

1 **Screening of populations of *Globodera pallida* selected for increased virulence on several potato**
2 **varieties**

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6 The potato cyst nematodes (PCNs) *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone) (family:
7 Heteroderidae) are major pests of potato causing annual losses of 9% in global production (Turner and
8 Subbotin, 2013). It is thought that PCN were introduced into Europe in the middle of 19th century when
9 wild potato collections were brought from several sites in South America to be used as breeding
10 material against late blight, *Phytophthora infestans* (Evans et al., 1975; Plantard et al., 2008).
11 Resistance from *Solanum tuberosum* ssp. *andigena* CPC1673 (*H1*) has been a durable and effective
12 way of controlling *G. rostochiensis* in the UK, however, the extensive use of varieties with this
13 resistance has resulted in selection in favour of *G. pallida*, which is now the most prevalent species
14 (Minnis et al., 2002).

15 Currently, several sources of partial resistance to *G. pallida* have been identified and are being used
16 in potato breeding programmes. Development of commercially viable varieties with durable
17 resistance to *G. pallida* has been challenging, since no single major resistance gene to *G. pallida* has
18 been identified and continuous use of resistance has been shown to select for populations with have
19 increased virulence (Turner et al., 1983; Benters et al., 1995; Phillips and Blok, 2008; Fournet et al.,
20 2016). Here we report the reproductive ability of *G. pallida* populations which had previously been
21 selected for increased virulence on *Gpa5* from *Solanum vernei* and *H3* from *S. tuberosum* ssp.
22 *andigena* CPC2802 (Phillips and Blok, 2008) on 5 commercial potato varieties with varying levels of
23 resistance to *G. pallida*.

24 Multiplication of two *G. pallida* populations from the James Hutton Institute PCN collection (Farcet
25 and Newton) along with their derived selected sub-populations was assessed on cvs Desirée, Royal,
26 Vales Everest, Innovator and Arsenal which have *G. pallida* (Pa 2/3, 1) resistance scores of 2, 3, 6, 8
27 and 9 respectively (scale 1-9 from low to high resistance <http://varieties.ahdb.org.uk>). Desirée is the
28 susceptible control, Royal has p55/7 (*S. multidissetum* (*H2*)), *S. tuberosum* ssp. *andigena* CPC2808 (*H3*)
29 and *S. vernei* in its pedigree (www.danespo.com) all possible sources of partial resistance to *G.*
30 *pallida*, Innovator and Arsenal have resistance derived from *S. vernei* and Vales Everest from *S.*
31 *tuberosum* ssp. *andigena* CPC2802 (*H3*). The Farcet and Newton founder populations had been
32 multiplied on the susceptible cv Desirée or on four partially resistant potato genotypes for 9 successive

33 generations (Phillips and Blok, 2008); two were derived from *S. vernei* (Sv_8906 - cv Guardian and
 34 Sv_11305 - cv Morag) and two from *S. tuberosum* ssp. *andigena* CPC2802 (H3) (Sa_11415 and
 35 Sa_12674).

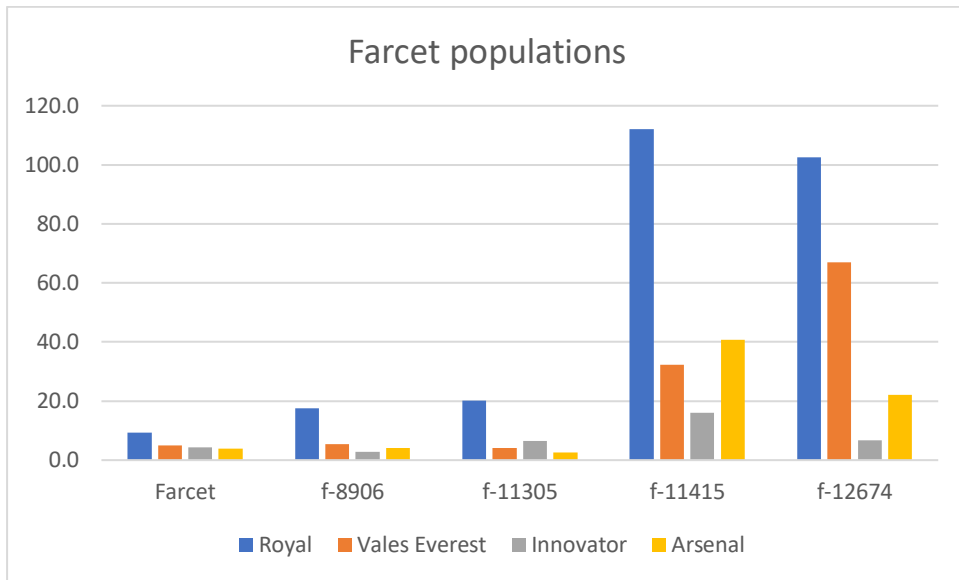
36 A sprouted tuber piece (size 1.5cm x 1.5cm) from each potato cultivar was planted in a deep root-
 37 trainer cell (Haxnicks, Bristol, UK) filled with insecticide-free compost containing 20 cysts from a
 38 specific nematode population. Each 'population x cultivar' combination consisted of 4 biological
 39 replicates in a completely randomised design and was performed twice. The screening tests were
 40 performed in a greenhouse (20°C / 16°C (average day/night temperature) with 16/8 h light/darkness).
 41 All cysts had previously been tested for hatching activity to ensure their vitality. Seven weeks post-
 42 inoculation, root trainers were opened and the number of females on each root surface were counted.

