SCEPTREPLUS

Final Trial Report

Trial code:	CP165 SP72
Title:	Development of an artificial inoculation method for cavity spot in pot grown carrots in the glasshouse
Crop	Carrot
Target	*Pythium violae*
Lead researcher:	John Clarkson
Organisation:	University of Warwick
Period:	March 2020 - May 2021
Report date:	31/07/21
Report authors:	John Clarkson & Nicole Pereira
ORETO Number: (certificate should be attached)	381

I the undersigned, hereby declare that the work was performed according to the procedures herein described and that this report is an accurate and faithful record of the results obtained

23/08/21 *John Clarkson*
Date Authors signature

Trial Summary

Introduction

Previous work in AHDB projects FV 391a and FV 391b developed artificial inoculation methods for *Pythium violae*, the main cause of cavity spot of carrots. In this project, pot-based glasshouse trials were carried out using two different kinds of *P. violae* inoculum to investigate if this approach could be successfully used to identify new crop protection products and sources of carrot resistance. This was done using metalaxyl-M (SL 567A) which is registered for use against cavity spot and four carrot varieties (two resistant, two susceptible) to confirm that the artificial inoculation techniques were effective in identifying treatments that reduce disease in future trials.

Methods

Two experiments were set up to evaluate i) a millet seed *P. violae* inoculum and ii) a compost/carrot *P. violae* inoculum for their ability to induce carrot cavity spot in glasshouse pot trials and to examine the effects of metalaxyl-M and carrot variety on disease development. Each experiment tested one of the inoculum types at two concentrations (10 and 30 mg g^{-1} growing medium) and used four carrot cultivars (Criolla and T1308403, susceptible; Nairobi, moderately resistant and Eskimo, resistant) each of which was either untreated or treated with two applications of metalaxyl-M (SL 567A at 1.3L/ha in 1000 L of water applied at sowing and 3 weeks later).

Results

- Both *P. violae* inoculum types induced cavity spot with disease incidence ranging from 4-84% carrots affected across the resistant and susceptible carrot varieties (no metalaxyl-M) with the higher concentrations (30 mg g^{-1} growing medium) resulting in more disease (Table 1).
- The millet *P. violae* inoculum generally resulted in greater incidence of cavity spot than the carrot compost inoculum. However, statistical comparisons between the two inoculum types cannot be made as they were tested in separate experiments.
- The carrot varieties Criolla and T1308403 were consistently more susceptible to cavity spot than Nairobi and Eskimo (Table 2).
- Metalaxyl-M treatments significantly reduced the incidence of cavity spot for the susceptible carrot varieties Criolla and T1308403 at both levels of *P. violae* inoculum and also reduced disease at the higher level of inoculum for Nairobi and Eskimo (Table 1).
- Results on cavity spot severity followed the same pattern as results above for disease incidence (see main report).

Table 1. Effect of carrot variety and metalaxyl-M treatment on cavity spot incidence for two different types and concentrations of *P. violae* inoculum (angular transformed values)

Treatment	Cavity spot incidence (% carrots affected)			
	***P. violae* millet**		***P. violae* carrot-compost**	
	10 mg/g	**30 mg/g**	**10 mg/g**	**30 mg/g**
Uninoc. control Criolla (S)	0.0	0.0	0.0	0.0
Uninoc. control T1308403 (S)	0.0	0.0	0.0	0.0
Uninoc. control Nairobi (R)	0.0	0.0	0.0	0.0
Uninoc. control Eskimo (R)	0.0	0.0	0.0	0.0
Inoc. Criolla (S)	27.3	69.0	20.9	27.1
Inoc. T1308403 (S)	21.9	83.7	40.9	50.6
Inoc. Nairobi (R)	9.0	40.1	0.0	34.9
Inoc. Eskimo (R)	7.4	25.1	22.1	4.4
Inoc. Criolla (S) + metalaxyl-M	13.4	32.1	8.8	13.4
Inoc. T1308403 (S) + metalaxyl-M	6.0	20.9	0.0	3.0
Inoc. Nairobi (R) + metalaxyl-M	12.0	9.0	0.0	0.0
Inoc. Eskimo (R) + metalaxyl-M	0	6.0	0.0	0.0
LSD inoc. vs uninoc. treatments	16.6		15.5	
LSD metalaxyl vs untreated	13.5		12.7	

Inoculated treatments for each carrot variety that are significantly different from the corresponding uninoculated control (P<0.001)
Inoculated treatments for each carrot variety that are not significantly different from the corresponding uninoculated control
Metalaxyl-M treatments for each inoculated carrot variety that are significantly different from corresponding inoculated untreated plants (P<0.001)
Metalaxyl-M treatments for each inoculated carrot variety that are not significantly different from corresponding inoculated untreated plants
Note: statistical comparisons of cavity spot incidence between carrot varieties are illustrated in Table 2.

Table 2. Effect of carrot variety on % cavity spot incidence (CS %) for two different types and concentrations of *P. violae* inoculum (angular transformed values)

	CS %	**Criolla**	**T1308403**	**Nairobi**	**Eskimo**	**CS %**	**Criolla**	**T1308403**	**Nairobi**	**Eskimo**
	10 mg g^{-1} *P. violae* millet inoculum					**10 mg g^{-1} *P. violae* carrot/compost inoculum**				
Criolla (S)	27.3					20.9				
T1308403 (S)	21.9					40.9				
Nairobi (R)	9.0					0.0				
Eskimo (R)	7.4					22.1				
	30 mg g^{-1} *P. violae* millet inoculum					**30 mg g^{-1} *P. violae* carrot/compost inoculum**				
Criolla (R)	69.0					27.1				
T1308403 (S)	83.7					50.6				
Nairobi (R)	40.1					34.9				
Eskimo (R)	25.1					4.4				
LSD	13.5					14.2				

Significant difference in cavity spot incidence between carrot varieties (P<0.05)
No significant difference in cavity spot incidence between carrot varieties

Conclusions

A millet-based *P. violae* inoculum has been proven to induce high levels of cavity spot and is suitable for use in glasshouse pot tests to identify crop protection products or carrot varieties that reduce disease.

