SCEPTREPLUS

Final Trial Report

Trial code:	SP69
Title:	Development of new strategies to control carrot cavity spot
Crop	Carrot
Target	*Pythium violae*
Lead researcher:	John Clarkson
Organisation:	University of Warwick
Period:	March 2020 - May 2021
Report date:	10/05/22
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ORETO Number: (certificate 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

16/05/22 *John Clarkson*
Date Authors signature

Trial Summary

Introduction

Cavity spot of carrot principally caused by *Pythium violae* and *P. sulcatum* has been a significant problem for over 40 years and continues to be one of the most unpredictable and economically important diseases for UK growers. Symptoms are observed as dark sunken lesions on the carrot root and growers often suffer severe losses as even roots with only a small number of superficial lesions are rejected by the market. Metalaxyl has been used routinely to control cavity spot for several decades and is still widely used to manage the disease. However, the level of control can prove variable and this has been attributed to enhanced microbial degradation in soil or pathogen resistance. There are also difficulties in optimising metalaxyl-M use for cavity spot control due to restrictions on application numbers and the complexity of factors affecting the timing of infection of carrot crops by *Pythium* spp. Moreover, the continued reliance on metalaxyl-M (SL567a) and hence a single mode of action, is therefore a major concern for long-term sustainability of the carrot industry. This project aimed to identify other crop protection products for cavity spot control.

Methods

The effect of four plant protection products (including the industry standard SL567a – metalaxyl-M; Table 1) on cavity spot disease was evaluated in a field 'macrocosm' system comprising sunken concrete pipes 1 m in diameter and 60 cm deep filled with sieved sandy loam soil previously inoculated with *P. violae* in 2016 and 2017. Successive carrots crops grown in these macrocosms were previously shown to have high levels of cavity spot (48% disease incidence in 2019).

Each treatment was replicated across four macrocosms in a randomised block design and compared with inoculated and uninoculated controls. Each macrocosm was sown with approximately 300 untreated seeds of the susceptible carrot variety cv. Criolla and seeds covered with a 1 to 2 cm layer of sieved soil. Single spray applications of the products (Table 1) were made to the soil in 1000 L water/ha at sowing. Macrocosms were watered regularly throughout the growing season via an oscillating line and appropriate control agents applied to reduce damage from carrot fly and slugs. Each macrocosm was covered with fleece and a thick layer of straw in November 2021 to prevent winter frost damage. Carrots were harvested in early March 2022 and all roots assessed for presence / absence of cavity spot lesions (incidence) and the number of lesions present (severity). Incidence and severity data was subjected to statistical analysis using ANOVA with no transformation necessary following examination of residual plot data.

Results

- Typical cavity spot lesions were observed on carrot roots in all treatments with some developed into particularly expanded cavities, destroying substantial areas of the root periderm.
- Cavity spot incidence ranged between 37.2 - 65.9% roots affected (Table 1) but there was no significant difference in cavity spot incidence between carrots treated with AHDB9726, AHDB9883 or AHDB9941 (56.3, 60.5 and 63.8% cavity spot incidence respectively) compared to the untreated control (65.9% incidence). However, plots treated with SL567a

resulted in carrots with significantly reduced incidence of cavity spot disease (37.2% incidence, P < 0.05) compared to the untreated control.

- Cavity spot severity across treatments ranged between 0.87-1.89 lesions per root (Table 1). Disease severity followed the same pattern as disease incidence across the different treatments, with no significant difference between untreated carrots (1.89 lesions / carrot) and those treated with AHDB9726, AHDB9883 or AHDB9941 (1.45, 1.54 and 1.54 lesions per carrot respectively). Again, only the SL567a treatment had significantly less lesions per carrot (0.87 lesions / carrot, P <0.05) than the untreated control.

Table 1. Effect of crop protection products on cavity spot disease incidence and severity

Treatment	Mean cavity spot incidence (% carrots affected)	Mean cavity spot severity (no. lesions per carrot)
Control (uninoculated)	38.58	0.84
AHDB9941	63.84	1.54
AHDB9883	60.45	1.54
SL567a	37.23	0.87
AHDB9726	56.29	1.45
Untreated (inoculated)	**65.90**	**1.89**
LSD	16.368	0.716
Treatments in which cavity spot incidence or severity was significantly less than in untreated, inoculated carrots (P<0.05)		

Conclusions

Macrocosms inoculated with *P. violae* resulted in high levels of cavity spot in the susceptible carrot cv. Criolla; however, no plant protection products tested significantly reduced disease with the exception of the industry standard SL567a.

