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DEVELOPMENT OF *Sclerotinia sclerotiorum* (Lib.) de Bary ON STORED CARROT TREATED WITH *Pythium oligandrum* Drechsler DETERMINED BY qPCR ASSAY

Anna Baturo-Cieśniewska✉, Aleksander Łukanowski, Katarzyna Koczwara, Leszek Lenc

Laboratory of Phytopathology and Molecular Mycology, UTP University of Science and Technology, Kordeckiego 20, 85-225 Bydgoszcz, Poland

ABSTRACT

Sclerotinia scletoriorum (Lib.) de Bary is a serious pathogen of carrots that can cause up to 50% losses of stored roots. Fungicides were found not to be completely effective for its control and due to residues they are not permitted for the use in storage. Biological control agents such as *Pythium oligandrum* may be a chance. The aim of research was to determine the level of carrot infection with *S. sclerotiorum* during few-month storage with qPCR and the potential control ability of this pathogen by Polyversum WP containing oospores of *P. oligandrum.* We analyzed carrot roots in combinations treated with Polyversum WP on the field, fumigated with biopreparation before storage and untreated control. *S. sclerotiorum* developed on carrots treated and untreated with Polyversum WP. During the storage, pathogen was isolated from 44.4% samples and *P. oligandrum* from 53.8% ones, respectively in the range from 0.0001 to 130 200.0; pg and from 0.004 to 0.3440 pg per sample. The number of roots with *S. sclerotiorum* and degree of their infection increased with prolonged storage. Analyses suggest that Polyversum WP may potentially limit the growth of *S. sclerotiorum*.

Key words: carrot, *Sclerotinia sclerotiorum*, *Pythium oligandrum*, storage

INTRODUCTION

Carrot (*Daucus carota* L.) has been cultivated since the tenth century for its taste and versatility [Kora et al. 2003]. It is characterized by the highest concentration of β-carotene among vegetables. Also other valuable ingredients such as fiber, vitamin C, vitamin E, B vitamins and carbohydrates can be found in the roots [Kunachowicz et al. 2017]. Carrot is a commercially important vegetable, the production of which in Poland according to the Central Statistical Office [2016] was 823 thousand tons in 2014 and 678 thousand tons in 2015. It is grown on the area of over 22 thousand hectares, making it the third largest crop in the country, after cabbage and onion. Roots in unprocessed form are consumed throughout the year, therefore they require proper storage conditions. They are generally kept at 0–1°C, with 95–98% relative air humidity, which provides the smallest losses due to respiration and evaporation [Adamicki and Nawrocka 2015]. Under such conditions, the development of storage diseases is significantly reduced, but despite this, fungi growing on roots can contribute to significant losses.

✉ baturo-a@utp.edu.pl

Sclerotinia scletoriorum (Lib.) de Bary, a polyphagous fungus of soil origin, infecting more than 450 plant species, develops both before harvest and after harvest, is a serious pathogen of carrots in many regions of the world [Purdy 1979, Boland and Hall 1994]. It is introduced into the storage with roots infected on the field. Even one infected root can be the source and the beginning of serious damage and losses up to 50% of stored carrots. Infected roots are susceptible to infection with other pathogens, which further increases the damage [Kora et al. 2008]. Fungus has the ability to grow at very low temperatures, thus the conditions in the storage do not fully protect the carrot against *S. sclerotiorum* [Finlayson et al. 1989, Pritchard et al. 1992]. Fungicides applied during the growing season were found not to be completely effective for the control of *S. sclerotiorum* rot, because of the wide host range of pathogen and long-term persistence of sclerotia in soil or development of pathogen resistance. Furthermore, due to residue problems, fungicides are not permitted for the use on postharvest roots. Chemical pesticides are frequently known for their negative impact on the environment and on the consumers. It encourages further investigation of alternative control methods [Cheah et al. 1997, Kora et al. 2008, Saharan and Mehta 2008, Gerbore et al. 2014]. Such methods include the use of biopreparations containing microorganisms antagonistic to pathogens. *Coniothyrium minitans* and several *Trichoderma* spp. can suppress development of the disease caused by *S. sclerotiorum* by parasitizing hyphae and sclerotia of the fungus [Inbar et al. 1996, McLaren et al. 1996, Kim and Knudsen 2008]. Also natural substances like chitosan may be useful to reduce *S. sclerotiorum* rot on carrot roots [Cheah et al. 1997]. Gerbore et al. [2014] noticed that *P. oligandrum*, as biological control agent, has received growing interest in the last decade. Numerous studies have shown that it is effective in reducing the infection of pathogens in the range of 15 to 100% depending on the host plant, target pathogen and application method.