Objectives

To evaluate i) a millet seed *P. violae* inoculum and ii) a compost/carrot *P. violae* inoculum for their ability to induce carrot cavity spot in glasshouse pot trials and to examine the effects of metalaxyl-M and carrot variety on disease development. This was to confirm that the artificial inoculation techniques were effective in identifying treatments that could reduce disease in future trials.

Trial conduct

Preparation of *P. violae* inoculum

<u>Millet seed inoculum:</u> A millet seed substrate was prepared by mixing 70 g white millet seed with water in 500 ml conical flasks to give a final moisture content of 62% w/w. Flasks were autoclaved three times at 121°C for 30 min with 24 h in-between each cycle. The sterile millet substrate was then inoculated with 14 agar plugs from actively growing cultures of *P. violae* isolate HL path 19 and incubated at 20°C in the dark for three weeks.

<u>Carrot/compost inoculum:</u> A compost / carrot substrate was prepared by mixing 120 g Levington F1 compost (sieved to 4 mm) with water in 500 ml conical flasks to give a final moisture content of 80% w/w. Finely grated organic carrot (50 g) was then added before the flasks were autoclaved twice at 121°C for 15 min with 24 h in-between each cycle. The sterile carrot / compost substrate was then inoculated with 14 agar plugs from actively growing cultures of *P. violae* isolate HL path 19 and incubated at 20°C in the dark for three weeks.

Prior to experimental set up, batches of both types of *P. violae* inocula were autoclaved for 1 h to provide 'dead inoculum' control treatments (uninoculated control treatments). Small samples of both live and dead (autoclaved) inocula were also plated onto corn meal agar and incubated for 5 days at 20°C to confirm *P. violae* had successfully colonised the substrates and that no live pathogen remained following autoclaving of inoculum for the dead inoculum controls.

Carrot cultivars and experiment treatment structure

Two experiments were set up using either millet seed or carrot / compost *P. violae* inoculum with the same structure. Each was set up with four carrot cultivars assessed previously for resistance to cavity spot in inoculated field 'macrocosms' as part of the Defra-funded VeGIN project. These were cv. Criolla (highly susceptible), T1308403 (highly susceptible), the industry standard cv. Nairobi (moderately resistant) and cv. Eskimo (highly resistant). Millet or carrot / compost *P. violae* inoculum concentrations of 10 mg and 30 mg g^{-1} growing medium were tested for each cultivar and in addition, the effect of the fungicide metalaxyl-M was also assessed across all carrot cultivar / inoculum concentration treatment combinations and compared with an untreated control. Each experiment consisted of 160 pots; this comprised 16 treatments (4 carrot varieties x 2 *P. violae* inoculum concentrations with and without metalaxyl-M application) with 8 replicate pots of 6 carrots per treatment (128 pots, 48 carrots per treatment) while uninoculated control treatments comprised four replicate pots of 10 mg and 30 mg g^{-1} dead (autoclaved) inoculum for each carrot variety (32 pots).

Inoculation of growing medium, experimental set-up and maintenance

A 50:50 v/v mix of sieved compost (John Innes No. 3, Erin, UK) and a horticultural grade sharp sand (Westland, UK) was used as the growing medium in both experiments. Appropriate amounts of each *P. violae* inoculum type were mixed with the compost/sand growing medium in a cement mixer to obtain concentrations of 10 and 30 mg g^{-1} growing medium. Tall plastic pots (5.5 L capacity) were filled with infested growing medium (5.65 kg per pot) at each inoculum concentration. Control pots received a mixture of 50:50 v/v compost/sand and autoclaved (dead) inoculum at the same concentrations of 10 and 30 mg g^{-1} growing medium. Pots were placed in deep saucers in a randomised block design within a 20 m^2 glasshouse compartment. (max 18°C, min 16°C). Supplementary lighting was used from 5:00 to 20:00 h to extend day length and pots watered to ensure a high moisture content before sowing with untreated carrot seed (25 seeds per pot). Spray applications of SL 567A (metalaxyl-M) were made at a rate of 1.3 L/ha in 1000 L water to the soil surface just after sowing and again 3 weeks post-sowing. The growing medium was kept damp by gentle overhead watering with additional watering into the saucers as carrots developed. After 5 weeks, seedlings were thinned out to six per pot.

Assessment of cavity spot symptoms

Mature carrot roots plants were harvested after 18 weeks (July 2020 and February 2021 for the millet and carrot/compost experiments, respectively) and each assessed for presence / absence of cavity spot symptoms (incidence) and also the number of lesions present per carrot (severity).

Statistical design and analysis

Each experiment occupied a single glasshouse compartment with the 160 pots per experiment organized into 8 blocks of 20 pots each, the blocks being arranged in 2 columns and 4 rows on two benches running along the length of the compartment. Within each block the 20 pots were arranged in 5 rows and 4 columns (i.e. 4 pots across the width of the bench). The four varieties were classified into two susceptible (Criolla, T1308403) and two resistant (Nairobi, Eskimo) types. The susceptible and resistant types were allocated to 4 blocks each, allowing for potential variation between the two sides of the glasshouse compartment and for variation along the length of the benches, with each block containing one pot of the inoculated control and two pots for each of the combinations of *P. violae* inoculum rate and +/- metalaxyl-M for each of the two varieties included.

Data from the experiment were analysed using ANOVA, taking account of the blocking structure described and extracting information about the differences between variety types (susceptible v. resistant), between varieties within type, and between the untreated control and the different combinations of inoculum rate and metalaxyl-M treatment (+/-). Disease incidence was expressed as the percentage of roots with cavities, and subjected to an angular transformation prior to analysis to satisfy the ANOVA assumptions of homogeneity of variance and normality. Disease severity was expressed as the mean number of cavities per root and subjected to a logarithm transformation prior to analysis, again to satisfy the ANOVA assumptions.