Objectives

To test plant protection products for control of cavity spot of carrot in field macrocosms artificially inoculated with *Pythium violae*.

Trial conduct

Macrocosm trial

The effect of four plant protection products (including the industry standard SL567a – metalaxyl-M; Table 1) on cavity spot disease was evaluated in a field 'macrocosm' system comprising sunken concrete pipes 1 m in diameter and 60 cm deep filled with sieved sandy loam soil previously inoculated with *P. violae* in 2016 and 2017 (4 macrocosms remained uninoculated). Successive carrots crops grown in these macrocosms were previously shown to have high levels of cavity spot (48% disease incidence in 2019).

Prior to sowing and treatment, the soil in each macrocosm was dug over to release compaction and a maintenance fertiliser dressing was applied (30 g 0:20:30 N:P:K). Each treatment was replicated across four macrocosms in a randomised block design and compared with inoculated and uninoculated controls. Each macrocosm was sown with approximately 300 untreated seeds of the susceptible carrot variety cv. Criolla and seeds covered with a 1 to 2 cm layer of sieved soil.

Single spray applications of the products were made to the soil in 1000 L water/ha at sowing. Macrocosms were watered regularly throughout the growing season via an oscillating line and appropriate plant protection products applied to reduce damage from carrot root fly and slugs. Each macrocosm was covered with fleece and a thick layer of straw in November 2021 to prevent winter frost damage.

Assessment of cavity spot symptoms and infection by *P. violae*

Carrots were harvested in early March 2022 and all roots assessed for presence / absence of cavity spot lesions (incidence) and the number of lesions present (severity). Cavities were only scored as typical lesions caused by *Pythium* spp. if they were sunken lesions or full cavities, dark, elliptical to round in shape and > 2mm in diameter on the root surface. Incidence and severity data was subjected to statistical analysis using ANOVA with no transformation necessary following examination of residual plot data.

Test site

Item	Details
Location address	Warwick Crop Centre, University of Warwick, Wellesbourne Campus
Crop	Carrot
Cultivar	Criolla
Soil or substrate type	Soil
Agronomic practice	N/A
Prior history of site	Soil in macrocosms artificially inoculated with *P. violae* in 2016/2017, and cropped repeatedly with carrot since then.

Trial design

Item	Details
Trial design:	Randomised block
Number of replicates:	4
Row spacing:	N/A
Plot size: (w x l)	1m diameter round macrocosms
Plot size: (m^2)	0.785
Number of plants per plot:	300 (seeds)

Treatment details

AHDB Code	Active substance	Product name/ manufacturers code	Formulation batch number	Content of active substance in product	Formulation type	Adjuvant
AHDB9941					Suspension concentrate	None
AHDB9883					Soluble liquid	None
Uncoded	Metalaxyl-M	SL567a	HEM9ED0003	465.2 g / L	Soluble liquid	None
AHDB9726					Wettable powder	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
1	AHDB9941	2650 g / 1550 g	5 L / ha [1]	A
2	AHDB9883	70.4 g	0.44 L / ha	A
3	SL567A	604.8 g	1.3 L / ha	A
4	AHDB9726	$5x10^{11}$ CFU	5 kg / ha	A

[1] Equivalent to two sprays at 2.5 L / ha

Application details

	Application A
Application date	25/5/2021
Time of day	11:30
Crop growth stage (Max, min average BBCH)	N/A
Crop height (cm)	N/A
Crop coverage (%)	N/A
Application Method	Spray
Application Placement	Soil
Application equipment	Berthoud Vermorel 2000HP
Nozzle pressure	2 Bar
Nozzle type	05F110
Nozzle size	05
Application water volume/ha	1000
Temperature of air - shade (°C)	N/A
Relative humidity (%)	N/A
Wind speed range (m/s)	N/A

Dew presence (Y/N)	N/A
Temperature of soil - 2-5 cm (°C)	Not recorded
Wetness of soil - 2-5 cm	Damp
Cloud cover (%)	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	N/A	N/A	66% disease incidence in untreated control

Assessment details

Evaluation date	Evaluation Timing	Crop Growth Stage (BBCH)	Evaluation type	Assessment
09/03/2022	41 weeks after sowing	49	Efficacy	Cavity spot incidence and severity

Results

Typical cavity spot lesions due to *Pythium* spp. were observed on carrot roots (cv. Criolla) in all treatments (including those harvested from control untreated plots; Fig. 1). Some lesions had developed into particularly expanded cavities, destroying substantial areas of the root periderm (Fig. 2).

