9 mg on
384 9260B-173A and 1179 mg on 'Cadillac'. Other isolates did not show such a stark difference
385 between virulence and avirulence, with some honeydew production and sclerotial weight values
386 being close to the demarcation points. For instance, isolates 102 and 172 had a number of
387 honeydew production and sclerotial weight per spike values which were close to being either
388 avirulent or virulent. Our knowledge of the genetics of the wheat – *C. purpurea* pathosystem is
389 very limited and the interaction of pathogen isolates with differential host lines yielding reactions
390 that are close to the avirulent/avirulent demarcation point is not uncommon in other
391 pathosystems such as the oat-crown rust pathosystem (Menzies, unpublished data). In the wheat
392 (*T. aestivum*) – leaf rust [*Puccinia recondita* Roberge ex Dezmaz. f. sp. *tritici* (Eriks. & Henn.)]
393 pathosystem, Kolmer & Dyck (1994) observed that the expression of resistance and avirulence
394 genes was highly dependent on the genotypes of the host lines and rust isolates involved in the
395 interactions. These two rust pathosystems have the luxury of near-isogenic host lines to study
396 these reactions. As previously mentioned, the genetics of the eight wheat differential lines used
397 in this study are not well known and it is possible that the genetics of resistance to *C. purpurea*
398 of these lines may be complex. Platford et al. (1977) studied the genetics of resistance to *C.*
399 *purpurea* in 'Kenya Farmer' and determined that a gene or genes for resistance which influence
400 honeydew production and frequency and size of sclerotia was located on chromosome 6b, but
401 genes on other chromosomes also influenced sclerotium size and honeydew production. Gordon
402 et al. (2015) studied the resistance to *C. purpurea* in the winter wheat line 'Robigus' and
403 identified interacting QTL that influence the size and weight of sclerotia, but the majority of the

404 genetic variation affecting sclerotium development was not detectable. They considered this lack
405 of genetic detection was the result of the presence of many small genetic differences affecting
406 sclerotium development.

407 The presence of different virulence phenotypes or races of *C. purpurea* has not been
408 previously reported. Our experiments were successful in identifying virulence phenotypes of *C.*
409 *purpurea* likely because of the use of wheat differential lines with known differences in reactions
410 to the pathogen.

411 In conclusion, our results show a wide range of genetic variability among isolates of *C.*
412 *purpurea* inoculated to wheat genotypes that were known to differ in their response to the
413 pathogen. Different virulence phenotypes were identified, and isolates varied in their virulence
414 depending on host of origin and geography. This information is important in development of
415 resistant germplasm to this pathogen. Knowledge of the virulence phenotypes is necessary to
416 effectively breed for resistance to this pathogen, and wheat genotypes with resistance to this
417 pathogen in one geographic area may not be resistant to virulence phenotypes of the pathogen
418 from other geographical areas. Nevertheless, this work confirms that resistant genotypes of
419 wheat do exist. The most effective resistance source in this study was 9260B-173A which
420 confirms the finding of Menzies (2004). The marked reduction in honeydew production and
421 weight of sclerotia of the *C. purpurea* isolates on this line warrants further genetic investigation.

422

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429

430 **References**

431 Bayles R, Fletcher M, Gladders P, Hall R, Hollins W, Kenyon D, Thomas J, 2009. *Towards a*
432 *sustainable whole-farm approach to the control of ergot.* Project Report No. 456 to the
433 Home Grown Cereals Authority, Stoneleigh Park, Warwickshire, U.K. 170 pages.

434 Cagaš B, Macháč R. 2002. Different pathogenicity of ergot isolates [*Claviceps purpurea* (Fr.)
435 Tul.] on Kentucky bluegrass (*Poa pratensis* L.). Plant Prot Sci. 38: 18-22.

436 Campbell WP, 1957. Studies on ergot infection in gramineous hosts. Can J Bot. 35: 315-320.

437 Canadian Grain Commission. 2012. The Official Grain Grading Guide.
438 http://grainscanada.gc.ca/oggg-gocg/04/oggg-gocg-4f-eng.htm (Accessed 08/05/2013).