Test site

Item	Details
Location address	Warwick Crop Centre, University of Warwick, Wellesbourne Campus
Crop	Carrot
Cultivar	Criolla, T1308403, Nairobi, Eskimo
Soil or substrate type	John Innes No. 3 + sand
Agronomic practice	N/A
Prior history of site	N/A

Trial design

Item	Details
Trial design:	Split factorial design
Number of replicates:	8 pots of 6 carrots per treatment (4 pots for uninoculated controls)
Row spacing:	N/A
Plot size: (w x l)	5.5 L pots
Plot size: (m^2)	N/A
Number of plants per plot:	6

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
N/A	Metalaxyl-M	S L567A	SM05K0046	465.2 g / L	Soluble liquid	None

Application schedule

Treatment number	Treatment: product name or AHDB code	Rate of active substance (ml or g a.s./ha)	Rate of product (l or kg/ha)	Application code
	Metalaxyl-M	604.8 g / ha	1.3 L / ha	A, B

Application details

	Application A	Application B	Application A	Application B
	Millet *P. violae* inoculum experiment		Carrot/compost *P. violae* inoculum experiment	
Application date	13/03/2020	31/03/2020	16/10/2020	5/11/2020
Time of day	10:30	10:30	10:30	10:30
Crop growth stage (Max, min average BBCH)	01	11	01	11
Crop height (cm)	N/A	2 cm	N/A	2 cm
Crop coverage (%)	0	10-20%	0	10-20%
Application Method	Spray	Spray	Spray	Spray
Application Placement	Soil	Soil/foliage	Soil	Soil/foliage
Application equipment	Bethoud Vermorel 2000 HP			
Nozzle pressure	2 Bar	2 Bar	2 Bar	2 Bar
Nozzle type	05F110	05F110	05F110	05F110
Nozzle size	05	05	05	05
Application water volume/ha	1000	1000	1000	1000
Temperature of air - shade (°C)	N/A	N/A	N/A	N/A
Relative humidity (%)	N/A	N/A	N/A	N/A
Wind speed range (m/s)	N/A	N/A	N/A	N/A
Dew presence (Y/N)	N/A	N/A	N/A	N/A
Temperature of soil - 2-5 cm (°C)	Not recorded	Not recorded	Not recorded	Not recorded
Wetness of soil - 2-5 cm	Damp	Damp	Damp	Damp
Cloud cover (%)	N/A	N/A	N/A	N/A

Untreated levels of pests/pathogens at application and through the assessment period

Common name	Scientific Name	EPPO Code	Infestation level pre-application	Infestation level at start of assessment period	Infestation level at end of assessment period
Cavity spot	*Pythium violae*	PYTHVI	Artificial inoculation at 10 or 30 mg g^{-1} growing medium	N/A	N/A

Assessment details

Evaluation date	Evaluation Timing	Crop Growth Stage (BBCH)	Evaluation type	Assessment
13/07/2020	18 weeks after sowing	49	Efficacy	Cavity spot incidence and severity
18/02/2021	18 weeks after sowing	49	Efficacy	Cavity spot incidence and severity

Results

Effect of *P. violae* inoculum concentration

Overall, there was a significant effect of pathogen inoculation (inoculated vs uninoculated treatments; $P<0.001$) for both types of *P. violae* inoculum and mean cavity spot incidence ranged from 4-84% carrots affected across the different varieties when not treated with metalaxyl-M (Table 3, Fig. 1). There was also a significant overall effect of inoculum concentration ($P<0.001$) for both *P. violae* inoculum types, with the higher concentration of 30 mg g^{-1} generally resulting in a greater incidence of cavity spot than the 10 mg g^{-1} concentration (Table 3, Fig. 1). The millet *P. violae* inoculum generally resulted in greater incidence of cavity spot and a more consistent effect than the carrot compost inoculum although statistical comparisons between inoculum types cannot be made as they were tested in separate experiments. Hence, descriptions of the effects of treatments below focus on the millet *P. violae* inoculum. The same pattern of results as observed for cavity spot incidence was also observed for disease severity (Table 4), where the mean number of cavity spot lesions ranged from 0.03-3 lesions per carrot across the different varieties not treated with metalaxyl-M (Fig. 2).

Effect of metalaxyl-M

Overall, metalaxyl-M treatments significantly reduced the incidence of cavity spot across the carrot varieties ($P<0.001$) for both *P. violae* inoculum types compared with untreated inoculated carrots. For instance for Criolla, disease was reduced from 27% to 13% carrots affected and from 69 to 32% carrots affected for the millet *P. violae* inoculum at the 10 and 30 mg g^{-1} concentrations, respectively (Table 3, Fig. 1). For T1308403, disease was reduced from 22% to 6% carrots affected and from 84 to 21% carrots affected for the low and high millet *P. violae* concentrations, respectively (Table 3, Fig. 1, Appendix Fig. 7). There were significant interactions between metalaxyl-M and carrot variety ($P<0.001$) and metalaxyl-M and *P. violae* inoculum rate ($P<0.001$) such that effect of the fungicide tended to be less evident or non-significant for the resistant varieties Nairobi and Eskimo at the lower inoculum level (Table 3, Fig. 1). However, at the higher *P. violae* millet inoculum level of 30 mg g^{-1}, metalaxyl-M significantly reduced cavity spot incidence from 40% to 9% for Nairobi and from 25% to 6% for Eskimo (Table 3, Fig. 1, Appendix Fig. 8). The same pattern of results as observed for cavity spot incidence was also observed for severity (Table 4, Fig. 2), where for instance metalaxyl-M reduced the mean number of cavity spot lesions from 3.1 to 0.2 lesions per carrot for the susceptible variety T1308403 using the millet inoculum at 30 mg g^{-1} (Fig. 2).