Across all the macrocosms inoculated with *P. violae*, cavity spot incidence ranged between 37.2 - 65.9% roots affected (Table 2, Fig. 3a). A background level of cavity spot was evident in uninoculated macrocosms (38.9% roots affected).

There was no significant difference in cavity spot incidence between carrots treated with AHDB9726, AHDB9883 or AHDB9941 (56.3, 60.5 and 63.8% cavity spot incidence respectively) compared to the untreated control (65.9% incidence; Table 2, Fig 3a). However, plots treated with SL567a resulted in carrots with significantly reduced incidence of cavity spot disease (37.2% incidence, P < 0.05) compared to the untreated control.

Similarly, cavity spot severity across treatments ranged between 0.87-1.89 lesions per root. Disease severity followed the same pattern as disease incidence across the different treatments, with no significant difference between untreated carrots (1.89 lesions / carrot) and those treated with AHDB9726, AHDB9883 or AHDB9941 (1.45, 1.54 and 1.54 lesions per carrot respectively). Again, only the SL567a treatment had significantly less lesions per carrot (0.87 lesions / carrot, P <0.05) than the untreated control (Table 2, Fig. 3b).

Table 2. Effect of crop protection products on cavity spot disease incidence and severity

Treatment	Mean cavity spot incidence (% carrots affected)	Mean cavity spot severity (no. lesions per carrot)
Control (uninoculated)	38.58	0.84
AHDB9941	63.84	1.54
AHDB9883	60.45	1.54
SL567a	37.23	0.87
AHDB9726	56.29	1.45
Untreated (inoculated)	**65.90**	**1.89**
LSD	16.368	0.716
Treatments in which cavity spot incidence or severity was significantly less than in untreated, inoculated carrots (P<0.05)		

Figure 1. Carrots (cv. Criolla) showing typical cavity spot lesions from field macrocosms inoculated with *P. violae*. Carrots from a) uninoculated control, b) inoculated control, c) AHDB9941, d) AHDB9883, e) SL467*a*, f) AHDB9726. Bar = 30 cm.

Figure 2. Extensive cavity spot lesions in carrots (cv. Criolla) from field macrocosms inoculated with *P. violae*. Carrots of varying sizes from a) AHDB9883 treatment showing typical, elliptical cavity spot lesions, b) AHDB9941 treatment showing expanded and deep cavities compared with c) inoculated control and d) uninoculated control. Bar = 10 cm.

