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Short Notes

Response of carrot seed germination to heat treatment, the emergency measure to reduce the risk of '*Candidatus* Liberibacter solanacearum' seed transmission

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Summary. In Europe and the Mediterranean region, '*Candidatus* Liberibacter solanacearum' (Lso) is associated with emerging diseases of *Apiaceae* crops, mainly carrot. Emergency measures for import of carrot seed were set, requiring seed to be heat-treated at 50°C or tested as Lso-negative by PCR. The germination response to heat treatment was assessed for 24 carrot cultivar and hybrid seed lots. Ten parsley, five fennel, and two celery seed lots were also analysed. Of these 41 seed lots, 21 were Lso-infected. Water heat treatment significantly decreased germinability compared to dry heat treatment, indicating that dry heat treatment is a cheaper and less detrimental procedure. However, the dry heat treatment significantly decreased seed germination compared to untreated controls in four of 24 seed lots of carrot, four of ten parsley seed lots, three of five fennel seed lots, and one of two celery seed lots. For parsley, the heat treatment reduced germinability to a lesser extent in Lso-infected than Lso-free seed lots. These data show that heat treatment can affect the germination of *Apiaceae* seeds to varying degrees, depending on species or variety, the type of heat treatment, and the sanitary status of the seeds.

Keywords. *Apiaceae*, *Daucus carota*, 50°C, seed import-export, FAO-IPPC emergency action.

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INTRODUCTION

'*Candidatus* Liberibacter solanacearum' (Lso) is a Gram-negative α proteobacterium, limited to the host plant phloem and psyllid vector haemolymph. Lso is associated with several severe plant diseases (Ilardi and Catara, 2013). The pathogen was first identified as associated with zebra chip in potato (*Solanum tuberosum*) (Munyaneza *et al*., 2007), and then with other diseases of solanaceous crops in Central and North America and Oceania. In Europe and the Mediterranean region, Lso was associated with vegetative disorders in *Apiaceae* crops, mainly carrot (*Daucus carota*) but also parsley (*Petroselinum*

crispum), celery (*Apium graveolens*), and fennel (*Foeniculum vulgare*) (EFSA, 2019; EPPO, 2020a; and references therein). In addition, Lso was detected in several commercial *Apiaceae* seeds marketed in Italy and the United Kingdom (Ilardi *et al*., 2016; Monger and Jeffries, 2016); in the United Kingdom, Lso was also found in seeds from an historical collection (Monger and Jeffries, 2018).

Twelve haplotypes of Lso have been described (EPPO, 2020a; Haapalainen *et al*., 2020 and references therein; Sumner-Kalkun *et al*., 2020). Haplotypes A and B are associated with diseases of potato and other solanaceous crops in the Americas and Oceania, and are included in the European Plant Protection Organization (EPPO) A1 quarantine list (EPPO, 2020b). Haplotypes C, D, E and H infect apiaceous hosts in Europe and the Mediterranean area, while haplotypes H(Con) and U mainly infect weeds. Cras1 and Cras2 are novel haplotypes recently found in psyllid *Craspedolepta* spp. (Sumner-Kalkun *et al*., 2020).