Effect of carrot variety

There was an overall significant effect of carrot variety across the *P. violae* inoculated treatments for both inoculum types ($P<0.001$). The susceptible carrot varieties Criolla and T1308403 consistently resulted in more disease than the resistant Nairobi and Eskimo and this was particularly apparent for *P. violae* inoculum levels of 10 mg g^{-1} (Table 5, Fig. 1, Appendix Fig. 4, Fig. 5, Fig. 6). For instance, for the millet *P. violae* inoculum at 10 mg g^{-1}, cavity spot incidence was 27 and 22% respectively for the susceptible varieties Criolla and T1308403 compared with 9% and 7% for Nairobi and Eskimo. At the 30 mg g^{-1} millet inoculum concentration, disease incidence was 69 and 84% respectively for the susceptible varieties Criolla and T1308403 compared with 40% and 25% for Nairobi and Eskimo (Table 5, Fig. 1, Appendix Fig. 4, Fig. 5). There was also a significant interaction between carrot variety and inoculum ($P<0.001$) such that the more resistant Eskimo and Nairobi resulted in fewer lesions compared to the corresponding uninoculated controls than the more susceptible Criolla and T1308403, especially at the lower rate of inoculum (Table 5). The same pattern of results was also observed for disease severity (Table 6) but it was also noted that both susceptible carrot varieties Criolla and T1308403 had larger and more expanded lesions than the resistant varieties Nairobi and Eskimo (Appendix Fig. 5).

Table 3. Effect of carrot variety and metalaxyl-M treatment on cavity spot incidence for two different types and concentrations of *P. violae* inoculum (angular transformed values)

Treatment	**Cavity spot incidence (% carrots affected)**			
	***P. violae* millet**		***P. violae* carrot-compost**	
	10 mg/g	**30 mg/g**	**10 mg/g**	**30 mg/g**
Uninoc. control Criolla (S)	0.0	0.0	0.0	0.0
Uninoc. control T1308403 (S)	0.0	0.0	0.0	0.0
Uninoc. control Nairobi (R)	0.0	0.0	0.0	0.0
Uninoc. control Eskimo (R)	0.0	0.0	0.0	0.0
Inoc. Criolla (S)	27.3	69.0	20.9	27.1
Inoc. T1308403 (S)	21.9	83.7	40.9	50.6
Inoc. Nairobi (R)	9.0	40.1	0	34.9
Inoc. Eskimo (R)	7.4	25.1	22.1	4.4
Inoc. Criolla (S) + metalaxyl-M	13.4	32.1	8.8	13.4
Inoc. T1308403 (S) + metalaxyl-M	6.0	20.9	0.0	3.0
Inoc. Nairobi (R) + metalaxyl-M	12.0	9.0	0.0	0.0
Inoc. Eskimo (R) + metalaxyl-M	0	6.0	0.0	0.0
LSD inoc. vs uninoc. treatments	16.6		15.5	
LSD metalaxyl-M vs untreated	13.5		12.7	

Inoculated treatments for each carrot variety that are significantly different from the corresponding uninoculated control ($P<0.001$)

Inoculated treatments for each carrot variety that are not significantly different from the corresponding uninoculated control

Metalaxyl-M treatments for each inoculated carrot variety that are significantly different from corresponding inoculated untreated plants ($P<0.001$)

Metalaxyl-M treatments for each inoculated carrot variety that are not significantly different from corresponding inoculated untreated plants

Note: statistical comparisons of cavity spot incidence between carrot varieties are illustrated in Table 5.

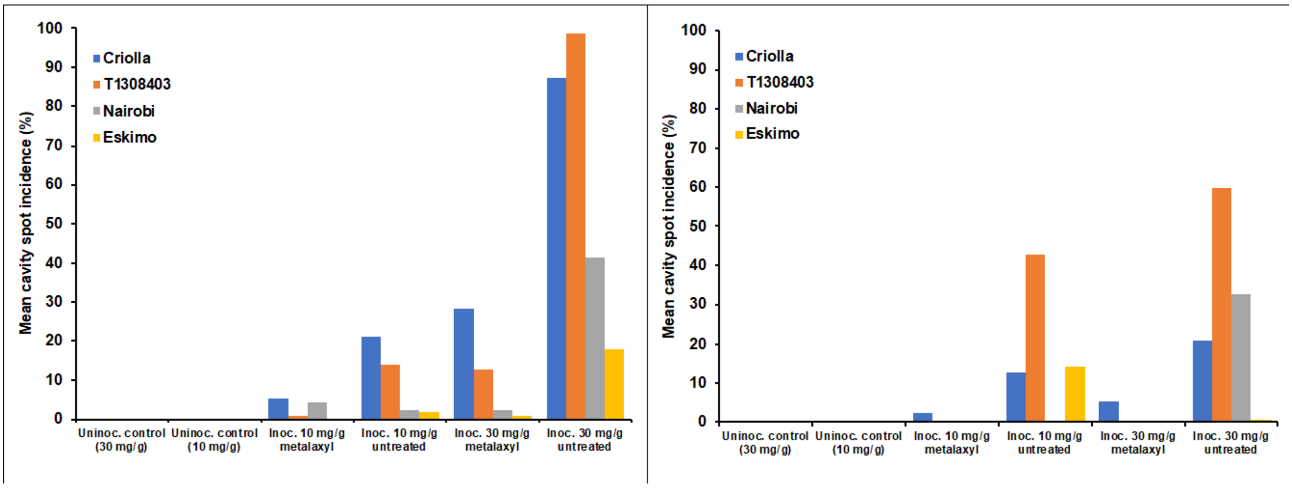

Figure 1. Effect of carrot variety and metalaxyl-M treatment on cavity spot incidence (back transformed values) for a) millet *P. violae* inoculum and b) carrot/compost *P. violae* inoculum

Table 4. Effect of carrot variety and metalaxyl-M treatment on cavity spot severity for two different types and concentrations of *P. violae* inoculum (log transformed values)

