



# **Final Report**


## **Evaluation of a method to assess varietal tolerance to potato cyst nematode**

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## SUMMARY (2015-2018)

Variety tolerance to PCN is an important element of pest management. The routine provision of tolerance information on new varieties is constrained by the difficulties of obtaining consistent information from field trials. A box growing system, with and without introduced PCN inoculum, was studied to determine if it would generate consistent information on variety tolerance. The ultimate aim was to assign new varieties into tolerance categories as used in the PCN management tool (<http://potatoes.ahdb.org.uk/online-toolbox/pcn-calculator>). The system was tested over 4 seasons. The results are summarised below and the full report for each year is also provided (Appendix 1-4).

### 2015

An initial scoping study was carried out using four varieties. For PCN-inoculated boxes, a small net “teabag” containing cysts of *Globodera pallida* (Pa2/3) was placed beneath each tuber. Egg numbers were determined by a hatch test, and gave 14.6 egg/larvae per g of soil in an approximate 1 litre of soil surrounding each tuber. Potato yields from the boxes were comparable with average commercial yields for the 2015 season. The behaviour of putative tolerant (Cara) and intolerant (Maris Peer) control varieties was as expected. Two test varieties were included (Markies, Melody) on the basis that they had produced variable results in previous (2010-2012) field-based trials to assess tolerance (Keer, 2013). Markies was confirmed as being intolerant showing a 16.8% yield loss in the presence of PCN. The corresponding loss for Maris Peer was 19.6%. The results for Melody indicate it to be more tolerant of PCN infestation (3.2% yield loss). Results reported from field-based trials to assess the tolerance of Melody have ranged from 3.8% to 19% yield loss (Keer 2007; Kerr 2013), with the highest yield losses being reported from un-irrigated field sites.

### 2016

The control (Cara, Maris Peer) and test varieties (Markies, Melody) from 2015 were included in the study and a further 8 varieties with reported resistance to PCN (*G. pallida*) were also evaluated (Lanorma, Divaa, Performer, Innovator, Camel, Eurostar, Arsenal, Panther). For the PCN-inoculated boxes, a small net “teabag” containing *G. pallida* (Pa2/3) cysts was placed beneath each tuber. The PCN species was originally confirmed by a PCR diagnostic. (Bates *et al.*, 2002). Egg numbers were determined by a hatch test, and gave 7.02 egg/larvae per g of soil calculated over the internal growing area of the box at the planting depth of 10 cm. This level was approximately three times that used in 2015 on a comparable basis. Potato yields from the boxes were comparable with average commercial yields for the 2016 season. The behaviour of the tolerant (Cara) and intolerant (Maris Peer) control varieties was again confirmed. Markies and Melody showed more variable results compared to 2015. Melody showed significant yield loss in the presence of PCN in contrast to the 2015 scoping study data, but in agreement with other studies (Keer, 2013). Markies showed a slight yield depression with PCN which contradicted the 2015 study. All the resistant varieties showed Pf/Pi values less than 1, as expected, but all appeared intolerant to varying degrees, with Eurostar showing the largest, and statistically significant, yield depression.

## 2017

An early accidental flooding of the boxes coupled with rainfall in 2017 resulted in poor initial growth which was not recoverable. Yields were low and highly variable. No conclusions could be drawn concerning tolerance to PCN, though varieties behaved as expected in terms of cyst and egg/larvae multiplication. The trial was repeated at the contractor's own cost in 2018.

## 2018

The test was repeated in 2018 with the almost same variety set as in 2016 (Cara, Maris Peer, Melody, Markies, Lanorma, Alcander, Performer, Innovator, Camel, Eurostar, Arsenal, Panther). For the PCN-inoculated boxes, a small net "teabag" containing *G. pallida* cysts was placed beneath each tuber. Cyst numbers were determined by a hatch test, and gave 7.06 egg/larvae per g of soil calculated over the internal growing area of the box at the planting depth of 10 cm. Yields were approximately similar to those achieved in 2016, and were much greater than those seen in 2017 when flooding occurred in the boxes. Plants developed well in 2018 and despite the very warm and dry conditions experienced, the irrigation regime maintained good top growth. Blight was well controlled. However, unlike 2016, there were no significant effects of PCN inoculation on yield or tuber number. It was not possible to identify any non-significant trends consistent with the 2016 data, except for slightly lower tuber numbers on PCN-inoculated boxes for Maris Peer, Innovator, Camel, Eurostar, Panther and Melody, whereas Cara and Alcander (putative tolerant types) had very similar tuber numbers.

The PCN multiplication was more erratic than seen in the scoping study in 2015, extended study in 2016 and even the flooding affected 2017 test. The reason for this is unknown. Extreme heat experienced in 2018 may have had an effect on nematode development, but there is no direct evidence for this.

## CONCLUSIONS

Initial results in the 2015 scoping study were encouraging, with Cara and Maris Peer behaving as expected (tolerant and intolerant varieties) while both multiplying the nematode. The 2016 test confirmed the Cara and Maris Peer result, indicating potential for box-type studies as a way forward for assessing tolerance, using a controlled inoculation level and obtaining yields comparable with those seen in commercial production. The 2017 test highlighted the sensitivity of the approach to adverse environmental influences. The 2018 test, despite achieving good growth, did not confirm previous data, and it must be concluded that the approach has proved too erratic to be considered as a future routine method for tolerance evaluation/updating the PCN calculator.

# **APPENDIX 1: 2015: EVALUATION OF A METHOD TO ASSESS VARIETAL TOLERANCE TO POTATO CYST NEMATODE (1 JUNE 2015 – 31 JANUARY 2016)**

## **1. INTRODUCTION**

Information on variety tolerance to potato cyst nematode (PCN) infection is a required element of the PCN Calculator tool developed through a number of research projects and available online from AHDB. Tolerance, defined as the ability to maintain production in the presence of a pest or disease, has been evaluated in field trials in the UK (e.g., Keer, 2007; Keer, 2013). While useful data was obtained in both projects, work during 2010-2012 was affected by multiple environmental stresses which precluded generation of tolerance data to update the PCN model for newer varieties. Glasshouse pot tests (Arntzen *et al*, 1994) over a short period (35 days), measuring root and shoot biomass, have also been used to indicate variety tolerance for breeding material. Arntzen and Wouters (1994) further demonstrated good correlation between pot and field tolerance tests. However, Been *et al*. (2005) pointed out that tolerance tests in pots may be biased by the size of pots available and restricted root growth. Keer (2013) also pointed out that while pot tests may be necessary, there were difficulties in relating outcomes to field behaviour.

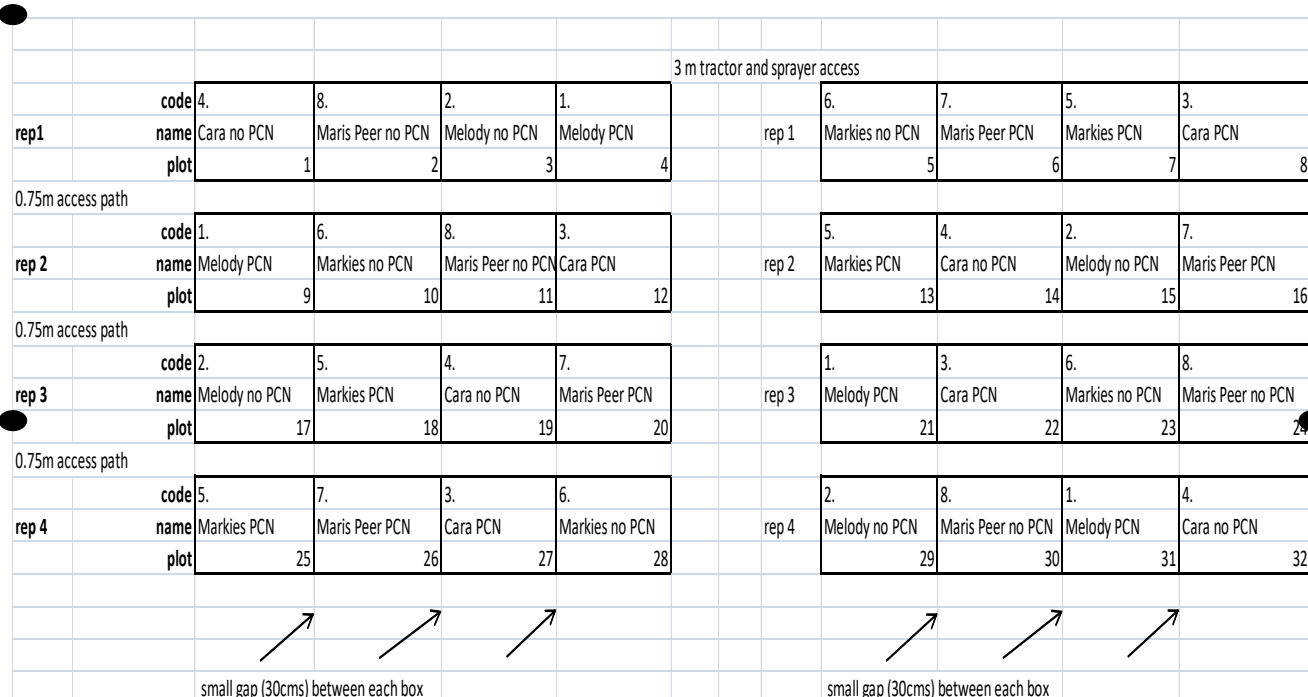
A recent investigation at NIAB into the tolerance of sugar beet varieties to the Beet Cyst Nematode (BCN) has used large boxes to grow beet roots, with standardised quantities of BCN introduced into the boxes, and non-inoculated boxes used as controls. Though several problems were encountered, particularly due to excess or insufficient water, the technique indicated potential for the evaluation of variety tolerance.