Lso is transmitted by psyllids (superfamily *Psylloideae*) in a persistent-propagative manner (Haapalainen, 2014), and species of *Trioza* and *Bactericera* (*Triozidae*) are known to transmit the most economically relevant Lso haplotypes worldwide (reviewed in Haapalainen, 2014). Recently, also *Craspedolepta* spp., in the family *Aphalaridae* were reported as vectors of Lso in Europe (Sumner-Kalkun *et al*., 2020). Transmission of Lso in true seed was suggested in carrot by Bertolini and colleagues (2015). This finding triggered activation of an emergency action by the Food and Agriculture Organization of the United Nations (FAO) - International Plant Protection Convention (IPPC) (FAO-IPPC Emergency actions, 2016). This measure requires carrot seed to be confirmed as Lso-negative by PCR, or to be heat-treated to inactivate the pathogen. Heat treatments are specified to be performed either in dry conditions, at a minimum temperature of 50°C for at least 72 continuous hours, or with hot water at a minimum temperature of 50°C for at least 20 continuous minutes. Other studies have reported that Lso seed transmission in carrot is not observed (Haapalainen *et al.* 2017; Oishi *et al*., 2017; Mawassi *et al*., 2018; Carminati *et al*., 2019; Fujikawa *et al*., 2020b; Nissinen *et al*., 2021). Based on these findings, Australia recently revised import conditions for apiaceous seeds, removing the requirement of heat treatment or Lso negative test (Australian Government, 2021). Nonetheless, some countries (e.g., Japan) still impose emergency measures and import restrictions for carrot and other *Apiaceae* seeds, and many countries reject imported *Apiaceae* Lso-infected seed lots (Italian National Plant Protection Organization, personal communication, 2022). Such measures have negative impacts on import stakeholders and seed producers. Moreover, the effect of heat treatment on the germinability of *Apiaceae* seeds has not been fully ascertained. Literature on this is limited, and the results have been contradictory.

Since a considerable share of carrot seed production is concentrated in the Mediterranean basin (Nissinen *et al*., 2021; Carosem GmbH, 2022), it is important to know the extent of heat treatment effects on carrot seed germination, for the varieties/hybrids cultivated in this area. The present study investigated the response to heat treatment of 24 carrot seed lots, including examples that were Lso-free and Lso-infected, and some relevant varieties (e.g., Nantese, Berlicum, Flakkée) which are extensively used by commercial growers and have major shares of the carrot seed market. In addition, parsley, fennel, and celery seed lots were also tested; the parsley seed lots included Lso-free and Lso-infected samples, while the fennel and celery seed lots were all Lso-free.

MATERIALS AND METHODS

Apiaceae *seed lots*

A total of 41 *Apiaceae* seed lots were used in this study (Table 1). These included: 24 carrot seed lots, belonging to six cultivars and two hybrids, plus 12 lots as blind samples; ten parsley seed lots, belonging to five cultivars, plus two seed lots as blind samples; five fennel seed lots, belonging to three cultivars and one hybrid; and two celery seeds lots of different cultivars. The commercial batches of seeds were purchased from nursery gardens, supermarkets, or plant shops. The blind seed lots were provided by the Plant Protection Service of Emilia Romagna region, Italy (SF-ER).

DNA extraction from Apiaceae *seeds and quantitative PCR for '*Candidatus *Liberibacter solanacearum' detection*

DNA extraction procedure and quantitative PCR test described by Li *et al.* (2009) and validated by Ilardi *et al.* (2019) were used to detect Lso in seeds. For each seed sample, the seeds were shaken for 30 min in 0.5% Triton X-100, rinsed and then left to soften in water overnight. The seeds were then crushed with a mechanical homogenizer (Interscience Bagmixer) for 10 min at maximum speed in plastic bags (Bioreba) with 1:10 (w/v) modified trimethylammonium bromide (CTAB) buffer (2.5% CTAB; 1.4 M NaCl; 0.1 M Tris-HCl, pH 8.0; 0.02 M EDTA, pH 8.0; 1% PVP-40; 0.53% ascorbic acid). 400 µg of RNase A were added to 500 µL of homogenate and, after incubation at 65°C for 30 min, genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen), follow-

Table 1a. Seed lots used in experiments, their germination rates after treatments (n.t. = no treatment, dry = dry heat treatment, water = water heat treatment), Chi-square and *P* values obtained by Chi-square tests. Germinability values are expressed as means ± standard deviations of n = 400 seeds. Significant differences (Chi-square tests) are indicated by * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$, *****= $P < 0.00001$, ns = not significant.