Treatment	Cavity spot severity (mean no. lesions/carrot)			
	P. violae millet		*P. violae* carrot-compost	
	10 mg/g	30 mg/g	10 mg/g	30 mg/g
Uninoc. control Criolla (S)	-2.30	-2.30	-2.30	-2.30
Uninoc. control T1308403 (S)	-2.30	-2.30	-2.30	-2.30
Uninoc. control Nairobi (R)	-2.30	-2.30	-2.30	-2.30
Uninoc. control Eskimo (R)	-2.30	-2.30	-2.30	-2.30
Inoc. Criolla (S)	-0.90	0.93	-1.23	-1.05
Inoc. T1308403 (S)	-1.27	1.15	-0.33	-0.04
Inoc. Nairobi (R)	-1.94	-0.25	-2.30	-0.83
Inoc. Eskimo (R)	-2.00	-1.18	-1.34	-2.12
Inoc. Criolla (S) + metalaxyl-M	-1.75	-0.82	-1.95	-1.75
Inoc. T1308403 (S) + metalaxyl-M	-2.06	-1.34	-2.30	-2.18
Inoc. Nairobi (R) + metalaxyl-M	-1.66	-1.81	-2.30	-2.30
Inoc. Eskimo (R) + metalaxyl-M	-2.30	-2.06	-2.30	-2.30
LSD inoc. vs uninoc. treatments	0.81		0.71	
LSD metalaxyl-M vs untreated	0.66		0.58	

Inoculated treatments for each carrot variety that are significantly different from the corresponding uninoculated control (P<0.001)

Inoculated treatments for each carrot variety that are not significantly different from the corresponding uninoculated control

Metalaxyl-M treatments for each inoculated carrot variety that are significantly different from corresponding inoculated untreated plants (P<0.001)

Metalaxyl-M treatments for each inoculated carrot variety that are not significantly different from corresponding inoculated untreated plants

Note: statistical comparisons of cavity spot severity between carrot varieties are illustrated in Table 6.

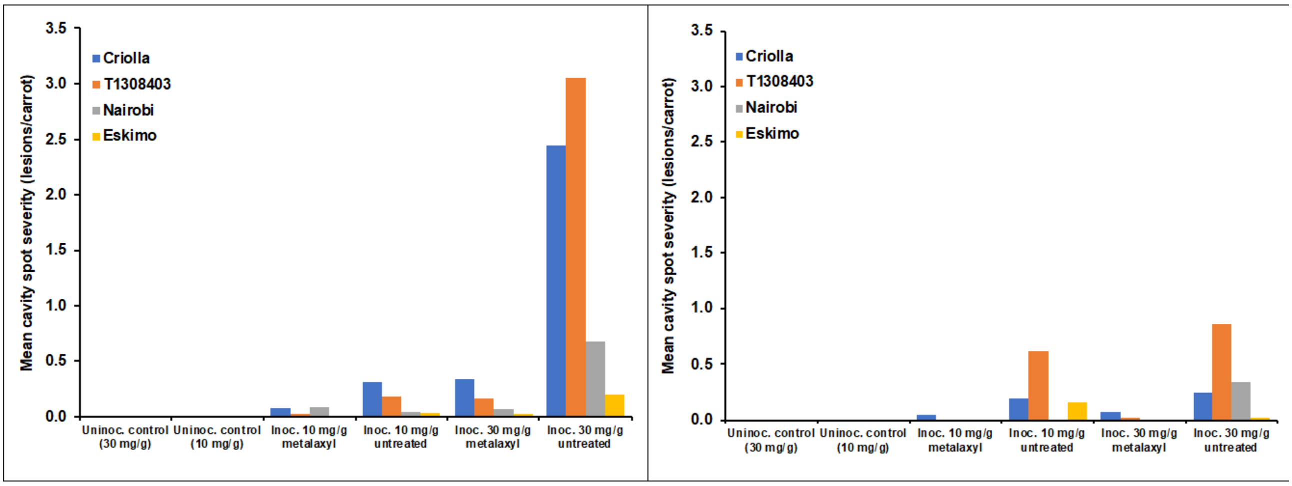

Figure 2. Effect of carrot variety and metalaxyl-M treatment on cavity spot severity (back transformed value) for a) millet *P. violae* inoculum and b) carrot/compost *P. violae* inoculum

Table 5. Effect of carrot variety on cavity spot incidence (CS %) for two different types and concentrations of *P. violae* inoculum (angular transformed values)

	CS %	Criolla	T1308403	Nairobi	Eskimo	CS %	Criolla	T1308403	Nairobi	Eskimo
	10 mg g^{-1} *P. violae* millet inoculum					10 mg g^{-1} *P. violae* carrot/compost inoculum				
Criolla (S)	27.3					20.9				
T1308403 (S)	21.9					40.9				
Nairobi (R)	9.0					0.0				
Eskimo (R)	7.4					22.1				
	30 mg g^{-1} *P. violae* millet inoculum					30 mg g^{-1} *P. violae* carrot/compost inoculum				
Criolla (R)	69.0					27.1				
T1308403 (S)	83.7					50.6				
Nairobi (R)	40.1					34.9				
Eskimo (R)	25.1					4.4				
LSD	13.5					14.2				

Significant difference in cavity spot incidence between carrot varieties (P<0.05)
No significant difference in cavity spot incidence between carrot varieties

Table 6. Effect of carrot variety on cavity spot severity (mean. no. of lesions per carrot, CS-S) for two different types and concentrations of *P. violae* inoculum (log transformed values)

	CS-S	Criolla	T1308403	Nairobi	Eskimo	CS-S	Criolla	T1308403	Nairobi	Eskimo
	10 mg g^{-1} *P. violae* millet inoculum					10 mg g^{-1} *P. violae* carrot/compost inoculum				
Criolla (S)	-0.90					-1.23				
T1308403 (S)	-1.27					-0.33				
Nairobi (R)	-1.94					-2.30				
Eskimo (R)	-2.00					-1.34				
	30 mg g^{-1} *P. violae* millet inoculum					30 mg g^{-1} *P. violae* carrot/compost inoculum				
Criolla (R)	0.93					-1.1				
T1308403 (S)	1.15					0.0				
Nairobi (R)	-0.25					-0.8				
Eskimo (R)	-1.18					-2.1				
LSD	13.5					14.2				

Significant difference in cavity spot incidence between carrot varieties (P<0.001)
No significant difference in cavity spot incidence between carrot varieties

Discussion

These results confirmed that artificial inoculation with a *P. violae* millet grain inoculum induces high levels of cavity spot and also showed that a carrot / compost inoculum could also initiate disease, although this was slightly less effective. These *P. violae* inocula primarily contain mycelium as well as some oospores and appear to induce cavity spot much more consistently than was observed previously in AHDB projects FV391a and FV391b for a *P. violae* sand / oat inoculum which largely contained only oospores. Importantly, the effects of both metalaxyl-M treatment and susceptible / resistant carrot varieties could be clearly observed using either *P. violae* millet grain or carrot / compost inocula which demonstrates for the first time that this type of inoculation approach is suitable for testing the activity of new crop protection products or carrot varieties for their ability to reduce cavity spot.