To monitor the development of pathogens, including storage ones, and to assess the effectiveness of treatments that limit their development molecular techniques based on PCR (polymerase chain reaction) and its modifications have been used [Capote et al. 2012]. One of them is the real-time PCR (qPCR-quantitative PCR) considered one of the most specific and sensitive tools used for quantitative determination of PCR product. Real-time PCR was shown to be a valuable method to detect and quantify fungal inoculum in plant tissues, soil and air [Lievens et al 2006, Rogers et al. 2009, Capote et al. 2012].

The objective of the current study was to determine the level of carrot infection by *S. sclerotiorum* during long-term storage and potential control ability of this pathogen by Polyversum WP (Biopreparaty spol. s.r.o.) containing oospores of *P. oligandrum*, using the simple, relatively inexpensive and reliable method. In addition, there was a need to verify the presence of *P. oligandrum* in the analyzed carrot samples because of confusing results of analyses for *S. sclerotiorum* in combinations treated with biopreparation.

MATERIAL AND METHODS

The roots of carrot cv. Komarno, harvested in the end of October 2014 from the field in Kołaczkowo (Kuyavian-Pomeranian province, Poland) were examined to determine *S. sclerotiorum* development.

Plants were sprayed with Polyversum WP on September 11 and 25 using 200 $g \cdot ha^{-1}$ every time. According to data of meteorological station in Kołaczkowo, mean air and soil temperatures and average rainfall on the days of biopreparation application in the field were respectively 14.3°C, 15.0°C and 0.025 mm; and 9.7°C, 11.3°C and 0.46 mm. The average values for these parameters for September were 14.6°C, 14.7°C and 0.16 mm, and for October 9.7°C, 11.8°C and 0.06 mm. Also azoxystrobin (Amistar 250 SC, Syngenta, 0.8 $l \cdot ha^{-1}$), metribusin (Sencor Liquid 600 SC, Bayer, 2 × 0,25 $l \cdot ha^{-1}$), linuron (Afalon 450 SC, Makhteshim-Agan, 2 $l \cdot ha^{-1}$) and tiacloprid with deltametrin (Proteus OD 110, Bayer, 0.25 $l \cdot ha^{-1}$) were applied. Dosage of fertilizers in $kg \cdot ha^{-1}$ was N – 200, P – 80, K – 137 and $CaCO_3$ – 800.

After harvest, roots were cooled down to ~4°C and fogged with Polyversum WP. Aliquots of 300 g biopreparation were applied per 1000 m^3 of separated storage chamber with a Pulsfog fogger for 30 min. After 6 h, roots were placed in glycol cooler at 0–1°C

and 99% humidity and stored until March 2015. Due to the fact that Polyversum WP has not been registered for the protection of carrots, the Department of Plant Breeding and Plant Protection of the Polish Ministry of Agriculture and Rural Development granted permission to use it in our research.

Sampling

Samples were collected in four (I–IV) dates: I – 02.12.2014, II – 02.01.2015, III – 03.02.2015, IV – 03.03.2015 from 4 combinations: C – control (carrot without protection with Polyversum WP), P1 – carrots sprayed with Polyversum WP on the field, P2 – carrot fogged with Polyversum WP in storage, P1 + P2 – carrots sprayed with Polyversum WP on the field and fogged with Polyversum WP in storage. Also, just after harvest, before fogging and storage, samples were taken from C and P1 (term 0). In all terms, 10 roots were collected from each combination resulting in 180 samples (roots).