439 Canadian Grain Commission. 28 May 2013. Harvest survey of Canadian grain quality [online].
440 Available from http://www.grainscanada.gc.ca/quality-qualite/hsp-per/hs-er-eng.htm
441 [accessed 30/09/2013].

442 Coley-Smith JR, Watkinson L. 1987. Susceptibility of durum wheat cultivars to ergot disease.
443 Ann Appl Biol. 110: 162-163.

444 Cooke RC, Mitchell DT. 1966. Sclerotium size and germination in *Claviceps purpurea*. Trans
445 Brit Mycol Soc. 49: 95-100.

446 Darlington LC, Mathre DE. 1976. Resistance of male sterile wheat to ergot as related to
447 pollination and host genotype. Crop Sci. 16: 728-730.

448 Darlington LC, Mathre DE, Johnston RH. 1977. Variation in pathogenicity between isolates of
449 *Claviceps purpurea*. Can J Plant Sci. 57: 729-733.

450 Gabbai, Lisbonne, Pourquier. 1951. Ergot poisoning at Pont St. Esprit. British Medical Journal 2
451 (4732): 650-651.

452 Gaudet D, Menzies J, Burnett P. 2000. Smuts, Bunts, and Ergot. Encyclopedia of Microbiology.
453 4. 2nd ed. San Diego, CA,USA: Academic Press; p. 297-315.

454 Gordon A, Basler R, Bonsept-Basler P, Fanston V, Harinarayan L, Grant PK, Birchmore R,
455 Bayles R, Boyd L, O'Sullivan DM. 2015. The identification of QTL controlling ergot
456 size in hexaploid wheat implicates a role for the *Rht* dwarfing alleles. Theor Appl Genet.
457 128: 2447-2460.

458 Kranz J. 2003. Comparative Epidemiology of Plant Diseases. Springer-Verlag, Berlin. 206 pp.

459 Linder DH. 1948. Fungi, Botany of the Canadian Eastern Artic. Part II. Thallophyta and
460 Bryophyta. Bulletin of the National Museum of Canada 97: 234-297.

461 Menzies JG. 2004. The reactions of Canadian spring wheat genotypes to inoculation with
462 *Claviceps purpurea*, the causal agent of ergot. Can J Plant Sci. 84: 625-629.

463 Menzies JG, Turkington TK. 2014. An overview of the ergot issue in western Canada:
464 Challenges and Solutions. Can J Plant Path. 37: 40-51.

465 Pageau D, Collin J, Wauthy J-M. 1994. Une note sure la résistance á l'ergot chez le blé tendre, le
466 blé dur et le triticale. Phytoprotection 75 : 45-49.

467 Pažoutová S, Pešicová K, Chudíčková M, Šrůtka P, Kolařík M. 2015. Delimitation of cryptic
468 species inside *Claviceps purpurea*. Fungal Biol. 119: 7-26.

469 Platford RG, Bernier CC. 1970. Resistance to *Claviceps purpurea* in spring and durum wheats.
470 Nature 226: 770.

471 Platford RG, Bernier CC. 1976 Reaction of cultivated cereals to *Claviceps purpurea*. Can J Plant
472 Sci. 56: 51-58.

473 Platford RG, Bernier CC, Evans LE. 1977. Chromosome location of genes conditioning
474 resistance to *Claviceps purpurea* in spring and durum wheat. Can J Genet Cytol. 19: 679-
475 682.

476 Popovic Z, Menzies JG. 2006. Intensive and extensive sampling techniques used to measure
477 genetic diversity of *Ustilago tritici*, using virulence and DNA polymorphism. Can J Plant
478 Path. 28: 197-207.

479 Puranik SB, Mathre DE. 1971. Biology and control of ergot on male sterile wheat and barley.
480 Phytopathology 61: 1075-1080.

481 R Core Team. 2016. R: A Language and Environment for Statistical Computing. R Foundation
482 for Statistical Computing. Vienna, Austria. http://www.R-project.org.

483 Rapilly F. 1968. Études sur l'ergot du blé : *Claviceps purpurea* (Fr.) Tul. Annales des épiphyties
484 19: 305-329.

485 Savary S, Madden LV, Zadoks JC, Klein-Gebbinck HW. 1994. Use of categorical information
486 and correspondence analysis in plant disease epidemiology. Adv Bot Res. 21: 213-240.