#	SPECIES (No. of analyzed lots) / Variety	Lso	Treatment	Germinability (%)	Chi-square value	*P* value	Significance
	CARROT (24 lots)						
7	Flakkée 2	+	n.t.	84.5 ± 1.73	11.46	0.000709	***
			dry	80.75 ± 4.79			
6	Berlicum 2	+	n.t.	86.75 ± 0.50	0.28	0.594553	ns
			dry	88.00 ± 2.16			
9	Nantese 2	+	n.t.	75.50 ± 5.74	11.52	0.000687	***
			dry	64.50 ± 1.73			
10	Flakkée 2	+	n.t.	80.25 ± 2.22	0.07	0.788421	ns
			dry	81.00 ± 1.41			
5	Nantese Migliorata 2	+	n.t.	82.25 ± 2.75	0.91	0.340838	ns
			dry	84.75 ± 1.71			
19	Parijse markt	-	n.t.	72.75 ± 4.79	10.99	0.000917	***
			dry	61.75 ± 4.43			
20	Nantese Clodia 2	+	n.t.	91.00 ± 3.74	11.32	0.000768	***
			dry	83.00 ± 3.56			
29	Kamilla F1	-	n.t.	90.75 ± 2.87	0.13	0.719126	ns
			dry	90.00 ± 1.41			
31	Rainbow F1	-	n.t.	39.50 ± 4.51	16.32	0.000054	****
			dry	53.75 ± 5.50			
5E	5E	+	n.t.	93.75 ± 1.26	0.56	0.757277	ns
			dry	93.50 ± 1.29			
			water	92.50 ± 1.91			
6F	6F	+	n.t.	92.00 ± 1.41	1.25	0.535037	ns
			dry	89.75 ± 3.86			
			water	90.50 ± 2.65			
38	Flakkèe 2	-	n.t.	88.75 ± 2.22	5.72	0.057303	ns
			dry	86.75 ± 2.99			
			water	83.00 ± 1.41			
39	Nantese 2	+	n.t.	89.50 ± 1.73	0	1	ns
			dry	89.50 ± 3.11			
40	Berlicum 2	+	n.t.	92.75 ± 0.96	2.99	0.083705	ns
			dry	89.25 ± 2.50			
196	196 (SF-ER)	-	n.t.	14.50 ± 4.36	2.2	0.137799	ns
			dry	11.00 ± 2.16			
198	198 (SF-ER)	-	n.t.	29.25 ± 4.65	0.48	0.488987	ns
			dry	31.50 ± 5.57			
199	199 (SF-ER)	-	n.t.	30.25 ± 3.86	2.49	0.114292	ns
			dry	25.25 ± 5.97			
201	201 (SF-ER)	-	n.t.	12.25 ± 1.71	0.05	0.827743	ns
			dry	11.75 ± 3.59			
202	202 (SF-ER)	-	n.t.	24.50 ± 5.32	3.55	0.059374	ns
			dry	19.00 ± 1.83			
204	204 (SF-ER)	+	n.t.	72.00 ± 1.15	0.01	0.937152	ns
			dry	72.25 ± 3.86			

(Continued)

Table 1a. (Continued).