DNA extraction from carrot roots, mycelium and Polyversum WP

The outer layer (1 mm) of carrot roots roughly cleaned from the soil using paper towel, after freeze-drying (CoolSAFE – Scanvac) and homogenization (2 min at 1800 rpm) into a fine powder in a homogenizer MM 400 (Retsch), was subjected to DNA isolation according to modified Doyle and Doyle method [1990]. Samples of 70 mg root powder were placed into 2.0 ml Eppendorf tubes with 900 µl of extraction buffer containing CTAB 5.0%, NaCl 5.0 M, EDTA 0.5 M, Tris-HCl (pH 8.0) 1.0 M, PVP, β-mercaptoethanol. In subsequent steps, chloroform, isoamyl alcohol and phenol were applied for DNA purification. The DNA was precipitated with 96% ethanol and suspended in 150 µl of ddH_2O. To get rid of PCR inhibitors, mainly phenolic compounds, the DNA was cleaned using the Anti-Inhibitor Kit (A&A Biotechnology).

Fungal genomic DNA from 20 mg of freeze-dried and homogenized (90 s at 5000 rpm) with quartz beads in MagnaLyser instrument (Roche) mycelium of reference isolates of *S. sclerotiorum* originated from carrot (CBS 141019), broccoli (CBS 140898), parsley (CBS 140896) and celery (CBS 140895) and *P. oligandrum* (CBS 109980) and also *Botrytis cinerea*, *Pythium debaryanum* and *Pythium ultimum* from own collection, was extracted to obtain material for the standard curves and control samples. Mycelium was grown for 3 days in 100 ml of potato broth (PB) in Erlenmeyer flask on rotary shaker (150 rpm). Additionally, DNA was isolated from 70 mg of Polyversum WP. For DNA extraction, the same DNA isolation protocol was used for mycelium and biopreparation as for carrot samples.

DNA concentrations in all samples were determined on a Quantus Fluorometer (Promega, USA) according to the manufacturer's procedure.

Verification of *S. sclerotiorum*-specific primers before qPCR analysis of carrot samples

During analyses, TMSCL2F/ TMSCL2R primers were applied [Kim and Knudsen 2008]. Due to the fact that the authors used them together with TaqMan probe, first their efficacy and specificity were tested in assay with SYBR Green dye with DNA of reference isolate *S. sclerotiorum* (CBS 141019). *B. cinerea* related to *S. sclerotiorum*, which may also be present on stored carrots, as well as *S. sclerotiorum* isolates from other vegetables, were used as a control. Real-time PCR amplifications were carried out according to the same protocol as for carrot samples.

Verification of *P. oligandrum*-specific primers and qPCR assay with DNA from Polyversum WP

Due to the fact that the P.OLIG.F1/P.OLIG.R04 primers were found to be specific to *P. oligandrum* on the basis of traditional PCR assay with DNA obtained from pure cultures of *P. oligandrum* and different fungal species [Godfrey et al. 2003] and because of confusing results concerning the presence of *P. oligandrum* in carrot samples in our qPCR assay, to be sure that reaction conditions and primers work properly and that *P. oligandrum* was present in biopreparation, qPCR assay with DNA extracted from Polyversum WP was carried out in three replications. As a control, DNA of *P. oligandrum* (CBS 109980), *P. debaryanum* and *P. ultimum* was used. Real-time PCR amplifications were carried out according to the same procedure as for carrot samples.

Quantification of *S. sclerotiorum* and *P. oligandrum* in carrot samples

QPCR amplifications were carried out in a total volume of 10 µl in LightCycler 480 II (Roche) in three replications for all samples. Prior to analyses, standard curves were prepared basing on 6 dilutions (22 500, 4500, 450, 45, 4.5 and 0.45 pg per reaction) of DNA extracted from reference isolates. The reaction conditions for *S. sclerotiorum* were as follows: 95°C for 10 min and 45 cycles (95°C for 20 s, 59°C for 1 min) and for *P. oligandrum*: 95°C for 10 min and 45 cycles (95°C for 10 s, 60 °C for 20 s, 72°C for 40 s). To verify the specificity of the reaction, a melting curve analyses were carried out under the following conditions: 95°C for 5 s, 65°C for 1 min, 95°C (with continuous fluorescence measurement in the range of 65°C to 95°C) and 40°C for 30 s. The reaction mixture consisted of 5 µl of 2× concentrated LightCycler 480 SYBR Green I Master (Roche), 0.25 $pM \cdot \mu l^{-1}$ of each primer: TMSCL2F/TMSCL2R [Kim and Knudsen 2008] for *S. sclerotiorum* or P.OLIG.F1/P.OLIG.R04 [Godfrey et al. 2003] for *P. oligandrum*, and 45 ng of template DNA per sample. In analyses of samples dilution of DNA from reference, isolates were applied as positive controls.