The scoping study (2015) reported here was designed to investigate the potential of a box system for future routine evaluation of PCN tolerance, with particular reference to a) the ability of box yields to represent commercial productivity and b) to determine whether the most consistent differences in tolerance described from field work could be reproduced in a box system.

## **2. METHODS**

Thirty-two pallet boxes, fully perforated and with external dimensions of 120 cm x 100 cm, and an internal depth of 72 cm (DOLAV Ltd, Watton, Norfolk) were lined with Mypex and filled with a sandy clay loam sub-soil from an archaeological spoil mound on NIAB trial ground, Huntingdon Road, Cambridge. The soil had previously been tested for PCN in 2014, and was tested again in 2015 by taking a trowel core (approximately 15 cm depth, and 5 cm diameter) from each box before planting. Cores were combined, mixed, and a 1 kg sample tested for PCN. Chitted tubers of the varieties Cara, Maris Peer, Markies and Melody were planted in each box “flat” at a depth of about 10 cm on 11/06/15. For PCN-inoculated boxes, a small net “teabag” containing equal numbers of cysts of *G. pallida* 2/3 originating from stock pots of Maris Piper and Desiree was placed beneath each tuber. Egg numbers were determined in

a hatch test, and gave 14.6 egg/larvae per g of soil in an approximate 1 litre of soil surrounding each tuber. Boxes were watered initially three times per week until emergence by a hand held sprinkler and then by 4 automatic sprinklers situated at each corner of the box layout. Individual sprinklers delivered 65 litres per minute for 20 minutes, at 06.30 and 18.30 daily. Prilled nitrogen fertiliser (34.5% N) was applied at 60 g per box on 21/07/15. Boxes were laid out on Mypex over grass in two blocks of 16, each block being 4 x 4 rows. A standard blight programme was applied with a tractor mounted sprayer, driving down a central pathway between the blocks. Each treatment (variety and PCN level) was replicated four times in a fully randomised design. An overview of the trial layout is shown in Figure 1 and Figure 2. The planting pattern per box is shown in Figure 3.



● = sprinkler position

**Figure 1.** Diagram of box trial layout

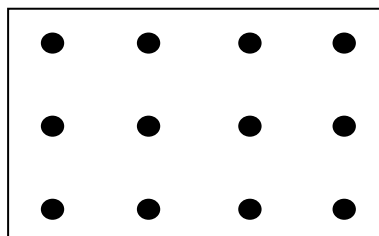








**Figure 2.** (Top) Overview of layout, with sprinkler position. (Middle) Close-up showing box detail. (Lower) Close-up of potato foliage on 15/08/15.



**Figure 3.** Planting configuration

Emergence (plant counts) was assessed on 08/07/15, 18/07/15 and 29/07/15. Haulm or top growth vigour was assessed visually on a 1 to 5 scale where 5 was most vigorous on 08/07/15, 18/07/15, 29/07/15 and 07/08/15. Canopy height was assessed on two occasions, 11/08/15 and 24/08/15 by measuring the distance between the soil level and the top leaf layer at 3 locations per box. The mean was analysed. The trial was harvested between the 8<sup>th</sup> and 14<sup>th</sup> of October. Total produce per box was washed, weighed and then tuber numbers counted in each of the following grade classes: 0-2 cm, 2.1-5 cm, 5.1 to 8 cm, 8.1-11 cm, and >11 cm. Just before harvest, 24 X 1 cm diameter soil cores were taken per inoculated box to a depth of approximately 25 cm. These were combined, dried, broken down and mixed thoroughly and a 1 kg sample extracted for cyst counting. A single box with no inoculation was also sampled.

### 3. RESULTS

Mean emergence over time is shown in Table 1. There was no effect of PCN level on plant number, though one replicate of Markies with PCN had only 8 plants emerged out of 12 planted, giving a low final mean plants emerged. This replicate was omitted from yield determinations. Haulm vigour/top growth was reduced significantly by PCN for Maris Peer and Markies on 29/07/15, and for Markies on 07/08/15. Height was reduced by PCN for Maris Peer, Markies and Melody at the first measurement, but only Maris Peer and Markies at the second measurement. None of the effects on height were significant except the reduction in Markies at the first measurement. (Table 3).

**Table 1.** Mean number of plants emerged

	PCN	No PCN	PCN	No PCN	PCN	No PCN
	08/07/15		18/07/15		29/07/15	
Cara	10.3	7.8	11.8	9.8	11.8	10.8
M Peer	9.5	9.5	11.0	11.0	11.5	11.8
Markies	7.8	8.3	9.0	10.0	9.3	11.0
Melody	7.5	9.0	10.0	10.8	10.8	10.8

**Table 2.** Haulm/top growth vigour 1-5, where 5 is most vigorous

	PCN	No PCN	PCN	No PCN	PCN	No PCN	PCN	No PCN
	08/07/15		18/07/15		29/07/15		07/08/15	
Cara	2.0	2.0	2.0	2.5	3.5	3.3	3.8	3.8
M Peer	3.0	3.5	4.0	4.0	3.8	4.5	4.8	5.0
Markies	1.8	2.8	1.5	3.0	3.3	4.0	3.5	4.8
Melody	3.3	4.0	3.3	4.0	4.0	4.3	3.8	4.3
Isd*(p<0.05)	1.39		1.19		0.69		0.71	

\*Isd is for comparison of PCN level within a variety

**Table 3.** Height measurement (cm) from soil level to top leaf layer

	PCN	No PCN	PCN	No PCN
	11/08/15		24/08/15	
Cara	47.5	43.9	78.8	76.8
M Peer	50.4	54.6	65.8	68.8
Markies	46.7	54.2	71.3	74.0
Melody	42.1	46.0	63.3	59.3

Isd for comparing PCN level within variety =6.50 on 11/08 and 7.10 on 24/08 (p<0.05)

Yield as kg per box was reduced significantly for Maris Peer and Markies, but not Cara or Melody. (Table 4). When calculated as kg/plant, the reduction was not significant for Markies. The growing area (ie internal dimensions) of each box was calculated as 1.1136 m<sup>2</sup>, and the corresponding tonnes/ha yield are also shown in

Table 4. One missing plot was used in the analyses (Markies replicate 4 with PCN) , where only 8 plants had emerged.

**Table 4** Yield: kg/box, kg/plant, and t/ha per box equivalent

	PCN	No PCN	PCN	No PCN	PCN	No PCN
	Yield kg/box		Yield kg/plant		Yield per box t/ha equivalent	
Cara	5.19	5.18	0.443	0.482	46.6	46.5
M Peer	5.41	6.73	0.471	0.573	48.6	60.4
Markies	5.25	6.31	0.523	0.574	47.1	56.7
Melody	7.30	7.54	0.685	0.699	65.6	67.7

Lsd for comparing +/- PCN kg/box = 1.134 and for kg/plant = 0.0967 ( $p < 0.05$ )

Size grade distribution (tuber number only) is shown in Table 5. Maris Peer inoculated with PCN showed a slightly higher proportion of smaller tubers than PCN free produce, and this was significant at  $p < 0.05$ , Lsd 12.04.

**Table 5** Size distribution (number of tubers) in cm

	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11
Cara PCN	3.25	22.2	36.0	7.25	0.00
Cara no PCN	4.50	27.0	38.2	5.00	0.00
M Peer PCN	1.75	66.2	55.5	3.50	0.00
M Peer no PCN	1.50	45.2	59.5	8.00	0.00
Markies PCN	2.75	20.2	34.2	13.00	0.50
Markies no PCN	3.75	25.8	42.0	13.00	1.00
Melody PCN	1.50	22.8	37.2	20.50	2.75
Melody no PCN	1.50	20.2	47.8	19.75	0.50

Produce from a replicate of each variety, with and without PCN, is shown in Figure 4.