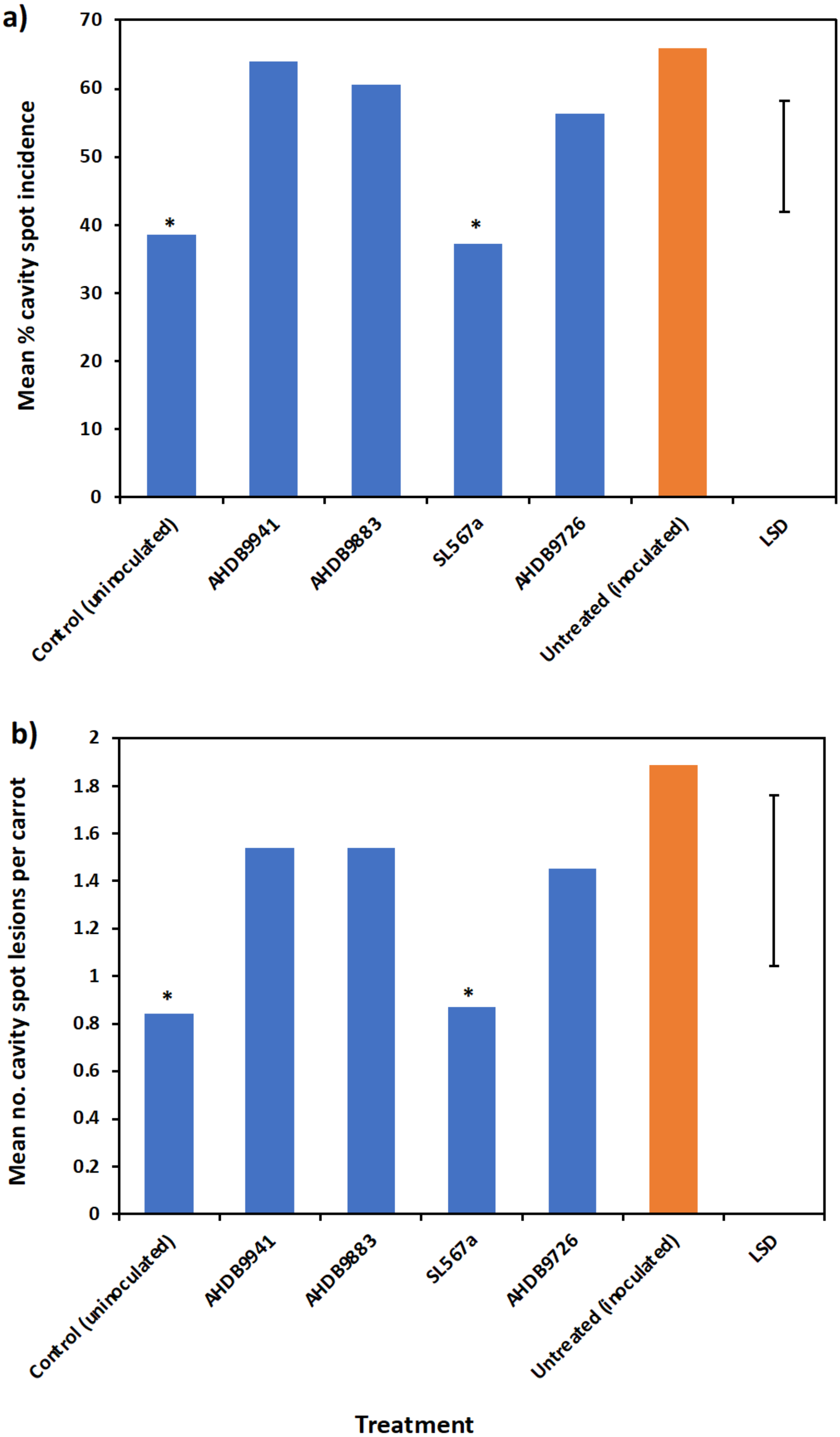

Figure 2: Effect of four treatments crop protection products on a) cavity spot incidence and b) severity for carrots grown in field macrocosms inoculated with *P. violae*. Error bar represents the LSD at the 5% level. Asterisks indicate significantly lower cavity spot incidence and severity relative to inoculated, untreated carrots.

Discussion

The field macrocosms inoculated with *P. violae* in 2016 and 2017 resulted in high level levels of cavity disease in the highly susceptible carrot cv. Criolla in this SCEPTREplus trial, with 66% roots affected in the untreated control. This represented a substantial increase in disease incidence from that observed in carrots cv. Nairobi in 2017 trial where 24% carrots were affected. This is likely due to the build-up of pathogen inoculum over several years of successive carrot growing and also that cv. Criolla is much more susceptible to cavity spot than cv. Nairobi as demonstrated in SCEPTREplus project SP72. A background level of cavity spot was evident in uninoculated macrocosms (38.9%), which was appreciably higher than that observed in the first year of cropping (cv. Nairobi) in 2017 (1.4%). This is likely due to the build-up of pathogen inoculum through successive carrot crops which probably originated from contamination via neighbouring inoculated macrocosms.

Only the industry standard SL567a (metalaxyl-M) significantly reduced cavity spot incidence and severity and confirms the utility of this product in controlling disease. The other products tested had little or no effect except for AHDB9726 which reduced cavity spot incidence by 10% (not significant). This demonstrates the challenge of identifying new products with efficacy against cavity spot and highlights the need for other long term management approaches for the disease. These could include plant resistance and improvement of soil structure and health, which are known to influence the severity of cavity spot.

A difficulty of testing product efficacy against cavity spot in field trials is that the uneven distribution of *Pythium* species inoculum and seasonal environmental variation can lead to inconsistent results. While the experimental set up (inoculated macrocosms) in this trial limited the number of treatments that could be tested, it represented a robust and reliable system for identifying control treatments as demonstrated by the clear activity of metalaxyl-M. A further experimental system (inoculated pots in a glasshouse) that could be used reliably for screening product efficacy for cavity spot control was also validated in SCEPTREplus trial SP 72.