Processing of results and statistical analyses

The amount of *S. sclerotiorum* and *P. oligandrum* DNA was calculated from C_T values using the standard curves and LightCycler software package (Roche). Quantity of fungal DNA in analyzed samples was calculated and expressed in pg per sample.

Statistical analyses were conducted using variance analysis for two-factor experiments for dates I–IV, where the first factor was the combination and the second was the date of sampling, or for one-factor experiment for term 0, where combination was the factor.

Root number with *S. sclerotiorum* and *P. oligandrum* for analyses was expressed in percent and converted to Bliss grades.

Honestly significant difference (HSD) at the $\alpha = 0.05$ significance level was established by Tukey's test in FR-ANALWAR 4.3. software based on Microsoft Excel [Rudnicki 2011].

RESULTS

Verification of primer specificity and correctness of reaction conditions

Preliminary qPCR analysis to verify the usefulness of TMSCL2F/TMSCL2R primers in SYBR Green assay and their specificity was successful. Product presence and single peak in melting curve analysis were obtained only for *S. sclerotiorum*. The species specificity of P.OLIG.F1/P.OLIG.R04 primers to *P. oligandrum* and its presence in the biopreparation were also revealed. The applied reaction conditions proved to be appropriate and allow to obtain reliable results in qPCR with SYBR. Also single peaks in melting curves analyses, specific for *S. sclerotiorum* (Fig. 1) and *P. oligandrum* (Fig. 2), were recorded in assays of carrot samples.

Pre-storage analyses of carrot samples

QPCR analyzes carried out before storage showed the presence of *S. sclerotiorum* in 2 samples in P1. Statistical analysis did not show significant differences between two combinations (C and P1) examined at that time. For *P. oligandrum*, significantly more carrot samples with this mycoparasite were revealed in P1 (7) than in C (2). However, the level of root colonization was similar (Tab. 1).

S. sclerotiorum occurrence in carrot samples during the storage

The presence of *S. sclerotiorum* was noted in 71 out of 160 samples (Tab. 2). The pathogen developed both on carrot derived from combinations where Polyversum WP was used and the combinations without biopreparation. The number of roots with *S. sclerotiorum* and degree of their infection increased with the storage duration. Despite the large differentiation in the DNA quantity of pathogen in individual combinations and terms, analysis of variance showed no significant differences between them. Tukey's HSD tests showed no interaction between the two factors analyzed. However, clear trends indicate a higher level of *S. sclerotinia* root infection in control combination (mean 3366.3258 pg) than in combinations where Polyversum WP was used (P1 9.2548, P2 970.8231, P1 + P2 949.7553 pg).