Overall, the millet grain *P. violae* inoculum resulted in slightly greater and more consistent disease development than the carrot / compost inoculum and hence this would the preferable approach for testing both crop protection products and potentially resistant carrot varieties. However, the concentration of this inoculum would need to be carefully considered in future tests. Concentrations of both 10 mg and 30 mg g^{-1} millet *P. violae* inoculum generally resulted in significant differences in disease being observed between resistant and susceptible carrot varieties, but the higher concentration allowed more differences to be observed within susceptible or resistant types which may be beneficial in ranking varieties more efficiently for cavity spot resistance in the future. Similarly, for the 10 mg g^{-1} concentration of the millet grain *P. violae* inoculum, the activity of metalaxyl-M was only significant for the susceptible varieties Criolla and T1308403 and was not evident for the moderately resistant Nairobi or highly resistant Eskimo. In contrast, the 30 mg g^{-1} concentration of inoculum resulted in significant reductions in disease levels being detected across all carrot varieties for the metalaxyl-M treatments. This therefore suggests that either very susceptible carrot varieties should be used to test crop protection products at the lower level of *P. violae* inoculum or that the higher level of inoculum should be used for more resistant varieties which includes the widely grown commercial variety Nairobi, which would have more commercial relevance.

Conclusions

A millet-based *P. violae* inoculum has been proven to induce high levels of cavity spot and is suitable for use in glasshouse pot tests to identify crop protection products or carrot varieties that reduce disease.

Acknowledgements

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Appendix

Trial diaries

Key dates for AHDB Sceptre+ cavity spot experiment with *P. violae* millet inoculum	
Date	**Experimental details**
18 Feb 2020	Millet seed inoculated with *P. violae* isolate HL Path 19
4 March 2020	Portion of millet inoculum autoclaved for dead inoculum controls
9 March 2020	Inoculation of sand/compost with dead inoculum and set up of 32 control pots
10-11 March 2020	Inoculation of sand/compost with live *P. violae* millet inoculum and set up of 128 live inoculum pots
12-13 March 2020	Control pots & *P.violae* inoculated pots sown with 4 carrot varieties
13 March 2020	First spray application of metalaxyl to 64 live inoculum pots
31 March 2020	Second spray application of metalaxyl to carrot seedlings
24 March -14 April 2020	Germination scored weekly
14 April 2020	Seedlings thinned to 6/pot
15 May 2020	Application of Movento to foliage to control aphids in compartment
18 May 2020	Liquid feed (2N:1P:4K) applied to carrots (weekly until harvest)
13-22 July 2020	Carrots harvested & scored for cavity spot incidence and severity

Key dates for AHDB Sceptre+ cavity spot experiment with *P. violae* carrot/compost inoculum	
Date	**Experimental details**
21 Sept 2020	Carrot/compost substrate inoculated with *P. violae* isolate HL Path 19
8 Oct 2020	Portion of carrot/compost inoculum autoclaved for dead inoculum controls
12 Oct 2020	Inoculation of sand/compost with dead inoculum and set up of 32 control pots
13-14 Oct 2020	Inoculation of sand/compost with live *P. violae* carrot/compost inoculum and set up of 128 live inoculum pots
15-16 Oct 2020	Control pots & *P.violae* inoculated pots sown with 4 carrot varieties
16 Oct 2020	First spray application of metalaxyl to 64 live inoculum pots
5 Nov 2020	Second spray application of metalaxyl to carrot seedlings
27 Oct – 9 Nov 2021	Germination scored weekly
16 Nov 2020	Seedlings thinned to 6/pot
14 Dec 2020	Liquid feed (2N:1P:4K) applied to carrots (weekly until harvest)
16-24 Feb 2020	Carrots harvested and scored for cavity spot incidence and severity

Trial images

Figure 3. Pot grown carrots in the glasshouse inoculated with *P. violae* millet inoculum (May 2020 at 9 weeks after sowing) and b) *P. violae* carrot/compost inoculum (Dec 2020 at 11 weeks after sowing)

Figure 4. Severe cavity spot symptoms observed in two the susceptible carrot varieties, a) T1308403 and b) Criolla, following inoculation with *P. violae* HL Path 19 millet inoculum at 30 mg g^{-1}. Less severe symptoms observed in the two resistant carrot varieties c) Nairobi and d) Eskimo.

Figure 5. Expanded, severe cavity spot lesions observed in a) susceptible carrot variety T1308403 T1308003 compared with b) more restricted lesions in resistant Eskimo variety following inoculation with *P.violae* HL Path 19 millet inoculum.

Figure 6. Cavity spot symptoms observed for the two susceptible carrot varieties, a) T1308403 and b) Criolla, following inoculation with *P. violae* HL Path 19 carrot/compost inoculum at 30 mg g^{-1}. Less severe symptoms were observed in the more resistant carrot varieties c) Nairobi and d) Eskimo.

Figure 7. Effect of metalaxyl-M on cavity spot symptoms observed for the susceptible carrot variety T1308403 following inoculation with *P. violae* HL path 19 millet inoculum at 30 mg g^{-1}; a) uninoculated carrots (dead inoculum control), b) treated with metalaxyl-M and c) untreated inoculated control (no metalaxyl-M).