**Figure 4.** Single replicate produce from Cara, Maris Peer, Markies and Melody with and without PCN inoculum.

PCN cyst numbers in the initial ( $P_i$ ) infection were calculated to give 14.6 egg/larvae per g of soil in a volume of 1 litre surrounding each plant. Final ( $P_f$ ) egg/larvae per g of sampled soil are shown in Table 6. The  $P_f/P_i$  ratio calculated on the basis of the discrete volume of soil around each plant at the start of the experiment was only just over 1 for each variety. However, this is misleading since the final sample cores were taken over the whole box. A better approximation of  $P_f/P_i$  can be calculated by averaging the initial value over the whole box, to a depth of 25 cm.  $P_f/P_i$  values for each variety using this method are also shown in Table 6. The values are much higher,

and typical of values reported in previous field studies on tolerance (Keer, 2013). There were no significant differences between varieties for the final cyst count or the egg/larvae count, as might be expected from their resistance ratings, all at 2. No PCN cysts were detected in either the soil tested from the archaeological dig spoil in 2014, or in the re-test of soil used in the boxes in 2015, or in the single non-inoculated box sample taken at the end of the experiment.

**Table 6.** Cysts/kg and egg/larvae per g of soil sampled at harvest

	Mean cyst count/kg	Mean egg/larvae count/g	Pf/Pi (egg/ larvae)
Cara	77	27.0	42.9
M Peer	77	22.9	36.4
Markies	94	39.1	62.1
Melody	54	15.8	25.1

#### 4. DISCUSSION

The box system gave an acceptable t/ha yield equivalent compared to reported commercial yields, which averaged 48.8 t/ha for the 2015 season (source AHDB Potatoes, December 2015 planting data). The high strength, fully perforated boxes did not split or break during the season of use. Lining with Mypex contained the field soil effectively. Boxes were observed after irrigation and periods of heavy rain, and showed no sign of surface pooling of water, indicating the perforated structure on all the surfaces was allowing effective drainage. Foliage blight was well controlled and harvested tubers were generally good quality, with a very low incidence of tuber blight developing during the period when tubers were washed and weighed.

The behaviour of the putative tolerant and intolerant varieties, Cara and Maris Peer, was as expected. Both have a rating of 2 for resistance to *G. pallida* but varied significantly in their productivity under the same level of PCN pressure introduced by the inoculum bags. There was no significant effect of *G. pallida* presence on the yield of Cara, while the nematode caused a yield depression of just under 12 tonnes/ha equivalent in Maris Peer. Yield loss calculated as: % yield loss = 100-((total yield in PCN inoculated plots/total yield in PCN free plots)\*100) for Maris Peer was 19.6%. In the previous field based studies (Kerr, 2013), the reported yield loss for Maris Peer in each of three years was 35%, 25% and 12%, respectively.

Yield loss for Markies in this study was 16.8% and it is categorised as intolerant showing a yield depression similar to that of Maris Peer. The previous field based trials (Kerr, 2013) had resulted in yield loss values of 8%, 13% and 9% over the three year project. Whilst a single year's data for Markies in earlier field work (Keer, 2007) indicated a yield loss of 6.8%. The yield loss for Maris Peer in the same trial was 15.4%.

In the current study, Melody appeared tolerant, with no significant effect on yield. The calculated yield loss was 3.2%. In the previous field based studies (Kerr, 2013), the reported yield loss for Melody was 19%, 15% and 6%, respectively. Whilst a single year's data for Melody in earlier work (Keer, 2007) indicated a yield loss of 3.8%. An additional year's data using the pallet box system will clarify the status of this variety.

The box system provides an intermediate method between field scale measurement of tolerance and pot-based systems. It has the advantage over the latter of allowing relatively unrestricted root growth, which is probably a major contributing factor to tolerance. Though field systems, by definition, provide a true growing environment, they can be confounded by variation in PCN distribution, seasonal weather conditions, non-target effects of nematicides used to give the comparison of lower PCN populations, and variable efficacy of nematicides. The box system delivers a highly controlled level of PCN inoculum to the root zone, in a way which parallels National List resistance testing. It also uses non-infested soil as the comparison for yield, eliminating variable nematicide effects. The environment can be controlled to an extent by means of irrigation and potentially, if needed, by temporary covers in very wet conditions. Potatoes can be grown successfully in a box environment as evidenced by comparable yields to average commercial outputs in the same season.

One of the main problems encountered during the study was the variable emergence of the seed stocks used. Most boxes (22 out of 32) produced 11 or 12 plants, but missing plants in some boxes may have affected results, though compensatory effects probably meant that overall box yields were a reliable indicator of productivity. However, missing plants within the two central “guarded” plants in some boxes precluded the separate analysis of guarded versus unguarded yields. In any future work, careful selection of seed tubers should improve uniformity of emergence.

The results from Cara and Maris Peer provide an encouraging basis for the further use of the box system. The more variable outcomes for Melody, compared to previous data from in-field experiments, can only be resolved by additional work. A similar approach using boxes to understand tolerance to beet cyst nematode in sugar beet varieties suffered from conditions being either too dry, or flooding in the boxes. The irrigation system used here, and the fully perforated boxes, successfully avoided these problems, and as a whole the box approach has merit for further investigation of tolerance to PCN.

## **5. CONCLUSION (2015)**

This scoping study has indicated that a box growing system could provide the basis for a future industry standard for determining variety tolerance to PCN. A minimum of two seasons testing will probably be required. The type of box was critical for good drainage, and the sprinkler irrigation used provided appropriate moisture levels as evidenced by commercially comparable yields.

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## **APPENDIX 2: 2016: EVALUATION OF A METHOD TO ASSESS VARIETAL TOLERANCE TO POTATO CYST NEMATODE (1 JUNE 2016 – 30 APRIL 2017)**

### **1. INTRODUCTION**

This study used a box growing system with and without introduced PCN inoculum, to ascertain tolerance of an extended set of varieties following an initial scoping study in 2015. The scoping study indicated that the box method did predict relative tolerances of two control varieties with the most consistent previous data. On this basis a further test was carried out in 2016 using the control varieties and further varieties reported to be PCN (*Globodera pallida*) resistors but with reputed intolerance to the nematode.

### **2. METHODS**

Ninety-six pallet boxes, fully perforated and with external dimensions of 120 cm x 100 cm, and an internal depth of 72 cm (DOLAV Ltd, Watton, Norfolk and Pallet Tower Ltd, Sale Cheshire) ) were lined with Mypex and filled with a sandy clay loam sub-soil from an archaeological spoil mound on NIAB trial ground, Huntingdon Road, Cambridge. The soil had previously been tested for PCN in 2015, and was tested again in 2016 by taking a trowel core (approximately 15 cm depth, and 5 cm diameter) from each box before planting. Cores were combined, mixed, and a 1 kg sample tested for PCN.

Chitted tubers of the varieties shown in Table 1 were planted flat in each box on 08/06/16 at a depth of about 10 cm in 3 rows of 4 tubers. For the PCN-inoculated boxes, a small net “teabag” containing 50 *G. pallida* cysts was placed beneath each tuber. Cyst numbers were determined by a hatch test, and gave 7.02 egg/larvae per g of soil calculated over the internal growing area of the box at the planting depth of 10 cm. Prilled nitrogen fertiliser (34.5% N) was applied at 500 kg/ha equivalent (60 g per box) on 14/06/16. Boxes were laid out on Mypex over grass in two blocks of 48, each block being 6 x 8 rows. Boxes were watered 1 week after planting by 8 automatic sprinklers situated at each corner of the box layout. Individual sprinklers delivered 65 litres per minute for 10 minutes, at 06.30 and 18.30 daily, but were switched off during periods of rain and after blight sprays. A late blight programme (Table 2) was applied with a tractor mounted sprayer, driving down a central pathway between the blocks. Each treatment (variety and PCN level) was replicated four times in a fully randomised design

Table 1 Varieties tested in 2016 with PCN (*G. pallida*) resistance ranking (1-9)

Variety	1-9 rating (1 = susceptible, from AHDB Potato Variety Database where available)
Cara	2
Maris Peer	2
Melody	2
Markies	2
Lanorma	5
Divaa	5
Performer	9
Innovator	Resistant (breeder data)
Camel	9
Eurostar	9
Arsenal	8 (breeder data)
Panther	8

Table 2 Late blight programme 2016

Date	Product	Rate l/ha
12/07/2016	Nando	0.3
22/07/2016	Ranman Twinpack A	0.2
22/07/2016	Ranman Twinpack B	0.15
18/07/2016	Infinito	1.6
27/07/2016	Ranman Twinpack A	0.2
27/07/2016	Ranman Twinpack B	0.15
05/08/2016	Infinito	1.6
12/08/2016	Nando	0.3

Emergence (plant counts) was assessed on 12/07/16 and 26/07/16. Canopy growth was assessed visually on a 1- 9 scale where 9 was the largest canopy on 12/07/16 and 26/07/16. Canopy height was assessed on two occasions, on 26/07/16 and 09/08/16 by measuring the distance between the soil level and the top leaf layer at 3 locations per box. The mean was analysed.

Haulm tops were cut off on 26/09/16 and the trial was harvested between 17/10/16 and 28/10/16. Total produce per box was washed, weighed and then tuber numbers counted in each of the following grade classes: 0-2 cm, 2.1-5 cm, 5.1 to 8 cm, 8.1-11 cm, and >11 cm. Just before harvest, 24 X 1 cm diameter soil cores were taken per inoculated box to a depth of approximately 30 cm. These were combined, dried, broken down and mixed thoroughly and a 1 kg sample extracted for cyst counting and subsequent egg/larvae counts. A single box with no inoculation was also sampled.