487 Watkins JE, Littlefield LJ. 1976. Relationship of anthesis in Waldron wheat to infection by
488 *Claviceps purpurea*. Trans Brit Mycol Soc. 66: 362-363.

489

490 Table 1. Single spore isolates of *Claviceps purpurea* (Fr.) Tul. assessed for pathogenic variation
491 on lines of hexaploid wheat (*Triticum aestivum* L.) and durum wheat (*T. turgidum* L. var.
492 *durum*).

Isolate	Year of isolation	Location of collection	Host source
7-1[a] (250621[b])	1996	MB07[c]	Triticale (X Triticosecale)
7-2 (250622)	1996	MB07	Triticale
7-3 (250623)	1996	MB07	Triticale
22 (250627)	1996	MB07	Barley
24 (250628)	1996	AB05	Barley
29-2 (250632)	1996	SK09	Barley
33-2 (250636)	2000	AB05	Barley
38	1997	MB07	Barley
40 (250638)	1997	SK08	Barley
44-2 (250641)	1997	MB09	Barley
77 (250646)	2000	SK03	Wheat
85 (250648)	2000	AB04	Wheat
89-1 (250649)	2000	SK01	Rye
90 (250650)	2000	SK08	Rye
93-2	2000	MB01	Rye
95 (250652)	2000	MB01	Rye
97	2000	AB07	Wheat

98 (250653)	2000	MB01	Durum Wheat
100	2000	MB01	Wheat
101	2000	MB01	Rye
102	2000	MB03	Wheat
115 (250657)	2000	MB07	Winter Wheat
125 (250658)	2000	SK01	Durum Wheat
128 (250659)	2000	SK02	Durum Wheat
138	2000	SK05	Wheat
148	2000	SK07	Rye (*Secale cereale* L.)
150 (250661)	2000	SK08	Durum Wheat
155	2000	SK08	Durum Wheat
166-1	2000	AB01	Durum Wheat
169-1 (250664)	2000	AB02	Durum Wheat
169-6 (250668)	2000	AB02	Durum Wheat
172	2000	AB02	Wheat
179-1 (250670)	2000	AB04	Rye
179-2 (250671)	2000	AB04	Rye
179-3 (250675)	2000	AB04	Rye
192-1 (250678)	2005	MB07	Oats
192-4 (250679)	2005	MB07	Oats
UK 03-20-1	2003	Cambridge U.K.	Wheat
UK 03-43-1 (250717)	2003	Cambridge, U.K.	Winter Wheat

UK 04-02-1	2004	Cambridge, U.K.	Blackgrass (*Alopecurus myosuroides* Huds.)
UK 04-97-1	2004	Cambridge, U.K.	Blackgrass

493 [a] More than one sclerotium could be obtained from one site. The first number represents the
494 collection number and the number following the hyphen represents the number of the sclerotium
495 sampled from that site in the instance of hyphenated isolate designations.

496 [b] Numbers in brackets are the DAOMC number for those isolates deposited at the Canadian
497 National Mycological Herbarium, Ottawa, ON, Canada.

498 [c] Unless otherwise indicated, the isolates originated in western Canada. Location of collection for
499 Canadian isolates indicates the provincial crop districts in Manitoba (MB), Saskatchewan (SK),
500 and Alberta (AB).

501

502 Table 2. The least squares means for the honeydew ratings[a] obtained from the inoculation of 41

503 single spore strains of *Claviceps purpurea* (Fr.) Tul. onto three lines of durum wheat (*T.*

504 *turgidum* L. var. *durum*) and five lines of hexaploid wheat (*Triticum aestivum* L.).