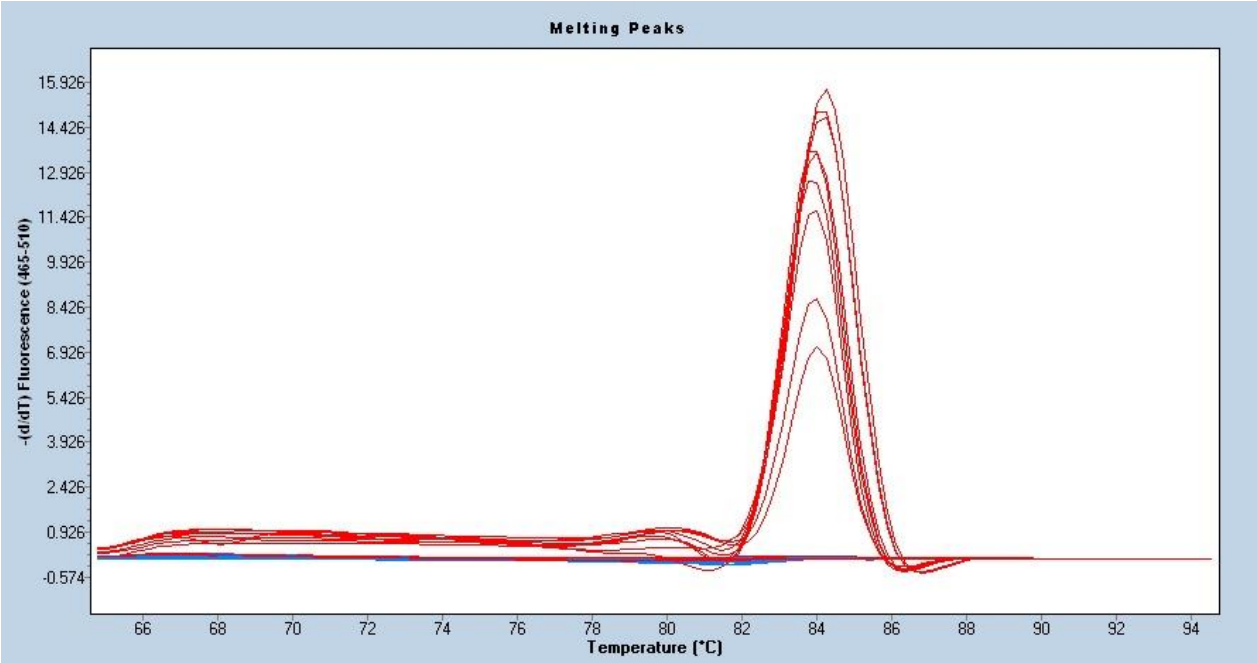

Fig. 1. Melting curves for *S. sclerotiorum* products in carrot samples

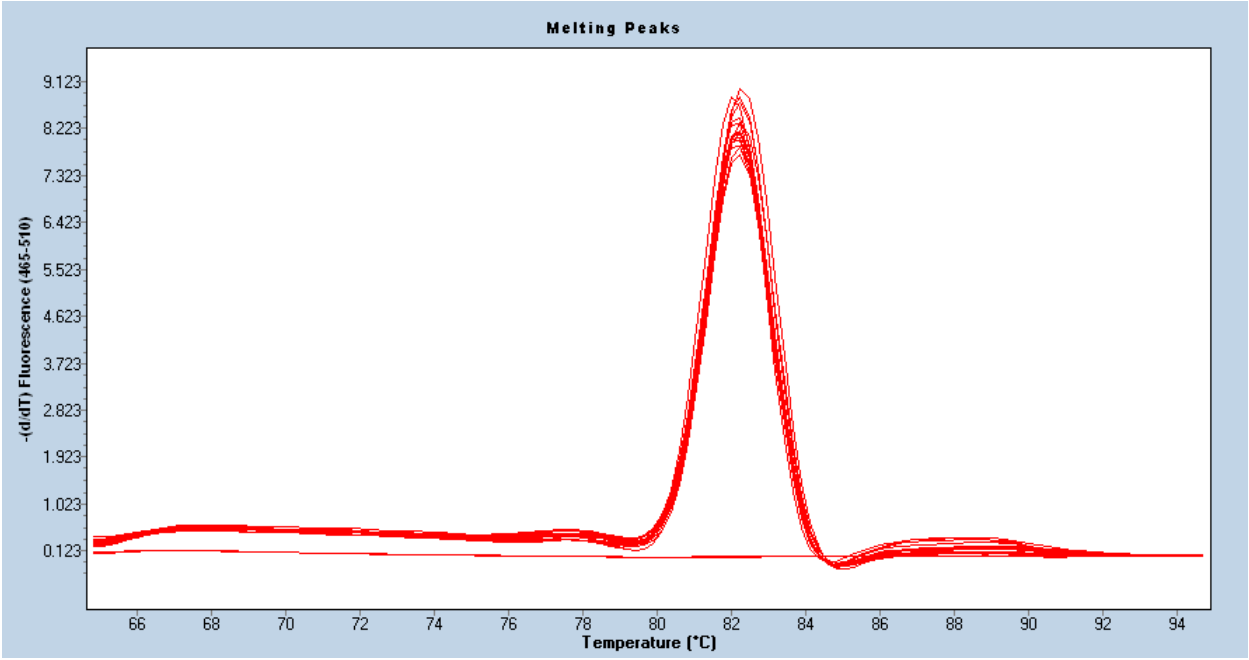

Fig. 2. Melting curves for *P. oligandrum* products in carrot samples

Table 1. Quantity of *S. sclerotiorum* and *P. oligandrum* in carrot samples measured in qPCR assay with SYBR Green before fogging and storage