### 3. RESULTS

Mean emergence over time is shown in Table 3. There was no effect of PCN level on plant number, and most boxes contained 12 plants at the end of July. Canopy growth was reduced significantly by PCN for Eurostar and Arsenal at the first scoring time,

and for Markies at the second score (Table 4). Height was significantly reduced by PCN for Markies and Eurostar at the second measurement (Table 5).

Table 3 Mean number of plants emerged

	PCN	No PCN	PCN	No PCN
	12/07/16		26/07/15	
Cara	11.75	11.75	11.75	11.75
M Peer	12	12	12	12
Markies	11.5	12	11.25	12
Melody	12	11.5	12	11.5
Lanorma	11.75	12	11.75	12
Divaa	12	12	12	12
Performer	12	12	12	12
Innovator	12	12	12	12
Camel	12	12	12	12
Eurostar	12	12	12	12
Arsenal	11.75	12	11.75	12
Panther	11.75	12	11.75	12

Table 4 Canopy growth, 1-9, where 9 is largest canopy

	PCN	No PCN	PCN	No PCN
	12/07/16		26/07/16	
Cara	3.75	3.5	4.5	4
M Peer	5.75	5.75	7.25	7
Markies	3.75	5.5	4.25	6.25
Melody	5	5.25	6	6.75
Lanorma	5.25	5.5	5.75	6
Divaa	5.5	5	6.25	5.5
Performer	6	6.25	7.25	7.75
Innovator	5	5.25	5.75	6.75
Camel	3.75	4.75	4.5	5.75
Eurostar	5	6.25	7	7.75
Arsenal	4.25	5.75	5.75	6.25
Panther	3.5	4.25	3.75	4.75
Isd (p=0.05)	1.091		1.634	
Isds are for comparison of PCN level within variety				

Table 5 Height measurement (cm) from soil level to top leaf layer

	PCN	No PCN	PCN	No PCN
	26/07/16		10/08/16	
Cara	46.3	43.3	54.1	50.4
M Peer	49.8	57.2	57.2	50.8
Markies	37.2	39.7	39.7	54.9
Melody	52.2	56.9	56.9	59.9
Lanorma	46.3	53.2	53.2	55.8
Divaa	43.9	49.8	49.8	38.4
Performer	57.0	58.3	58.3	61.4
Innovator	45.6	50.3	50.3	52.8
Camel	44.8	45.8	45.8	52.5
Eurostar	53.9	54.4	54.4	68.1
Arsenal	46.8	51.3	51.3	53.6
Panther	34.2	37.1	37.1	41.2
Isd(p=0.05)	9.14		8.34	

Isds are for comparison of PCN level within variety

PCN reduced yield (kg/box) significantly for Melody and Eurostar (Table 6). The growing area (ie internal dimensions) of each box was calculated as 1.1136 m<sup>2</sup>, and the corresponding tonnes/ha yield are also shown in Table 6. Average yield per plant is included, though plant numbers were almost identical in each box.

Table 6 Yield (kg/box, kg/plant, and t/ha per box equivalent

	PCN	No PCN	PCN	No PCN	PCN	No PCN
	Yield kg/box		Yield kg/plant		Yield per box t/ha equivalent	
Cara	4.25	4.16	0.36	0.35	38.13	37.33
M Peer	5.98	6.69	0.50	0.56	53.68	60.08
Markies	5.13	5.42	0.46	0.45	46.03	48.68
Melody	3.88	6.22	0.32	0.54	34.85	55.85
Lanorma	5.35	6.23	0.46	0.52	48.08	55.90
Divaa	5.21	4.23	0.43	0.35	46.78	37.97
Performer	6.11	6.89	0.51	0.57	54.83	61.86
Innovator	5.79	6.22	0.48	0.52	51.98	55.89
Camel	4.82	5.82	0.40	0.49	43.30	52.29
Eurostar	5.88	7.25	0.49	0.60	52.84	65.06
Arsenal	4.93	5.81	0.42	0.48	44.25	52.19
Panther	3.61	4.04	0.31	0.34	32.41	36.30
Isd(p=0.05)	1.218					

Isd is for comparison of PCN level within variety

Examples of produce (single replicate) from the resistors Eurostar, Innovator and Arsenal are shown in Fig 1. Size grade distribution for tuber number is shown in Table 7, and tuber weight in Table 8.

Fig 1 Comparison of produce sorted in size grades for Eurostar, Innovator and Arsenal





Table 7 Size distribution (cm) for tuber numbers

	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11	Total
Cara PCN	2.3	38.8	39.0	0.5	0.3	80.8
Cara no PCN	2.3	33.3	38.3	1.7	0.0	75.7
M Peer PCN	3.5	60.8	66.8	3.5	0.0	134.5
M Peer no PCN	3.0	83.7	81.7	1.7	0.0	170.0
Markies PCN	6.0	43.5	60.5	6.0	0.0	116.0
Markies no PCN	6.0	48.5	49.0	8.5	0.3	112.2
Melody PCN	6.0	44.5	42.3	2.0	0.0	94.8
Melody no PCN	7.0	64.8	58.5	7.3	0.0	137.5
Lanorma PCN	2.8	27.0	45.0	8.8	0.3	83.8
Lanorma no PCN	4.3	27.8	48.5	10.8	1.0	92.2
Divaa PCN	3.8	22.0	44.5	10.8	0.0	81.0
Divaa no PCN	3.8	26.0	41.3	6.8	0.0	77.8
Performer PCN	1.0	14.0	37.3	19.0	1.0	72.2
Performer no PCN	0.8	18.5	34.3	20.8	2.5	76.8
Innovator PCN	1.8	17.0	33.5	15.0	0.5	67.8
Innovator no PCN	3.5	23.3	37.5	16.0	1.3	81.5
Camel PCN	4.3	47.5	41.8	4.0	0.0	97.5
Camel no PCN	5.5	61.5	53.0	5.5	0.0	125.5
Eurostar PCN	4.8	34.8	49.5	6.5	0.0	95.5
Eurostar no PCN	10.3	48.0	58.3	10.0	0.0	126.5
Arsenal PCN	2.8	56.3	40.5	0.5	0.0	100.0
Arsenal no PCN	3.8	59.3	42.3	3.3	0.0	108.5
Panther PCN	4.3	30.8	32.5	4.0	0.3	71.8
Panther no PCN	2.5	43.8	37.0	5.0	0.0	88.2
Isd(p=0.05) between PCN level	3.82	16.51	15.29	5.09	0.83	20.94

Table 8 Size distribution (cm) tuber weight in kg

	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11
Cara PCN	0.01	0.96	3.20	0.08	0.00
Cara no PCN	0.01	0.75	3.09	0.31	0.00
M Peer PCN	0.01	1.48	4.09	0.40	0.00
M Peer no PCN	0.01	1.87	4.65	0.17	0.00
Markies PCN	0.01	0.81	3.59	0.71	0.00
Markies no PCN	0.01	0.94	3.36	1.06	0.05
Melody PCN	0.02	0.91	2.71	0.25	0.00
Melody no PCN	0.02	1.31	3.76	1.13	0.00
Lanorma PCN	0.01	0.56	3.42	1.30	0.07
Lanorma no PCN	0.01	0.59	3.69	1.67	0.27
Divaa PCN	0.01	0.43	2.88	1.89	0.00
Divaa no PCN	0.01	0.45	2.94	0.82	0.00
Performer PCN	0.00	0.26	2.83	2.79	0.22
Performer no PCN	0.00	0.30	2.94	3.03	0.62
Innovator PCN	0.01	0.39	2.64	2.54	0.21
Innovator no PCN	0.01	0.50	2.86	2.49	0.37
Camel PCN	0.01	1.16	3.15	0.51	0.00
Camel no PCN	0.02	1.38	3.69	0.74	0.00
Eurostar PCN	0.01	0.70	4.17	0.91	0.10
Eurostar no PCN	0.02	1.03	4.61	1.58	0.00
Arsenal PCN	0.01	1.73	3.13	0.06	0.00
Arsenal no PCN	0.01	1.73	3.55	0.52	0.00
Panther PCN	0.01	0.61	2.33	0.62	0.05
Panther no PCN	0.01	0.74	2.54	0.75	0.00
Isd(p=0.05) between PCN level	0.010	0.384	1.031	0.874	0.199

Final (Pf) egg/larvae per g of sampled soil are shown in Table 9 with final cyst numbers and Pf/Pi for egg larvae. Pi was calculated by assuming equal distribution of the original egg/larvae counts through the 30 cm depth that was finally sampled. Resistors had Pf/Pi values of less than 1, while varieties with lower ratings all increased the nematode. No PCN cysts were detected in the initial soil sample taken from an aggregate of all the boxes or in the single non-inoculated box sample taken at the end of the experiment.