43 The highest numbers of females were observed on the susceptible control Desirée (average 119.1 -
 44 51.3 and 150.4 - 84.4), however, for f-11415 and f-12674 there were no significant differences in the
 45 numbers of females for Desirée and Royal, and for Desirée, Royal and Vales Everest respectively)
 46 (Table 1).

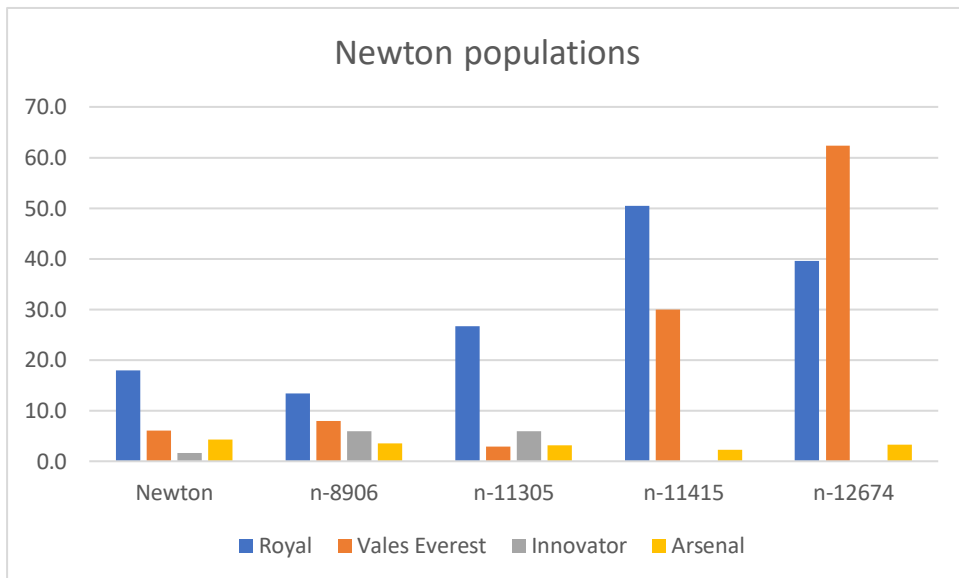
47 Table 1 The mean number of females of *G. pallida* populations Newton and Farcet and their associated selected
 48 populations counted on the roots of Desirée, Royal, Vales Everest, Arsenal or Innovator. The underlined numbers show the
 49 mean number of females when the nematode populations were selected on the same resistance source as the tested
 50 potato variety. The superscripted letters stand for the least significant difference (LSD) (p -value = 0.05).

	Desirée	Royal	Vales Everest	Arsenal	Innovator
Farcet	76.0 ^{fg}	7.1 ^{abc}	3.8 ^{ab}	3.3 ^a	3.0 ^{ab}
f-8906	119.1 ^h	20.8 ^{abcd}	6.4 ^{ab}	<u>3.5^a</u>	<u>4.9^{ab}</u>
f-11305	93.0 ^{gh}	18.8 ^{abcd}	3.8 ^{ab}	<u>6.0^{ab}</u>	<u>2.4^{ab}</u>
f-11415	92.8 ^{gh}	104.0 ^h	<u>29.9^{bcde}</u>	15.0 ^{abc}	37.9 ^{de}
f-12674	51.3 ^{ef}	52.6 ^{ef}	<u>34.3^{cde}</u>	3.5 ^a	11.4 ^{abcd}
Newton	141.0 ^f	25.3 ^{abc}	8.4 ^a	2.3 ^a	6.0 ^a
n-8906	144.8 ^f	15.4 ^{ab}	11.6 ^a	<u>8.6^a</u>	<u>5.1^a</u>
n-11305	150.4 ^f	40.1 ^{bc}	4.4 ^a	<u>8.8^a</u>	<u>4.8^a</u>
n-11415	84.4 ^{de}	42.5 ^c	<u>25.3^{abc}</u>	0.3 ^a	1.9 ^a
n-12674	119.8 ^e	47.5 ^c	<u>74.6^d</u>	0.6 ^a	4.0 ^a