Fungal Strain	Durum Wheat line			Hexaploid Wheat Line				
	9260B-173A	'Kyle'	'Melita'	HY630	'Kenya Farmer'	'Lee'	'Vista'	'Cadillac'
7-1	1	3.2	3.7	1.9	1.4	2.7	2.7	2.9
7-2	1	2.7	3.9	1.6	1.2	3.4	2.9	2.4
7-3	2.2	1.9	3.7	2	1.7	2.9	1.9	2.9
22	1	1.4	2.7	1	1	1.4	1.4	1.7
24	1.7	2.2	2.9	1.3	2.4	1.2	1.7	2.9
29-2	1.4	2.4	3.4	1	1.2	2.4	1.7	1.9
33-2	1	1.4	3.4	1.9	1.4	2.7	1.4	2.7
38	1	1	3.2	1	1	1.4	1.9	1
40	1	2.7	2.7	1.9	1	1.4	2.7	1.9
44-2	1	2.2	3.4	3	2.2	2.9	3.4	2.9
77	1	2.5	3.9	1	1	1.5	2.6	2.1
85	1.9	1.9	3.9	2.3	1.7	2.9	2.7	2.9
89-1	1.7	2.7	3.9	3.4	3.2	3.7	3.7	3.9
90	1	2.9	3.4	3.4	2.9	2.9	3.4	3.7
93-2	1	2.9	3.4	3.7	3.4	3.7	3.7	3.9
95	1	2.9	3.7	3.7	2.9	3.7	3.9	3.7

97	1	1.2	1.7	2.5	1	1.2	1.4	1
98	2.4	1.9	2.7	1.5	1	1.7	1.9	2.2
100	1	2.7	2.9	1.5	1	1.2	2.2	1.2
101	1	3.4	3.7	3.4	3.2	3.9	3.9	3.9
102	1	2.2	3.2	2.3	1	2.7	2.7	2.9
115	1	1.2	2.7	1.5	1	1.7	2.7	2.7
125	1	2.4	3.9	2	1.9	3.2	2.9	2.9
128	1	2.4	3.2	1	1.9	2.4	2.7	2.9
138	2.9	1.4	2.2	2.9	1	2.2	2.4	1.9
148	1	3.4	3.4	3.4	3.2	3.4	2.7	3.7
150	1	1.9	3.7	1	1.2	1.4	1	2.7
155	1.2	3.4	3.9	3	2.7	2.7	3.9	3.9
166-1	1	3.4	3.9	1.9	1.7	3.2	3.2	2.9
169-1	3.2	3.2	3.9	3.9	3.2	3.4	3.9	3.9
169-6	1	3.4	3.4	3.7	3.2	3.2	3.4	3.2
172	1	2.4	2.9	2	1	1	1.2	1.2
179-1	1	2.2	3.4	3	2.2	2.9	3.4	2.9
179-2	1	3.9	3.9	3.9	3.4	3.7	3.9	3.9
179-3	2.4	3.5	3.9	2.7	1	1.9	2.2	1.9
192-1	1.9	2.9	3.4	1.9	1	1.2	1.7	1
192-4	1	1.2	3.2	2.9	1.4	2.4	2.9	1.9
UK 03-20-1	2	2.9	3.9	3.4	2.7	2.9	3.7	3.4

UK 03-43-1	3.6	2.6	3.9	2.8	2.9	3	3.6	3
UK 04-02-1	3.5	3	3.8	3.2	2.8	3.4	4	3.6
UK 04-97-1	3.2	2.4	3.7	2.2	2.4	3.2	2.7	3.7

505 [a] Honeydew Rating Scale was 1 = none visible, 2 = honeydew confined within the glumes, 3 =
506 honeydew exuding from the florets in small drops, 4 = large drops of honeydew running down
507 the spikes (Menzies, 2004). An isolate of *C. purpurea* was considered virulent on a wheat line if
508 it caused a honeydew production rating of 2.1 or greater.

509

510

511 Table 3. The least squares means of the weight (mg) of sclerotia per spike obtained from the

512 inoculation of 41 single spore strains of *Claviceps purpurea* (Fr.) Tul. onto three lines of

513 durum wheat (*T. turgidum* L. var. *durum*) and five lines of hexaploid wheat (*Triticum*

514 *aestivum* L.).