Table 6. Cysts/kg and egg/larvae per g of soil sampled at harvest

	Mean cyst count/kg	Mean egg/larvae count/g	Pf/Pi (egg/ larvae)
Cara	183	41.95	17.93
M Peer	208	51.57	22.04
Markies	113	25.29	10.81
Melody	238	42.83	18.30
Lanorma	155	27.31	11.67
Divaa	50	7.87	3.36
Performer	13	0.00	0.00
Innovator	5	0.09	0.04
Camel	23	0.04	0.02
Eurostar	33	0.91	0.39
Arsenal	35	0.24	0.10
Panther	20	0.99	0.42

#### 4. DISCUSSION

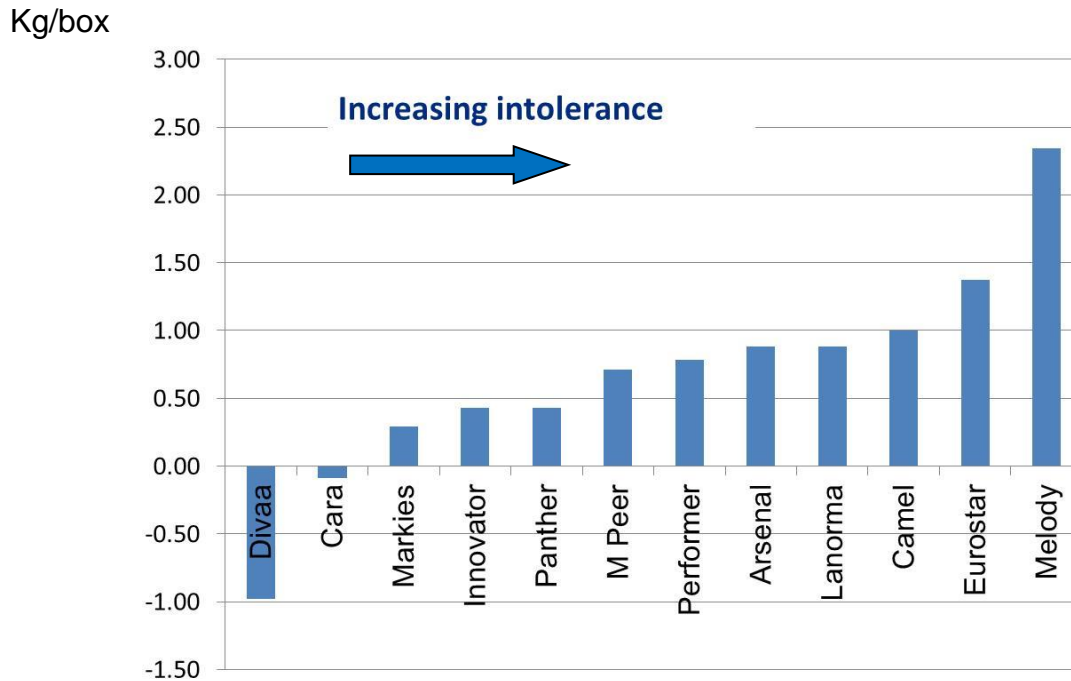
The box system gave an acceptable t/ha yield equivalent compared to reported commercial yields, which averaged 45 t/ha for the 2016 season (source AHDB Potatoes, November 2016 market data). Foliage blight in the boxes was relatively well controlled, though some small foci did develop. Harvested tubers were generally good quality, with a low incidence of tuber blight developing during the period when tubers were washed, weighed and graded. Some slug damage was observed.

The behaviour of the two putative tolerant and intolerant varieties, Cara and Maris Peer, was as expected and agreed with 2015 data, though the yield depression for Maris Peer in 2016 was not significant. Cara showed slightly higher yields with PCN than without. All the resistant varieties showed Pf/Pi values less than 1, as expected, but all appeared intolerant to varying degrees, with Eurostar showing the largest, and statistically significant, yield depression. Of the intermediate resistant varieties, Divaa showed a yield increase, and Lanorma a decrease. Neither differences were statistically significant. Melody was the only susceptible variety to show significant yield loss, in contrast to the 2015 scoping study data, but in agreement with other studies (Keer, PCL Report R432). Markies showed a slight yield depression with PCN which contradicted the 2015 study.

#### 5. CONCLUSION (2016)

The consistent behaviour of Cara and Maris Peer suggests the box system has merit as a means of comparing the effects of PCN on yield without use of nematicide. Based on this single year with an extended variety set, tolerance rankings (yield without PCN less yield with PCN) are shown in Fig 2.

Fig 2 Tolerance ranking based on 2016 box yields



There was some degree of agreement between the box trial outcomes and those seen in the Elveden 2016 SPot Farm plots, where nematicide was used to control PCN. Outcomes for Cara and Maris Peer were similar, but the Elveden data indicated Eurostar was more tolerant than Innovator. Multi-year tests for traits such as pest or disease tolerance are essential to discern consistent trends which could be used as part of PCN management systems. Varieties in the 2016 box trial are being tested for a second year in 2017. A statistical investigation of available tolerance test data from various sources is ongoing, and will include 2017 box trial outcomes.

## 6. REFERENCES

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## **APPENDIX 3: 2017: EVALUATION OF A METHOD TO ASSESS VARIETAL TOLERANCE TO POTATO CYST NEMATODE (1 JUNE 2017 – 30 APRIL 2018)**

### **1. INTRODUCTION**

An initial scoping study in 2015 with standardised quantities of PCN introduced into large boxes, and non-inoculated boxes used as controls, indicated that the box method did predict relative tolerances of two control varieties. On this basis, a further test was carried out in 2016 including an additional 8 varieties reported to be PCN (*G. pallida*) resistors but with reputed intolerance to the nematode. This test indicated that the putative controls, Maris Peer (intolerant) and Cara (tolerant) were again behaving consistently, and that high resistors to *G. pallida* multiplication were intolerant. The 2017 test used the same variety set with one addition (Alcander) to replace an unobtainable variety (Divaa).

### **2. METHODS**

Ninety-six pallet boxes, fully perforated and with external dimensions of 120 cm x 100 cm, and an internal depth of 72 cm (DOLAV Ltd, Watton, Norfolk and Pallet Tower Ltd, Sale Cheshire) were lined with Mypex and filled with a sandy clay loam sub-soil from an archaeological spoil mound on NIAB trial ground, Huntingdon Road, Cambridge. The soil had previously been tested for PCN in 2015, 2016 and was tested again in 2017 by taking a trowel core (approximately 15 cm depth, and 5 cm diameter) from each box before planting. Cores were combined, mixed, and a 1 kg sample tested for PCN.

Chitted tubers of the varieties shown in Table 1 were planted flat in each box on 08/05/17 at a depth of about 10 cm in 3 rows of 4 tubers. For the PCN-inoculated boxes, a small net “teabag” containing 60 *G. pallida* (Pa2/3) cysts was placed beneath each tuber. Cyst numbers were determined by a hatch test, and gave 11.1 egg/larvae per g of soil calculated over the internal growing area of the box at the planting depth of 10 cm. Prilled nitrogen fertiliser (34.5% N) was applied at 500 kg/ha equivalent (60 g per box) on 15/05/17. Boxes were laid out on Mypex over grass in two blocks of 48, each block being 6 x 8 rows. Boxes were watered 1 X per week after planting by 8 automatic sprinklers situated at each corner of the box layout. Individual sprinklers delivered 65 litres per minute for 10 minutes, at 06.30 and 18.30 daily, but were switched off during periods of rain and after blight sprays. An accidental timing error in early June combined with a period of rainfall resulted in excessive “pooling” in the boxes, and after they had drained irrigation times were subsequently adjusted to maintain a water content between 24-25%, with an application rate between 3mm and 5mm per day, turning off completely during rain. A late blight programme (Table 2) was applied with a tractor mounted sprayer, driving down a central pathway between the blocks. Each treatment (variety and PCN level) was replicated four times in a fully randomised design

Table 1 Varieties tested in 2017 with PCN resistance ranking (1-9)

Variety	1-9 rating (1 = susceptible, from AHDB Potato Variety Database where available)
Cara	2
Maris Peer	2
Melody	2
Markies	2
Lanorma	5
Alcander*	Resistant (breeder data)
Performer	9
Innovator	Resistant (breeder data)
Camel	9
Eurostar	9
Arsenal	8 (breeder data)
Panther	8

\* to replace Divaa

Table 2 Late blight programme 2017

Date	Product	Rate litres or kg/ha
13/06/17	Fubol Gold	1.9 (kg)
24/06/17	Nando	0.3
30/06/17	Option	0.15
06/07/17	Infinito	1.6
13/07/17	Nando	0.3
21/07/17	Infinito	1.6
27/07/17	Revus	0.6
06/08/17	Infinito	1.6
10/08/17	Nando/Tizca	0.3
17/08/17	Infinito	1.6
26/08/17	Nando/Tizca	0.3

Emergence (plant counts) was assessed on 01/06/17 and 19/06/17. Canopy growth (ground cover) was assessed visually on a 1- 5 scale where 5 was the largest canopy/greatest ground cover on 20/07/17. Canopy height was assessed on 20/07/17 by measuring the distance between the soil level and the top leaf layer at 3 locations per box. The mean was analysed.