Combination	Parameter	*S. sclerotiorum*	*P. oligandrum*
C	n[1]	0 A[2]	2 B
	min. [3]	0	0.0062
	max.	0	0.0263
	mean	0	0.0032
P1	n	2 A	7 A
	min.	0.1302	0.0011
	max.	0.1384	0.0051
	mean	0.0269	0.0020
	Total n	2	9
	Mean (pg)	0.0134	0.0026

[1] number of samples with *S. sclerotiorum* or *P. oligandrum*
[2] varied capital letters in the columns indicate significant differences between the combinations
[3] minimal / maximal / mean quantity of *S. sclerotiorum* or *P. oligandrum* in combination or term (pg)

Analyzing the sampling dates, it was shown that on average, the highest amount of DNA, i.e. 5233.0164 pg, was found in IV term, and the lowest, 0.0482 pg and 0.0816 pg, respectively, in I and II dates. In all of 160 samples tested, average of 1324.0398 pg of *S. sclerotiorum* was found ranging from 0 to 130 200.0 pg. The lowest DNA value (0.0001 pg) in the pathogen-identified samples was found in IV term in P2 where carrot roots were fogged with the Polyversum WP in the storage. At the same time, but in combination C, the highest value – 130 200.0 pg was noted. Statistical analysis revealed significant differences between the number of samples with *S. sclerotiorum*. The differences were particularly evident in the case of dates. Significantly the largest number of samples with *S. sclerotiorum* was in term IV (mean 6.8 samples) and the least in date I (2.2 samples). Comparing the number of infected roots in combinations, the highest number of pathogenic roots was observed in the combinations C and P1 + P2 (5.0 and 5.25, respectively), and the least in combination P2 (3.25 samples). In the first term, the lowest number of roots with *S. sclerotiorum* was found in combinations C and P1 – in both cases one, but in the same combinations in dates III and IV, their number increased significantly, respectively to 8 and 6, and 8 and 7.

P. oligandrum occurrence in carrot samples during the storage

P. oligandrum was revealed in 86 samples (Tab. 3) in all combinations regardless of the application or not using the biopreparation. It was identified in very small quantities. Statistical analysis showed no significant differences in the quantity of isolated DNA in terms and combinations. The range of DNA was from 0.004 pg in the sample in P2 in III to 0.3440 pg in combination C in II. The average quantity of *P. oligandrum* in all samples was 0.0077 pg. The highest amount of its DNA was in C – 0.0121 pg where no Polyversum WP was used, whereas in combinations P1, P2 and P1 + P2 with biopreparation, respectively 0.0079, 0.0063 and 0.0043 pg were noted. Most of the roots with *P. oligandrum* were found in P2 in II and in P1 + P2 in I and IV – 8 samples in all cases. These values in dates I and II were significantly higher in comparison with other combinations in these terms. On average, in P2 and P1 + P2 there was more roots

colonized by *P. oligandrum*, respectively 6.25 and 6.0 samples, than in C and P1 (4.47 and 4.5 samples), but statistical analysis showed no significant differences between combinations.

DISCUSSION

During the storage, we isolated *S. sclerotiorum* from over 44% samples in total. Despite the diverse results in the level of root infection, there was no interaction between combinations and dates. This was probably due to the high variability between values in the individual repetitions caused by the presence of single roots that were very strongly infected. Root damage increased with prolonged storage, as indicated by the number of infected samples. After four months of storage, 67% of roots were with *S. sclerotiorum*, which is 45% more than after one month. As noted by Kora et al. [2003], this pathogen is commonly found on the roots of carrots and can develop

Table 2. Quantity of *S. sclerotiorum* in carrot samples measured in qPCR assay with SYBR Green during storage