Fungal Strain	Durum Wheat line			Hexaploid Wheat Line				
	9260B-173A	'Kyle'	'Melita'	HY630	'Kenya Farmer'	'Lee'	'Vista'	'Cadillac'
7-1	3[a]	80	78	8	30	93	244	188
7-2	3	10	139	21	34	132	271	97
7-3	48	14	288	104	31	152	192	260
22	0	2	41	13	0	23	95	102
24	22	16	66	9	209	55	155	132
29-2	3	35	155	12	36	186	144	128
33-2	6	6	93	15	46	281	130	99
38	0	4	33	6	4	23	184	19
40	2	10	169	54	33	74	190	75
44-2	71	43	319	93	169	291	536	306
77	2	44	169	2	8	17	125	47
85	10	18	84	60	44	424	489	357
89-1	5	27	185	297	604	368	514	714
90	9	124	337	412	691	538	734	1179

93-2	2	102	260	472	747	444	682	1150
95	18	39	641	218	253	257	579	870
97	17	4	54	94	19	40	82	50
98	20	17	57	92	134	295	246	75
100	18	41	158	28	6	21	191	83
101	40	98	630	323	278	505	543	619
102	0	7	61	29	7	127	57	183
115	0	10	47	19	43	145	149	250
125	23	174	674	25	81	217	380	497
128	3	6	96	24	46	225	231	259
138	80	6	141	146	16	324	109	190
148	2	169	550	371	681	543	335	978
150	0	17	101	0	14	52	75	101
155	2	48	304	184	156	345	397	557
166-1	9	424	777	9	224	371	400	524
169-1	92	77	997	440	399	426	842	911
169-6	16	33	272	290	617	168	580	454
172	4	40	90	113	28	50	130	62
179-1	17	125	638	481	449	420	765	1201
179-2	13	400	925	257	469	535	704	853
179-3	99	206	514	126	4	49	55	204
192-1	59	133	195	24	2	41	83	31
192-4	27	25	287	139	25	82	164	49

UK 03-20-1	59	103	320	118	49	91	420	13
UK 03-43-1	212	31	356	238	272	188	282	250
UK 04-02-1	195	142	278	115	133	130	457	531
UK 04-97-1	129	31	377	107	57	143	64	316

515 [a]An isolate of *C. purpurea* was considered virulent on a wheat line if it caused a sclerotial weight

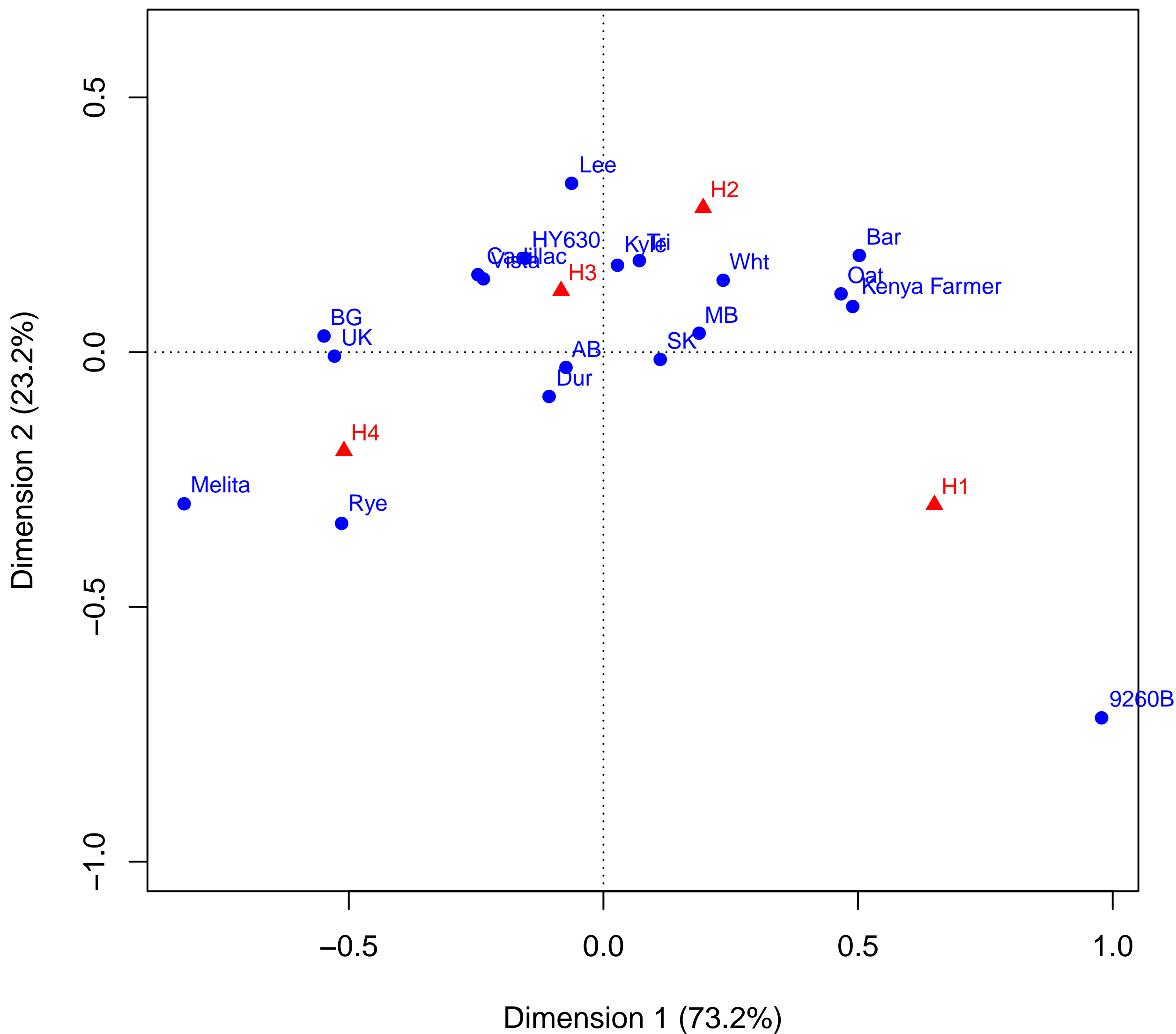
516 per spike of 81 mg or greater.