Haulm tops were cut off on 28/08/17 and the trial was harvested between 20/09/17 and 2/10/17. Total produce per box was washed, weighed and then tuber numbers counted in each of the following grade classes: 0-2 cm, 2.1-5 cm, 5.1 to 8 cm, 8.1-11 cm, and >11 cm. Just before harvest, 24 X 1 cm diameter soil cores were taken per inoculated box to a depth of approximately 30 cm. These were combined, dried, broken down and mixed thoroughly and a 1 kg sample extracted for cyst counting and subsequent egg larvae counts. A single box with no inoculation was also sampled.

### 3. RESULTS

Mean emergence over time is shown in Table 3. There was no effect of PCN level on plant number, and most boxes contained 12 plants by mid -June with the exception of one box of Cara. There was no significant effect of PCN level within variety on canopy growth (Table 4). Height was significantly reduced by PCN for Cara only (Table 5).

Table 3 Mean number of plants emerged

	PCN	No PCN	PCN	No PCN
	01/06/17		19/06/17	
Cara	11.25	10.75	11.25	10.75
M Peer	11.25	11.5	11.75	12
Markies	12	11.5	12	11.75
Melody	12	11.25	12	12
Lanorma	10.75	11.5	11	11.75
Alcander	12	11.25	12	11.25
Performer	11.5	11.25	11.5	11.25
Innovator	11.75	12	12	12
Camel	11.25	11	12	11.5
Eurostar	10.75	12	12	12
Arsenal	11.75	11.25	11.75	11.25
Panther	11.5	11.75	11.5	12

Table 4 Canopy growth, 1-5, where 5 is largest canopy

	PCN	No PCN
	20/07/17	
Cara	3.25	4.50
M Peer	3.00	3.25
Markies	2.75	2.75
Melody	2.75	3.50
Lanorma	2.50	2.50
Alcander	3.75	3.50
Performer	3.75	3.75
Innovator	2.50	2.50
Camel	2.50	3.25
Eurostar	2.50	3.25
Arsenal	3.75	3.50
Panther	2.00	2.25

Isd (p<0.05) 1.667

Isds are for comparison of PCN level within variety

Table 5 Height measurement (cm) from soil level to top leaf layer

	PCN	No PCN
	20/07/17	
Cara	32.5	44.2
Maris Peer	30.2	26.8
Melody	22.3	29.8
Markies	27.2	24.3
Lanorma	22.5	24.1
Alcander	34.9	30.4
Performer	30.6	30.3
Innovator	16.8	19.8
Camel	21.0	25.0
Eurostar	22.6	25.8
Arsenal	31.3	27.6
Panther	13.1	12.6

lsd(p<0.05) 10.05

lsds are for comparison of PCN level within variety

There were no significant effects of PCN on yield (kg/box) of varieties (Table 6). The growing area (ie internal dimensions) of each box was calculated as 1.1136 m<sup>2</sup>, and the corresponding tonnes/ha yield are also shown in Table 6. Average yield per plant is included, though plant numbers were almost identical in each box.

Table 6 Yield (kg/box, kg/plant, and t/ha per box equivalent

	PCN	No PCN	PCN	No PCN	PCN	No PCN
	Yield kg/box		Yield kg/plant		Yield per box t/ha equivalent	
Cara	3.81	4.55	0.34	0.43	34.2	40.9
M Peer	4.10	4.25	0.36	0.38	36.8	38.2
Markies	2.55	2.53	0.21	0.22	22.9	22.7
Melody	3.54	4.08	0.31	0.36	31.8	36.6
Lanorma	2.87	3.43	0.27	0.30	25.8	30.8
Alcander	3.73	3.53	0.31	0.31	33.5	31.7
Performer	4.61	4.26	0.40	0.38	41.4	38.3
Innovator	3.26	3.53	0.28	0.29	29.3	31.7
Camel	3.67	4.01	0.33	0.38	33.0	36.0
Eurostar	3.02	3.98	0.28	0.33	27.1	35.7
Arsenal	4.68	4.25	0.40	0.38	42.0	38.2
Panther	3.23	3.72	0.40	0.38	29.0	33.4

lsd (p<0.05) 1.015

lsd is for comparison of PCN level within variety

Size grade distribution for tuber number is shown in Table 7, and tuber weight in Table 8. There were significant reductions in total tuber number when PCN was present for

Cara, Innovator, Eurostar and Panther. However, PCN significantly increased total tuber number in some varieties.

Table 7 Size distribution (cm) for tuber numbers

	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11	Total number
Cara PCN	4.5	44.7	34.2	1.0	0.0	84.5
Cara no PCN	6.8	54.5	32.5	2.5	0.0	96.3
M Peer PCN	7.3	80.3	39.3	1.0	0.0	127.8
M Peer no PCN	11.0	80.5	39.5	2.3	0.0	133.3
Markies PCN	6.0	34.3	21.3	1.5	0.0	63.0
Markies no PCN	5.3	32.8	21.3	1.8	0.3	61.3
Melody PCN	7.3	39.5	31.0	4.3	0.0	82.0
Melody no PCN	4.8	23.3	37.0	5.5	0.0	70.5
Lanorma PCN	3.3	23.5	21.0	3.8	0.5	52.0
Lanorma no PCN	3.5	23.3	29.3	2.5	0.0	58.5
Alcander PCN	8.3	99.3	18.0	0.3	0.0	125.8
Alcander no PCN	10.0	66.5	25.0	0.8	0.0	102.3
Performer PCN	2.3	20.8	33.0	10.8	0.3	67.0
Performer no PCN	2.8	22.3	24.5	11.0	1.0	61.5
Innovator PCN	1.8	31.3	26.3	3.5	0.5	63.3
Innovator no PCN	5.0	34.3	30.0	4.0	0.0	73.3
Camel PCN	4.5	41.3	31.0	3.0	0.0	79.8
Camel no PCN	6.8	41.8	31.8	6.0	0.0	86.3
Eurostar PCN	4.8	28.0	23.8	3.3	0.0	59.8
Eurostar no PCN	16.5	44.5	28.0	7.3	0.3	96.5
Arsenal PCN	6.5	66.0	33.8	0.3	0.0	106.5
Arsenal no PCN	10.0	66.5	25.0	0.8	0.0	102.3
Panther PCN	4.0	27.0	30.5	1.8	0.0	63.3
Panther no PCN	7.5	54.0	31.0	3.5	0.0	96.0
Isd (p<0.05) between PCN level						9.26

Table 8 Size distribution (cm) tuber weight in kg

	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11
Cara PCN	0.01	1.17	2.47	0.16	0.00
Cara no PCN	0.02	1.54	2.40	0.59	0.00
M Peer PCN	0.02	1.77	2.17	0.13	0.00
M Peer no PCN	0.04	1.71	2.24	0.26	0.00
Markies PCN	0.02	0.84	1.48	0.21	0.00
Markies no PCN	0.01	0.82	1.44	0.20	0.06
Melody PCN	0.01	0.86	2.12	0.55	0.00
Melody no PCN	0.01	0.42	2.86	0.79	0.00
Lanorma PCN	0.01	0.49	1.70	0.54	0.14
Lanorma no PCN	0.01	0.46	2.56	0.41	0.00
Alcander PCN	0.02	2.46	1.21	0.04	0.00
Alcander no PCN	0.03	2.04	1.42	0.04	0.00
Performer PCN	0.00	0.46	2.52	1.57	0.06
Performer no PCN	0.01	0.43	1.83	1.70	0.31
Innovator PCN	0.01	0.73	1.93	0.49	0.11
Innovator no PCN	0.01	0.75	2.20	0.57	0.00
Camel PCN	0.01	1.07	2.15	0.44	0.00
Camel no PCN	0.01	0.95	2.21	0.83	0.00
Eurostar PCN	0.01	0.68	1.83	0.50	0.00
Eurostar no PCN	0.04	0.83	2.05	1.03	0.03
Arsenal PCN	0.02	1.91	2.71	0.04	0.00
Arsenal no PCN	0.03	1.91	2.18	0.13	0.00
Panther PCN	0.01	0.58	2.38	0.25	0.00
Panther no PCN	0.02	0.93	2.26	0.52	0.00

Final (Pf) egg/larvae per g of sampled soil are shown in Table 9 with final cyst numbers and Pf/Pi for egg larvae. Pi was calculated by assuming equal distribution of the original egg/larvae counts through the 30 cm depth that was finally sampled. Resistors had Pf/Pi values of less than 1, while varieties with lower ratings all increased the nematode. No PCN cysts were detected in the initial soil sample taken from an aggregate of all the boxes or in the single non-inoculated box sample taken at the end of the experiment.