Conclusions

Macrocosms inoculated with *P. violae* resulted in high levels of cavity spot in the susceptible carrot cv. Criolla; however, no plant protection products tested significantly reduced disease with the exception of the industry standard SL467a.

Acknowledgements

We thank AHDB for funding and Syngenta UK for providing SL 567a. We also thank Andrew Mead (Rothamsted UK) for statistical analyses and Horticultural Services for plant maintenance and help with experiment set up.

Appendix

Trial diary

Date	Experimental details
25/05/21	Carrot seed (cv Criolla) sown
25/05/21	Application of test products at recommended rates
15/11/21	Application of straw to macrocosms for frost protection
09/03/22	Harvest and assessment of carrots for cavity spot

Macrocosm field trial November 2021

Raw data

		No. of carrots with no. lesions																			
Plot	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**No. carrots**	**No. carrots with lesions**	**Total no. lesions**	**CS Incidence**	**CS Severity**
4	Uninoc. control	17	1	1	2	1	0	0	0	0	0	0	0	0	0	0	80	22	35	27.50	0.44
9	Uninoc. control	28	6	10	1	2	0	0	0	0	0	0	0	0	0	0	120	47	84	39.17	0.70
18	Uninoc. control	14	7	2	1	1	1	2	0	0	0	0	0	0	0	0	79	28	63	35.44	0.80
20	Uninoc. control	15	11	7	6	5	1	2	0	0	0	0	0	0	0	0	90	47	127	52.22	1.41
5	AHDB9941	28	9	9	5	6	1	0	0	0	0	0	0	0	0	0	91	58	129	63.74	1.42
8	AHDB9941	28	20	7	7	0	3	3	2	0	1	0	0	0	0	0	101	71	182	70.30	1.80
16	AHDB9941	21	13	7	5	3	2	2	0	1	0	0	0	0	0	0	87	54	138	62.07	1.59
22	AHDB9941	18	13	9	3	3	1	1	0	0	0	0	0	0	0	0	81	48	111	59.26	1.37
1	AHDB9883	33	21	25	11	5	2	3	3	0	0	2	1	0	0	1	161	107	325	66.46	2.02
12	AHDB9883	17	10	6	5	2	1	0	0	0	0	0	0	0	0	0	72	41	91	56.94	1.26
14	AHDB9883	35	18	11	5	8	2	2	2	0	0	0	0	0	0	0	133	83	206	62.41	1.55
19	AHDB9883	23	17	3	8	2	0	1	2	0	0	0	0	0	0	0	100	56	131	56.00	1.31
6	SL567a	12	10	10	3	2	0	0	0	1	0	0	0	0	0	0	98	38	93	38.78	0.95
10	SL567a	12	6	7	1	3	0	0	0	0	0	0	0	0	0	0	87	29	64	33.33	0.74
17	SL567a	24	11	9	8	3	4	1	1	0	0	0	0	0	0	0	130	61	159	46.92	1.22
21	SL567a	12	6	2	1	1	1	0	0	0	0	0	0	0	0	0	77	23	45	29.87	0.58
2	AHDB9726	19	13	16	7	7	4	2	2	0	0	0	0	0	0	0	98	70	210	71.43	2.14
11	AHDB9726	21	16	5	3	1	1	1	0	0	0	0	0	0	0	0	101	48	98	47.52	0.97
13	AHDB9726	17	8	6	5	2	1	1	1	0	1	1	0	0	0	0	61	43	123	70.49	2.02
24	AHDB9726	31	12	3	6	3	0	0	0	0	0	0	0	0	0	0	154	55	103	35.71	0.67
3	Inoc. control	23	17	6	4	5	2	3	0	0	1	0	1	0	0	0	105	62	171	59.05	1.63
7	Inoc. control	25	26	18	9	8	8	3	4	2	1	0	0	0	0	0	131	104	336	79.39	2.56
15	Inoc. control	19	11	7	2	3	2	1	0	0	0	0	1	0	0	0	81	46	116	56.79	1.43
23	Inoc. control	28	21	9	6	7	2	4	1	0	2	0	0	0	0	0	117	80	224	68.38	1.91

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

Date of issue: **6 October 2017**
Effective date: **20 March 2017**
Expiry date: **19 March 2022**

Signature Alison Richardson
Authorised signatory

Certification Number
ORETO 381

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