Combination	Parameter	Sampling term				Total	Mean
		I	II	III	IV		
C	n[1]	1 B[2] b[3]	5 A a	8 A a	6 A a	20 A	5.0 A
	min.[4]	0.1038	0.0688	0.0408	0.4830	–	–
	max.		1.0140	2288.0000	130 200.0000	–	–
	mean	0.0104	0.1436	229.0063	13 236.1431	13 465.3034	3366.3258
P1	n	1 B b	1 B b	8 A a	7 A a	17 AB	4.25 AB
	min.	0.3328	0.1944	0.3220	6.5200	–	–
	max.			82.0000	80.1000	–	–
	mean	0.0333	0.0194	12.5231	24.4432	37.0190	9.2548
P2	n	2 AB b	2 AB b	3 B ab	6 A a	13 B	3.25 B
	min.	0.3570	0.0819	0.0596	0.0001	–	–
	max.	0.4600	0.2712	0.73000	38 760.0000	–	–
	mean	0.0817	0.0353	0.1289	3883.0464	3883.2923	970.8231
P1 + P2	n	5 A ab	5 A ab	3 B b	8 A a	21 A	5.25 A
	min.	0.0933	0.0326	2.5640	2.2740	–	–
	max.	0.1920	0.8385	97.8000	36 420.0000	–	–
	mean	0.0672	0.1279	10.3934	3788.4328	3799.0213	949.7553
	Total n	9 b	13 ab	22 ab	27 a	71	–
	Mean n	2.2 b	3.2 ab	5.5 ab	6.8 a	–	4.43
	Mean (pg)	0.0482	0.0816	63.0129	5233.0164	21 184.6360	1324.0398

[1] number of samples with *S. sclerotiorum*
[2] varied capital letters in the columns indicate significant differences between the combinations
[3] varied small letters in the lines indicate significant differences between the terms
[4] minimal / maximal / mean quantity of *S. sclerotiorum* in combination or term (pg)

after harvest. Disease in storage is a direct consequence of foliar and crown infection in the field. Mycelium grows from the crowns and spreads to adjacent roots. Pathogen can grow at temperatures as low as 0°C, which explains its development in glycol cooler conditions, and can cause extensive decay [Geary 1978, Finlayson et al. 1989, McDonald 1994].

To identify *S. sclerotiorum* in carrot samples, we used primers designed by Kim and Knudsen [2008] for assays with probe. Our studies showed that they can be successfully applied with SYBR Green. Optimization of the reaction conditions allows to obtain reliable results. SYBR Green real-time PCR with melting curve analysis has been described as a simple, rapid and cheaper technique than analyses with specific molecular probes for the detection and identification of plant pathogens [Capote et al. 2012, Fraga et al. 2014]. As expected, high levels of DNA of *S. sclerotiorum* was observed in root with visible signs of severe infection, but small amount of the

Table 3. Quantity of *P. oligandrum* in carrot samples measured in qPCR assay with SYBR Green during storage

Combination	Parameter *	Sampling term				Total	Mean
		I	II	III	IV		
C	n[1]	5 AB[2] a[3]	4 AB a	5 A a	5 A a	19 A	4.75 A
	min.[4]	0.0011	0.0012	0.0016	0.0011	–	–
	max.	0.0123	0.3440	0.0241	0.0204	–	–
	mean	0.0027	0.0367	0.0051	0.0037	0.0482	0.0121
P1	n	3 B a	3 B a	6 A a	6 A a	18 A	4.5 A
	min.	0.0053	0.0050	0.0048	0.0022	–	–
	max.	0.0085	0.0121	0.0268	0.1034	–	–
	mean	0.0020	0.0027	0.0116	0.0153	0.0316	0.0079
P2	n	7 AB a	8 A a	6 A a	4 A a	25 A	6.25 A
	min.	0.0018	0.0014	0.0004	0.0007	–	–
	max.	0.0297	0.0532	0.0127	0.0256	–	–
	mean	0.0099	0.0077	0.0034	0.0043	0.0254	0.0063
P1 + P2	n	8 A a	4 AB a	4 A a	8 A a	24 A	6.0 A
	min.	0.0026	0.0012	0.0015	0.0010	–	–
	max.	0.0282	0.0102	0.0036	0.0162	–	–
	mean	0.0095	0.0020	0.0009	0.0047	0.0172	0.0043
	Total n	23 a	19 a	21 a	23 a	86	–
	Mean n	5.8 a	4.8 a	5.2 a	5.8 a	–	5.40
	Mean (pg)	0.0060	0.0123	0.0053	0.0070	0.1224	0.0077