Table 9. Cysts/kg and egg/larvae per g of soil sampled at harvest

	Mean cyst count/kg	Mean egg/larvae count/g	Pf/Pi (egg/ larvae)
Cara	38.0	93.4	25.2
M Peer	32.4	62.1	16.8
Markies	39.5	68.5	18.5
Melody	25.5	44.8	12.1
Lanorma	19.5	44.6	12.1
Alcander	2.8	0.40	0.1
Performer	1.8	0.13	0.0
Innovator	1.3	0.19	0.1
Camel	1.8	2.06	0.6
Eurostar	0.3	0.21	0.1
Arsenal	2.5	0.60	0.2
Panther	2.0	1.24	0.3

#### 4. DISCUSSION

Yields were approximately 60% of those achieved in 2016, and reflected the effects of early flooding and late blight which became established despite the blight programme. Though there were some non-significant effects of PCN on yield, these did not reflect the 2016 data, or previous indications of tolerance to PCN infection. Cara (tolerant control), and Maris Peer (intolerant control) did not show the relative yield differences expected, though both multiplied cysts according to their susceptibility rankings. There were significant effects of PCN level on tuber number, but again these did not reflect previous data and in some cases PCN increased tuber number. Despite the flooding, tubers were of relatively good quality with little tuber blight at harvest, but weights within grade classes were all lower than 2016.

#### 5. CONCLUSION (2017)

The 2017 data illustrates the risks attached to box type trials for tolerance testing, where an early environmental effect such as flooding has an over-riding impact on future growth and yield, despite cyst and egg larvae multiplication occurring in line with known variety rankings. The experiment was repeated in 2018 with improved irrigation management.

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## **APPENDIX 4: 2018: EVALUATION OF A METHOD TO ASSESS VARIETAL TOLERANCE TO POTATO CYST NEMATODE (1 JUNE 2018 – 31 JULY 2019)**

### **1. INTRODUCTION**

The 2017 trial was repeated in 2018 with the same variety set.

### **2. METHODS**

Ninety-six pallet boxes, fully perforated and with external dimensions of 120 cm x 100 cm, and an internal depth of 72 cm (DOLAV Ltd, Watton, Norfolk and Pallet Tower Ltd, Sale Cheshire) ) were lined with Mypex and filled with a sandy clay loam top-soil from Tim O'Hare Associates, Telford Way, Kettering. The company normally provides top-soil for amenity and landscape areas, and it was declared free from noxious weeds and contaminants. The supplier reported there was no known history of potato growing on the soil source. It was tested for PCN before use by taking a trowel core (approximately 15 cm depth, and 5 cm diameter) from each box before planting. Cores were combined, mixed, and a 1 kg sample tested for PCN. Chitted tubers of the varieties shown in Table 1 were planted flat in each box on 18/05/18 at a depth of about 10 cm in 3 rows of 4 tubers. Each treatment (variety and PCN level) was replicated four times in a fully randomised design.

Maris Peer tubers failed to emerge, with the issue traced to the seed stock supplied to NIAB. A further stock was sourced and planted on 08/06/18. For the PCN inoculated boxes, a small net "teabag" containing 60 *G. pallida* (Pa2/3) cysts was placed beneath each tuber. Cyst numbers were determined by a hatch test, and gave 7.06 egg/larvae per g of soil calculated over the internal growing area of the box at the planting depth of 10 cm. A fresh batch of net bags of the same cyst stock was used under the replanted Maris Peer tubers. Prilled nitrogen fertiliser (34.5% N) was applied at 500 kg/ha equivalent (60 g per box) on 01/06/18. Boxes were laid out on Mypex over grass in two blocks of 48, each block being 6 x 8 rows. Boxes were watered 1 week after planting by 8 automatic sprinklers situated at each corner of the box layout. Individual sprinklers were timed to switch on at 06.30 and 18.30 daily for 1 or 2 minute periods to maintain a soil water content between 24-25%, with an application rate between 3mm and 5mm per day. A late blight programme (Table 2) was applied with a tractor mounted sprayer, driving down a central pathway between the blocks.

Table 1 Varieties tested in 2018 with PCN resistance ranking (1-9)  
 Variety 1-9 rating (1 = susceptible) from AHDB  
 Potato Variety Database where  
 available

Cara	2
Maris Peer	2
Melody	2
Markies	2
Lanorma	5
Alcander	Resistant (breeder data)
Performer	9
Innovator	Resistant (breeder data)
Camel	9
Eurostar	9
Arsenal	8 (breeder data)
Panther	8

Table 2 Late blight programme 2018

Date	Product	Rate litres or kg/ha
07/06/18	Valbon	1.6 kg
17/06/18	Invader	2.4 kg
23/06/18	Valbon	1.6 kg
07/07/18	Valbon	1.6 kg
14/07/18	Curzate +Tizca	2 kg +0.3 l
26/07/18	Curzate +Tizca	2 kg +0.3 l
02/08/18	Infinito	1.6 l
10/08/18	Curzate +Tizca	2 kg +0.3 l
17/08/18	Infinto	1.6 l
28/08/18	Curzate +Tizca	2 kg +0.3 l
05/09/18	Revus +Tizca	0.6 l +0.3 l
13/09/18	Infinito	1.5 l

Emergence (plant counts) was assessed on 15/06/18 and 29/06/18. Canopy growth (ground cover) was assessed visually as a percentage cover per box on 06/07/18 and 03/08/18. Canopy height was assessed on the same dates by measuring the distance between the soil level and the top leaf layer at 3 locations per box. The mean was analysed.

Haulm was desiccated on 12/09/18 (Roquat and Warrior at 2 and 1.5 l respectively). Maris Peer boxes were covered with polythene due to the re-planting required, and allowed to grow on for a further four weeks. Tops were removed by hand before harvest. Produce was harvested over two weeks beginning 08/10/18.

Total produce per box was washed, weighed and then tuber numbers counted in each of the following grade classes: 0-2 cm, 2.1-5 cm, 5.1 to 8 cm, 8.1-11 cm, and >11 cm. Just before harvest, 24 X 1 cm diameter soil cores were taken per inoculated box to a

depth of approximately 30 cm. These were combined, dried, broken down and mixed thoroughly and a 1 kg sample extracted for cyst counting and subsequent egg larvae counts. A single box with no inoculation was also sampled.

### 3. RESULTS

Twelve tubers in every box had emerged by 15/06/18 with the exception of the Maris Peer boxes. However, all of these had emerged to give small, healthy plants by 06/07/18 from the new stock planted on 08/06/18. There was no significant effect of PCN level within variety on canopy growth (Table 3). Maris Peer plants had lower canopy cover estimates than the rest of the varieties at both dates due to re-planting. There was some indication that PCN was reducing ground cover in Maris Peer Markies, and Lanorma compared to Cara, but surprisingly the suspected intolerant varieties such as Innovator were almost identical in ground cover between the infection levels.

Table 3 Mean % ground cover on and 06/07/18 and 03/08/18

	PCN	No PCN	PCN	No PCN
	06/07/18		03/08/18	
Cara	95.0	93.5	72.5	71.3
M Peer	80.0	80.0	47.5	57.5
Markies	95.0	92.3	70.0	80.0
Melody	95.0	95.0	63.8	58.8
Lanorma	95.0	91.3	51.3	61.3
Alcander	92.5	92.5	51.3	53.8
Performer	92.0	91.3	45.0	51.3
Innovator	95.0	93.8	60.0	62.5
Camel	95.0	95.0	60.0	60.0
Eurostar	93.8	95.0	57.5	57.5
Arsenal	92.5	92.5	65.8	63.8
Panther	92.5	91.3	46.3	45.0
Isd (p<0.05)	4.28		15.56	

Isds are for comparison of PCN level within variety

Canopy heights were very similar between PCN treatments within varieties (Table 4) with no significant differences at either measurement date.

Table 4. Height measurement (cm) from soil level to top leaf layer  
on 06/07/18 and 03/08/18

	PCN	No PCN	PCN	No PCN
	06//07/18		03/08/18	
Cara	58.1	62.4	63.7	65.8
Maris Peer	24.9	23.8	42.7	37.7
Melody	64.7	54.8	49.3	44.9
Markies	53.8	56.9	65.2	62.9
Lanorma	48.1	46.4	37.7	34.3
Alcander	53.1	55.5	43.7	43.3
Performer	67.8	63.4	36.3	46.0
Innovator	51.1	45.3	34.5	34.2
Camel	57.2	56.1	51.8	49.7
Eurostar	64.3	59.5	51.5	41.1
Arsenal	58.7	58.9	55.2	57.6
Panther	40.3	41.0	29.0	30.1
Isd(p<0.05)	8.96		10.62	
Isds are for comparison of PCN level within variety				

There were no significant effects of PCN on yield (kg/box) of varieties (Table 5). The growing area (ie internal dimensions) of each box was calculated as 1.1136 m<sup>2</sup>, and the corresponding tonnes/ha yield are also shown in Table 5. Average yield per plant is included, though all boxes contained twelve plants. Plant growth in the boxes and examples of harvested tuber quality are shown in Figures 1 and 2.