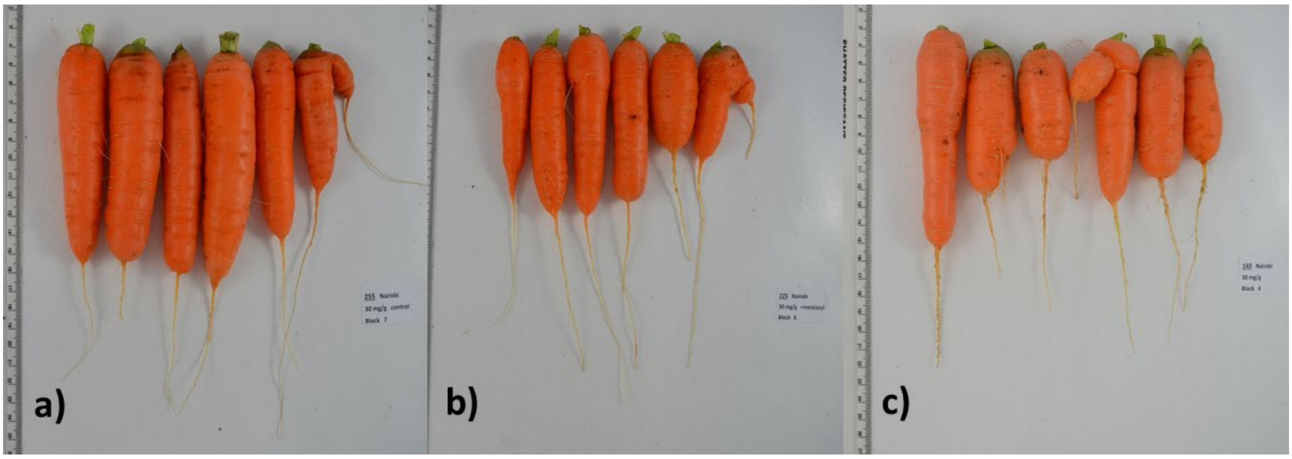

Figure 8. Effect of metalaxyl-M on cavity spot symptoms observed for the partially resistant carrot variety Nairobi following inoculation with *P. violae* HL path 19 millet inoculum at 30 mg g^{-1}; a) uninoculated carrots (dead inoculum control), b) treated with metalaxyl-M and c) untreated inoculated control (no metalaxyl-M).

Raw data

P. violae millet inoculum								
block!	var_type!	variety!	rate!	inoc_type!	treat!	Number	No_carrots_ with_cavities	Total_cavities
1	resistant	Eskimo	10 mg/g	Dead	control	6	0	0
1	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	1	1
1	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Eskimo	30 mg/g	Dead	control	6	0	0
1	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	1	1
1	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	1	1
1	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	1	1
1	resistant	Nairobi	10 mg/g	Dead	control	6	0	0
1	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	1	1
1	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	1	1
1	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	1	1
1	resistant	Nairobi	30 mg/g	Dead	control	6	0	0
1	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	3	5
1	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	5	14
1	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	1	2
1	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	1	2
1	susceptible	T1308403	10 mg/g	Dead	control	6	0	0
1	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	1
1	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	1
1	susceptible	T1308403	10 mg/g	Live	+metalaxyl	7	0	0
1	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	T1308403	30 mg/g	Dead	control	6	0	0
1	susceptible	T1308403	30 mg/g	Live	-metalaxyl	1	1	1
1	susceptible	T1308403	30 mg/g	Live	-metalaxyl	5	5	45
1	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	1	1
1	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	Criolla	10 mg/g	Dead	control	6	0	0
1	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	0	0
1	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	3	7
1	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	1	1
1	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	2	2
1	susceptible	Criolla	30 mg/g	Dead	control	6	0	0
1	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	6	16
1	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	5	14
1	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1
1	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	2	2
2	resistant	Eskimo	10 mg/g	Dead	control	6	0	0
2	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
2	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
2	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	30 mg/g	Dead	control	6	0	0
2	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	1	1
2	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	1	2
2	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Dead	control	6	0	0
2	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	1	1
2	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	30 mg/g	Dead	control	6	0	0

2	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	4	10
2	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	2	3
2	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	10 mg/g	Dead	control	6	0	0
2	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	1
2	susceptible	T1308403	10 mg/g	Live	-metalaxyl	7	2	3
2	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	T1308403	30 mg/g	Dead	control	5	0	0
2	susceptible	T1308403	30 mg/g	Live	-metalaxyl	4	4	25
2	susceptible	T1308403	30 mg/g	Live	-metalaxyl	5	4	10
2	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	Criolla	10 mg/g	Dead	control	6	0	0
2	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	0	0
2	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	1
2	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	Criolla	30 mg/g	Dead	control	6	0	0
2	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	5	39
2	susceptible	Criolla	30 mg/g	Live	-metalaxyl	2	1	1
2	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	2	2
3	resistant	Eskimo	10 mg/g	Dead	control	6	0	0
3	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	2	2
3	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Dead	control	6	0	0
3	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	1	1
3	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	2	3
3	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Dead	control	6	0	0
3	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	1	5
3	resistant	Nairobi	30 mg/g	Dead	control	6	0	0
3	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	1	2
3	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	1	1
3	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	10 mg/g	Dead	control	6	0	0
3	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	1
3	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	3
3	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	30 mg/g	Dead	control	6	0	0
3	susceptible	T1308403	30 mg/g	Live	-metalaxyl	3	3	9
3	susceptible	T1308403	30 mg/g	Live	-metalaxyl	3	3	5
3	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	1	2
3	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	1	1
3	susceptible	Criolla	10 mg/g	Dead	control	6	0	0
3	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	5	14
3	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	2
3	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	Criolla	30 mg/g	Dead	control	6	0	0
3	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	6	26