[1] number of samples with *P. oligandrum*
[2] varied capital letters in the columns indicate significant differences between the combinations
[3] varied small letters in the lines indicate significant differences between the terms
[4] minimal / maximal/ mean quantity of *P. oligandrum* in combination or term (pg)

pathogen was identified in samples without symptoms. Suarez et al. [2005] noted that DNA-based techniques, which include qPCR, are particularly useful in the detection of pathogen development in plants, before symptoms become visible.

To identify *P. oligandrum* in carrot samples and in Polyversum WP, we successfully used specific primers P.OLIG.F1/P.OLIG.R04 with SYBR Green. Their designers, Godfrey et al. [2003] have shown their effectiveness in identifying this fungus in traditional PCR. They were also successfully applied in qPCR with hybridization probes by Takenaka et al. [2008] for *P. oligandrum* quantification in tomato rhizosphere.

Our studies revealed the presence of *P. oligandrum* DNA in samples treated with Polyversum WP, but also in combinations where the biopreparation was not used. According to Van der Plaats-Niterink [1981], Ribeiro and Butler [1992] and Rey et al. [2008], non-plant-pathogenic oomycete *P. oligandrum* is a common inhabitant of soils and, as Gerbore et al. [2014] showed based on numerous literature data, it has been isolated from the rhizosphere of many plants. This explains its presence in combinations untreated with biopreparation.

Despite the ambiguity of the results and poor development of *P. oligandrum* on stored carrots, there are, however, some tendencies indicating the limiting effect of *P. oligandrum* on *S. sclerotiorum*. Ribeiro and Butler [1992] and Madsen and de Neergaard [1999] observed mycoparasitism of *P. oligandrum* towards sclerotia of *S. sclerotiorum*. According to Rey et al. [1998], *P. oligandrum* can penetrate rapidly into the root tissues without damaging the plant and thus protect it from infection by pathogens. To our best knowledge, literature contains no information on the effect of *P. oligandrum* on carrot storage quality and also on fogging efficacy in control of pathogens of stored vegetable roots. Therefore, it is difficult to verify our results. According to the distributor, Polyversum WP applied in the form of fogging, can improve the quality of plant raw materials stored for a long time. Such efficiency is declared, for example, in the case of celery purified from the soil [Bartczak 2015]. However, we have not found any detailed data, including results of mycological studies, on this subject. Cheah et al. [1997] who applied chitosan, as a post-harvest treatment for the control of *S. sclerotiorum* in storage, observed that coating the carrot roots decreased disease incidence and inhibited development of the fungus. It is possible that low storage temperatures, which are likely to significantly reduce *P. oligandrum* activity, may not be as significant in naturally derived polysaccharide. Al-Rawahi and Hancock [1997] observed higher frequency of isolation of *P. oligandrum* from soil at the temperature higher than 25°C. *P. oligandrum* was not detected in the soil incubated at 16°C. According to the manufacturer's information label, Polyversum WP works most effectively at 12–25°C. In spite of this, in case of protection of vegetables against storing diseases, the manufacturer recommends using the preparation after placing e.g. root celery or red cabbage in the storage chamber and cooling down to 3–5°C [https://bip.minrol.gov.pl/ content/download/45979/261667/version/1/file/Polyversum%20WP_zast.profesjonalne.pdf]. Our carrot was fogged at such temperature. In addition to temperature, other factors could also affect our results, because varied biotic and abiotic factors may regulate the efficacy of biocontrol agents [Vallance et al. 2009].

CONCLUSIONS

S. sclerotiorum developed on both carrot derived from combinations where Polyversum WP was used and in combinations without biopreparation. Root damage increased with prolonged storage. Our preliminary results suggest that Polyversum WP may limit the growth of *S. sclerotiorum* on stored carrots. Poor development of *P. oligandrum* on carrots probably could be due to extremely low temperature. It is needed to carry out further studies to optimize the parameters of the biopreparation application.

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