Figure 1 Plant growth in box test, 21/06/18



Figure 2 Harvest quality

Table 5. Yield (kg/box, kg/plant, and t/ha per box equivalent)

	PCN	No PCN	PCN	No PCN	PCN	No PCN
	Yield kg/box		Yield kg/plant		Yield per box t/ha equivalent	
Cara	6.00	6.72	0.50	0.56	53.88	60.34
M Peer	4.70	5.36	0.39	0.45	42.21	48.13
Markies	8.41	6.60	0.70	0.55	75.52	59.27
Melody	8.79	9.96	0.73	0.83	78.93	89.44
Lanorma	7.57	7.54	0.63	0.63	67.98	67.71
Alcander	6.89	6.31	0.57	0.53	61.87	56.66
Performer	8.10	7.40	0.68	0.62	72.74	66.45
Innovator	7.90	7.10	0.66	0.59	70.94	63.76
Camel	7.57	7.75	0.63	0.65	67.98	69.59
Eurostar	7.82	7.42	0.65	0.62	70.22	66.63
Arsenal	8.09	9.62	0.67	0.80	72.65	86.39
Panther	5.79	6.38	0.48	0.53	51.99	57.29
lsd (p<0.05)	1.906					
lsd is for comparison of PCN level within variety						

Size grade distribution for tuber number is shown in Table 6, and tuber weight in Table 7. Differences in total tuber number between PCN level were not significant, though most varieties had slightly fewer tubers in the PCN infected treatment. There did not appear to be any differences in size grade distribution for PCN level within variety for either tuber weight or number.

Table 6. Size distribution (cm) for tuber numbers

Size class	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11	Total number
Cara PCN	17.0	71.3	50.0	1.0	0.0	139.3
Cara no PCN	15.3	70.0	56.5	4.3	0.0	146.0
M Peer PCN	39.0	201.8	20.3	0.0	0.0	261.0
M Peer no PCN	47.0	218.8	17.5	0.0	0.0	283.3
Markies PCN	15.8	61.8	64.5	10.5	1.0	153.3
Markies no PCN	17.8	63.3	54.8	10.8	1.0	147.5
Melody PCN	33.0	107.3	73.0	6.5	0.0	219.8
Melody no PCN	37.0	102.3	95.3	12.0	0.0	246.5
Lanorma PCN	17.5	40.5	51.3	14.8	1.3	125.3
Lanorma no PCN	19.0	46.0	52.0	13.0	1.0	131.0
Alcander PCN	21.0	84.8	51.3	1.0	0.0	158.0
Alcander no PCN	19.0	86.8	51.3	1.5	0.0	158.5
Performer PCN	4.8	28.5	32.3	22.5	7.8	95.8
Performer no PCN	7.0	30.0	31.5	20.8	6.8	96.0
Innovator PCN	17.0	44.5	41.5	19.3	6.8	129.0
Innovator no PCN	27.8	67.0	43.5	14.0	4.8	157.0
Camel PCN	29.0	106.5	53.5	3.8	0.3	193.0
Camel no PCN	28.3	111.0	82.0	4.3	0.3	225.8
Eurostar PCN	27.5	47.0	45.3	17.3	2.8	139.8
Eurostar no PCN	30.8	74.0	47.5	16.0	0.5	168.8
Arsenal PCN	15.0	95.8	65.8	1.8	0.0	178.3
Arsenal no PCN	19.8	86.0	65.3	5.3	0.0	176.3
Panther PCN	14.5	56.5	36.8	8.8	0.5	117.0
Panther no PCN	20.0	61.5	48.5	10.5	0.3	140.8
Isd (p<0.05) between PCN level						44.16

Table 7. Size distribution (cm) tuber weight in kg

Size class	0-2.0	2.1-5.0	5.1-8.0	8.1-11.0	>11
Cara PCN	0.04	1.71	4.06	0.18	0.00
Cara no PCN	0.03	1.50	4.45	0.73	0.00
M Peer PCN	0.11	3.40	1.19	0.00	0.00
M Peer no PCN	0.36	3.42	1.58	0.00	0.00
Markies PCN	0.04	1.19	5.14	1.76	0.29
Markies no PCN	0.04	1.01	3.52	1.79	0.25
Melody PCN	0.07	1.97	5.69	1.06	0.00
Melody no PCN	0.07	1.53	6.26	2.09	0.00
Lanorma PCN	0.19	0.75	3.81	2.39	0.43
Lanorma no PCN	0.08	0.85	3.91	2.34	0.36
Alcander PCN	0.05	2.13	4.54	0.17	0.00
Alcander no PCN	0.04	1.83	4.15	0.29	0.00
Performer PCN	0.01	0.45	2.50	3.38	1.76
Performer no PCN	0.01	0.43	2.10	3.18	1.68
Innovator PCN	0.03	0.62	2.75	2.83	1.67
Innovator no PCN	0.06	1.01	3.01	2.02	0.91
Camel PCN	0.05	1.95	4.37	1.12	0.08
Camel no PCN	0.05	2.23	4.65	0.77	0.04
Eurostar PCN	0.06	0.93	3.35	2.83	0.64
Eurostar no PCN	0.07	1.28	3.45	2.51	0.11
Arsenal PCN	0.04	2.09	5.55	0.40	0.00
Arsenal no PCN	0.06	2.04	6.48	1.04	0.00
Panther PCN	0.04	1.00	3.24	1.36	0.14
Panther no PCN	0.04	1.09	3.51	1.66	0.08

Final (Pf) egg/larvae per g of sampled soil are shown in Table 8 with final cyst numbers and Pf/Pi for egg larvae. Pi was calculated by assuming equal distribution of the original egg/larvae counts through the 30 cm depth that was finally sampled. Most resistors had Pf/Pi values of approximately 1, though Camel and Panther were higher than expected. Susceptible varieties increased the nematode to a greater extent, but with less agreement to their resistance ratings than previously observed (eg Lanorma with a rating of 5 had a much higher Pf/Pi than Cara, rated 2).



No PCN cysts were detected in the initial soil sample taken from an aggregate of all the boxes or in the single non-inoculated box sample taken at the end of the experiment. Some grass cysts (possibly *Heterodera graminis*) were found, and all cyst counts were subsequently checked to ensure only *Globodera pallida* had been counted.

Table 8. Cysts/kg and egg/larvae per g of soil sampled at harvest

	Mean cyst count/kg	Mean egg/larvae count/g	Pf/Pi (egg/ larvae)
Cara	5.8	7.9	15.58
M Peer	8.5	7.2	14.20
Markies	12.8	29.0	57.20
Melody	4.5	7.3	14.40
Lanorma	25.7	34.3	67.65
Alcander	2.0	0.6	1.18
Performer	2.0	0.8	1.58
Innovator	2.3	0.5	0.99
Camel	2.8	3.1	6.11
Eurostar	1.0	1.1	2.17
Arsenal	2.0	0.3	0.59
Panther	2.0	2.4	4.73

#### 4. DISCUSSION

Yields were approximately similar to those achieved in 2016, and were much greater than those seen in 2017 when flooding occurred in the boxes. Plants developed well in 2018 and despite the very warm and dry conditions experienced, the irrigation regime maintained good top growth. Blight was well controlled. However, unlike 2016, there were no significant effects of PCN level on yield or tuber number. In 2016, tuber numbers were approximately equally distributed between the 2.1 to 5.0 and 5.1 to 8.0 cm size categories, whereas for several varieties in 2018, the distribution was less equal, and more tubers were seen in the smaller size category. This may have been a reflection of the very high seasonal temperatures affecting bulking, and potentially this could have limited differentiation in yield between infected and non-infected boxes. It was not possible to identify any non-significant trends consistent with the 2016 data, except for slightly lower total tuber numbers on PCN infected boxes for Maris Peer, Innovator, Camel, Eurostar, Panther and Melody, whereas Cara and Alcander (putative tolerant types) had very similar tuber numbers.

The PCN multiplication was more erratic than seen in the scoping study in 2015, extended study in 2016 and even the flooding affected 2017 test. The reason for this is unknown. Extreme heat experienced in 2018 may have had an effect on nematode development, but there is no direct evidence for this. There was a high Pf/Pi ratio for Markies and Lanorma, but the former yielded more with PCN infection than without, and Lanorma gave almost exactly the same yields whether infected or not.

## **5. CONCLUSION (2015-2018)**

Initial results in the 2015 scoping study were encouraging, with Cara and Maris Peer behaving as expected (tolerant and intolerant varieties) while both multiplying the nematode. The 2016 test confirmed the Cara and Maris Peer result, indicating potential for box-type studies as a way forward for assessing tolerance, using a controlled infection level and obtaining yields comparable with those seen in commercial production. The 2017 test highlighted the sensitivity of the approach to adverse environmental influences. The 2018 test, despite achieving good growth, did not confirm previous data, and it must be concluded that the approach has proved too erratic to be considered as a future routine method for tolerance evaluation.

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