517

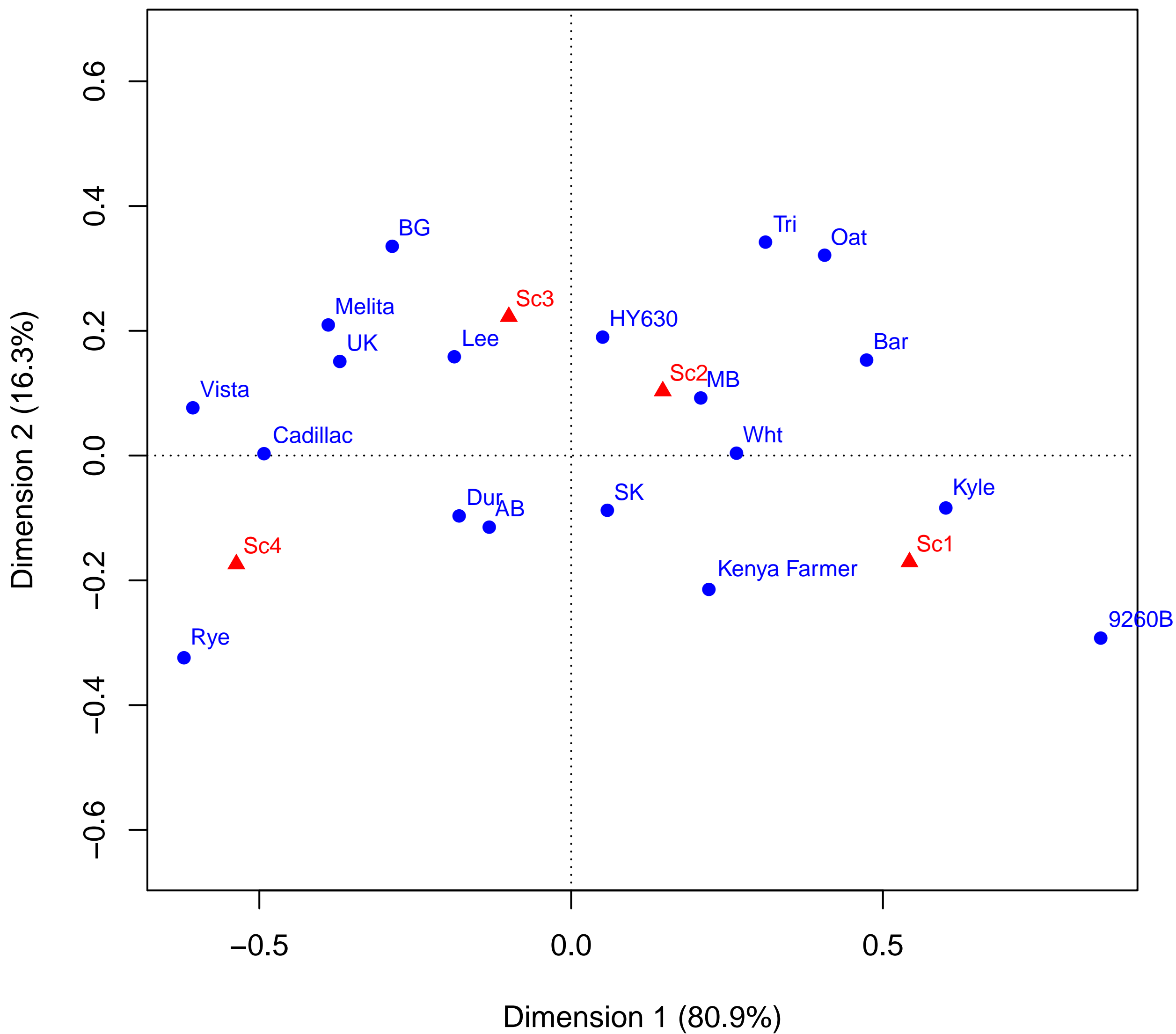
518

519 Figure 1. Correspondence analysis biplot of the contingency table involving wheat differential
520 line, isolate sampling location and isolate host source for honeydew production (H1 to
521 H4). The graph is derived from the eigenanalysis of these contingency tables. The first
522 eigenvector accounted for 73.2% of the variation in the contingency tables, and the
523 second eigenvector accounted for 23.2% of the variation. The path from H1 to H4
524 indicates increasing honeydew production with means of 1.0, 2.0, 3.0 and 4.0 for H1, H2,
525 H3 and H4, respectively. Wheat differential lines; 9260B-173A (9260B), 'Kyle',
526 'Melita', HY630, 'Kenya Farmer', 'Lee', 'Vista', 'Cadillac'. Isolate sampling location;
527 United Kingdom (UK), Alberta (AB), Saskatchewan (SK), Manitoba (MB). Isolate host
528 source; rye (Rye), blackgrass (BG), triticale (Tri), durum wheat (Dur), hexaploid wheat
529 (Wht), oats (Oat), barley (Bar).

530 Figure 2. Correspondence analysis biplot of the contingency table involving wheat differential
531 line, isolate sampling location and isolate host source for sclerotial weight per spike (Sc1