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55 Figure 1 Percentage of females counted on the root surfaces of Royal, Vales Everest, Innovator and
56 Arsenal after 8 weeks of *G. pallida* populations (a) Farcet and (b) Newton and their corresponding
57 selected populations (f-8906, f-11305, f-11415 and f-12674, and n-8906, n-11305, n-11415 and n-
58 12674) relative to Desirée. Results are from 2 experiments with 4 reps/experiment.

59 As expected from the AHDB resistance scores, Royal was overall the second most relatively susceptible
60 variety followed by Vales Everest. Both had higher relative susceptibility to f-11415, f-12674, n- 11415
61 and n-12674, lines selected on the *S. tuberosum* spp. *andigena* CPC 2808 derived genotypes compared
62 to f-8906, f-11305, f-8906 and f-11305 which were selected on *S. vernei* derived genotypes (Fig 1 a,
63 b). Innovator and Arsenal had similarly low relative susceptibility except for f-11415 and f-12674 which
64 had relatively higher multiplication (>20 and 40% respectively) particularly with Arsenal (Fig 1a).

65 The relatively higher multiplication rates of f-11415, f-12674, n-11415 and n-12674 on Vales Everest
66 confirms the results of Phillips and Blok, 2008 who found that resistance from *S. tuberosum* spp.
67 *andigena* CPC 2808 was more readily overcome than resistance from *S. vernei*. Currently, more
68 varieties with *G. pallida* resistance derived from *S. vernei* such as Ambassador, Arsenal, Divaa, Elland,
69 Eurostar, Mistay, Panther and Performer are available than those with *S. tuberosum* spp. *andigena*
70 CPC2808 in their pedigrees. Since breakdown of the *S. vernei* resistance is expected to occur more
71 slowly than H3, these varieties could be useful to suppress *G. pallida* when used in a control program.
72 The selected lines used in this study, particularly f-11415 and f-12674, showed relatively increased
73 multiplication on Innovator and Arsenal, however, these lines had undergone 9 multiplication rounds
74 of selection to achieve increased virulence. Others have reported that 6 successive generations of
75 continuous multiplication of *G. pallida* populations on *S. vernei* resistance was sufficient to generate
76 highly virulent lineages (Benters et al., 1995; Fournet et al., 2016;). It is therefore expected that *S.*
77 *vernei* resistance to *G. pallida* is unlikely to be as durable as the H1 resistance to *G. rostochiensis* and
78 breakdown of resistance is already reported in the North-East of the Netherlands where varieties for
79 starch production are grown (pers comm L. Molendijk). It is curious that f-11415 and f-12674, which
80 had undergone selection on Sa_11415 and Sa_12674 with *S. tuberosum* ssp. *andigena* CPC2802 (H3)
81 in their background, had increased virulence towards Innovator and Arsenal.

82 A further aspect to be considered with regard to the durability of resistance to *G. pallida* is that
83 different sources of *S. vernei* resistance have been used in and between breeding programs and
84 pedigrees are often complex making it difficult to determine which gene(s) is/are responsible for the
85 phenotype in the final variety. Several *S. vernei* sources were used in the breeding of Innovator with
86 *S. vernei* acc LGU 8 (vrn) (*Gpa5*) expected to be the primary source of the resistance whereas 62.33.3
87 (*S. vernei* acc V24/20) which was also used for Innovator is likely to be the source of the resistance
88 Sv_8906 and Sv_11305 (Phillips and Blok, 2008; van Eck et al., 2017).

89 In conclusion, continuous cultivation of potato varieties with resistance to *G. pallida* imposes a high
90 selection pressure and is likely to be detrimental to the durability of the resistance. While varieties
91 with high levels of resistance to *G. pallida* are now available, further control strategies will likely be
92 needed to prevent the emergence of highly virulent PCN populations. Pyramiding different sources of
93 resistance is currently underway in the expectation that this will improve the durability of cultivars
94 with *G. pallida* resistance in the future.

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