3	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	4	8
3	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	3
3	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Dead	control	6	0	0
4	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Dead	control	6	0	0
4	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	1	1
4	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	3	3
4	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Dead	control	6	0	0
4	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	1	1
4	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	1	1
4	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	30 mg/g	Dead	control	6	0	0
4	resistant	Nairobi	30 mg/g	Live	-metalaxyl	5	3	7
4	resistant	Nairobi	30 mg/g	Live	-metalaxyl	4	2	3
4	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	10 mg/g	Dead	control	6	0	0
4	susceptible	T1308403	10 mg/g	Live	-metalaxyl	7	1	1
4	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	0	0
4	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	30 mg/g	Dead	control	4	0	0
4	susceptible	T1308403	30 mg/g	Live	-metalaxyl	4	4	19
4	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	5	16
4	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	2	2
4	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	2	3
4	susceptible	Criolla	10 mg/g	Dead	control	6	0	0
4	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	2	4
4	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	1
4	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	1	1
4	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	Criolla	30 mg/g	Dead	control	5	0	0
4	susceptible	Criolla	30 mg/g	Live	-metalaxyl	5	3	7
4	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	6	40
4	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	6	39
4	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1
P. violae carrot/compost inoculum								
bench!	**var_type!**	**variety!**	**rate!**	**inoc_type!**	**treat!**	**Number**	**No_carrots_ with_cavities**	**Total_cavities**
1	resistant	Eskimo	10 mg/g	Dead	Dead	6	0	0
1	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Eskimo	30 mg/g	Dead	Dead	6	0	0
1	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	2	2
1	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Nairobi	10 mg/g	Dead	Dead	6	0	0
1	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Nairobi	10 mg/g	Live	+metalaxyl	5	0	0
1	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0

1	resistant	Nairobi	30 mg/g	Dead	Dead	6	0	0
1	resistant	Nairobi	30 mg/g	Live	-metalaxyl	7	2	2
1	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	0	0
1	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
1	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	T1308403	10 mg/g	Dead	Dead	7	0	0
1	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	3	6
1	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	4	10
1	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	T1308403	10 mg/g	Live	+metalaxyl	7	0	0
1	susceptible	T1308403	30 mg/g	Dead	Dead	6	0	0
1	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	3	10
1	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	4	8
1	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	Criolla	10 mg/g	Dead	Dead	6	0	0
1	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	0	0
1	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	1
1	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	Criolla	30 mg/g	Dead	Dead	6	0	0
1	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	2	2
1	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	0	0
1	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	0	0
1	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	10 mg/g	Dead	Dead	6	0	0
2	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	1	2
2	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	1	1
2	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	30 mg/g	Dead	Dead	6	0	0
2	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
2	resistant	Eskimo	30 mg/g	Live	-metalaxyl	7	0	0
2	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Dead	Dead	7	0	0
2	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Live	-metalaxyl	7	0	0
2	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	30 mg/g	Dead	Dead	6	0	0
2	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	4	5
2	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	3	4
2	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
2	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	10 mg/g	Dead	Dead	6	0	0
2	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	1	1
2	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	0	0
2	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	30 mg/g	Dead	Dead	6	0	0
2	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	0	0
2	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	0	0
2	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	Criolla	10 mg/g	Dead	Dead	6	0	0
2	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	2	3
2	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	4
2	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
2	susceptible	Criolla	30 mg/g	Dead	Dead	6	0	0

2	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	2	3
2	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	0	0
2	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1
2	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	10 mg/g	Dead	Dead	6	0	0
3	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	1	1
3	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	2	2
3	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Dead	Dead	6	0	0
3	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Eskimo	30 mg/g	Live	+metalaxyl	7	0	0
3	resistant	Nairobi	10 mg/g	Dead	Dead	5	0	0
3	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Nairobi	30 mg/g	Dead	Dead	6	0	0
3	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	2	2
3	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	3	4
3	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
3	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	10 mg/g	Dead	Dead	6	0	0
3	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	4	4
3	susceptible	T1308403	10 mg/g	Live	-metalaxyl	7	4	10
3	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	10 mg/g	Live	+metalaxyl	7	0	0
3	susceptible	T1308403	30 mg/g	Dead	Dead	6	0	0
3	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	2	2
3	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	6	15
3	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
3	susceptible	Criolla	10 mg/g	Dead	Dead	6	0	0
3	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	1
3	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	2	3
3	susceptible	Criolla	10 mg/g	Live	+metalaxyl	7	0	0
3	susceptible	Criolla	10 mg/g	Live	+metalaxyl	7	1	1
3	susceptible	Criolla	30 mg/g	Dead	Dead	6	0	0
3	susceptible	Criolla	30 mg/g	Live	-metalaxyl	7	1	1
3	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	4	8
3	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1
3	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Dead	Dead	6	0	0
4	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	1	1
4	resistant	Eskimo	10 mg/g	Live	-metalaxyl	6	3	3
4	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Dead	Dead	6	0	0
4	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Eskimo	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Dead	Dead	6	0	0
4	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Live	-metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	10 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	30 mg/g	Dead	Dead	6	0	0
4	resistant	Nairobi	30 mg/g	Live	-metalaxyl	7	1	1

4	resistant	Nairobi	30 mg/g	Live	-metalaxyl	6	3	3
4	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
4	resistant	Nairobi	30 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	10 mg/g	Dead	Dead	6	0	0
4	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	4	5
4	susceptible	T1308403	10 mg/g	Live	-metalaxyl	6	3	7
4	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	30 mg/g	Dead	Dead	6	0	0
4	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	6	33
4	susceptible	T1308403	30 mg/g	Live	-metalaxyl	6	6	26
4	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	T1308403	30 mg/g	Live	+metalaxyl	6	1	1
4	susceptible	Criolla	10 mg/g	Dead	Dead	6	0	0
4	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	0	0
4	susceptible	Criolla	10 mg/g	Live	-metalaxyl	6	1	1
4	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	0	0
4	susceptible	Criolla	10 mg/g	Live	+metalaxyl	6	1	1
4	susceptible	Criolla	30 mg/g	Dead	Dead	6	0	0
4	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	1	1
4	susceptible	Criolla	30 mg/g	Live	-metalaxyl	6	3	5
4	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	2	2
4	susceptible	Criolla	30 mg/g	Live	+metalaxyl	6	1	1

Certificate of

Official Recognition of Efficacy Testing Facilities or Organisations in the United Kingdom

This certifies that

Warwick Crop Centre, School of Life Sciences

complies with the minimum standards laid down in Regulation (EC) 1107/2009 for efficacy testing.

The above Facility/Organisation has been officially recognised as being competent to carry out efficacy trials/tests in the United Kingdom in the following categories:

Agriculture/Horticulture
Biologicals and Semiochemicals

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Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 381

HSE
Chemicals Regulation Division

Department of Agriculture and Rural Development