532 to Sc4). The graph is derived from the eigenanalysis of these contingency tables. The first
533 eigenvector accounted for 80.9% of the variation in the contingency tables, and the
534 second eigenvector accounted for 16.3% of the variation. The path from Sc1 to Sc4
535 indicates increasing sclerotial weight with means of 11.2, 80.1, 226.9 and 610.5 for Sc1,
536 Sc2, Sc3 and Sc4, respectively. Wheat differential lines; 9260B-173A (9260B), 'Kyle',
537 'Melita', HY630, 'Kenya Farmer', 'Lee', 'Vista', 'Cadillac'. Isolate sampling location;
538 United Kingdom (UK), Alberta (AB), Saskatchewan (SK), Manitoba (MB). Isolate host
539 source; rye (Rye), blackgrass (BG), triticale (Tri), durum wheat (Dur), hexaploid wheat
540 (Wht), oats (Oat), barley (Bar).



Lee
H2
HY630
Kyle
Tri
Bar
Cadillac
Vista
Wht
H3
Oat
Kenya Farmer
BG
UK
MB
AB
SK
Dur
H4
Melita
Rye
H1
9260B
0.5
0.0
−0.5
−1.0
−0.5
0.0
0.5
1.0
Dimension 1 (73.2%)
Dimension 2 (23.2%)


Dimension 2 (16.3%)
Dimension 1 (80.9%)
0.6
0.4
0.2
0.0
−0.2
−0.4
−0.6
−0.5
0.0
0.5
BG
Melita
UK
Lee
Sc3
Vista
Cadillac
Sc4
Rye
Dur
AB
HY630
Sc2
MB
Tri
Oat
Bar
Wht
SK
Kyle
Sc1
Kenya Farmer
9260B