#	SPECIES (No. of analyzed lots) Variety	Lso	Treatment	Germinability (%)	Chi-square value	*P* value	Significance
205	205 (SF-ER)	+	n.t.	89.25 ± 1.71	74.16	<0.00001	*****
			dry	89.25 ± 2.63			
			water	69.25 ± 5.44			
942	942 (SF-ER)	+	n.t.	87.25 ± 2.22	103.82	<0.00001	*****
			dry	88.25 ± 4.03			
			water	62.50 ± 3.51			
943	943 (SF-ER)	+	n.t.	81.75 ± 5.79	2.76	0.096867	ns
			dry	77.00 ± 2.71			
944	944 (SF-ER)	+	n.t.	81.50 ± 0.58	0.01	0.927261	ns
			dry	81.75 ± 2.50			
	PARSLEY (10 lots)						
P2	P2 (SF-ER)	+	n.t.	96.5 ± 2.65	21.47	0.000022	****
			dry	92.5 ± 1.91			
			water	87.75 ± 1.71			
P3	P3 (SF-ER)	+	n.t.	95.00 ± 1.15	1.79	0.408199	ns
			dry	92.75 ± 5.19			
			water	93.50 ± 1.29			
12	Gigante di Napoli - A	+	n.t.	62.75 ± 6.70	15.89	0.000067	****
			dry	48.75 ± 6.50			
18	Gigante di Napoli - B	-	n.t.	84.75 ± 2.63	0.04	0.843022	ns
			dry	85.25 ± 1.50			
22	Nano Ricciuto 2	+	n.t.	61.00 ± 3.56	54.17	<0.00001	*****
			dry	35.00 ± 4.24			
23	Comune 2	+	n.t.	92.75 ± 1.50	1.06	0.303925	ns
			dry	90.75 ± 4.57			
25	Comune	-	n.t.	94.50 ± 2.65	0.35	0.551515	ns
			dry	93.50 ± 2.52			
26	Halflange	-	n.t.	83.00 ± 3.16	0.68	0.408801	ns
			dry	80.75 ± 2.06			
27	Gigante di Napoli	-	n.t.	91.25 ± 1.71	5.47	0.019366	*
			dry	86.00 ± 5.35			
28	Nano Ricciuto 2	+	n.t.	90.00 ± 2.58	1.5	0.22062	ns
			dry	87.25 ± 1.50			
	CELERY (2 lots)						
17	d'Elne	-	n.t.	96.00 ± 0.82	3.46	0.062749	ns
			dry	93.00 ± 3.16			
24	Dorato d'Asti	-	n.t.	76.00 ± 2.16	239.99	<0.00001	*****
			dry	21.25 ± 4.99			
	FENNEL (5 lots)						
21	Romanesco	-	n.t.	86.00 ± 1.83	151.91	<0.00001	*****
			dry	44.50 ± 7.33			
30	Romanesco	-	n.t.	86.50 ± 3.42	256.27	<0.00001	*****
			dry	30.75 ± 5.68			
32	Selvatico	-	n.t.	64.25 ± 4.27	0.14	0.711143	ns
			dry	65.50 ± 6.45			
33	Waden Romen	-	n.t.	90.25 ± 0.50	12.62	0.000381	***
			dry	81.50 ± 5.80			
34	Amedeus F1	-	n.t.	84.00 ± 3.74	0.32	0.569983	ns
			dry	82.50 ± 3.32			

ing the manufacturer's instructions. Quantitative PCR tests was carried out according to Ilardi *et al*. (2019) and using TaqMan Universal Master Mix II no UNG (Applied Biosystem). After 45 cycles, samples were considered positive if an exponential amplification curve was produced with a cycle threshold (Ct) value <40. For each amplification event, the following controls were included: a negative extraction control, represented by a sample of uninfected matrix; a positive amplification control, represented by DNA extracted from infected carrot; and a negative amplification control, i.e., nuclease-free water. All samples were tested in two technical repetitions.

Heat treatments of Apiaceae *seeds*

Dry heat treatment is generally considered as more feasible by seed producers and seed companies than water heat treatment. Dry treatments avoid the need for subsequent drying step and the risk of mould development. For this reason, the *Apiaceae* seed lots analysed in the present study were mainly tested by dry heat treatment. Comparisons of dry and water treatments were also carried out on a smaller scale, for five carrot seed lots (# 5E, 6F, 38, 205 and 942) and two parsley seed lots (# P2 and P3) (Table 1, Figure 1). For dry treatment, the seed samples (10 g, corresponding to approx. 10,000 seeds) were placed in 50 mL capacity polypropylene tubes, and these were heated in an oven at 50°C for 72 continuous hours. After heating, seed samples were stored at 4°C until used.

For water treatment, the seed samples (each of 10 g each contained in a woven cotton bag) were immersed in a 50°C water bath for 20 min, and then immediately cooled with cold tap water. The seeds were then air-dried on filter paper and then stored at 4°C until used.

Germination of Apiaceae *seeds*

For each lot, 400 heat-treated and 400 untreated control seeds (four biological replicates, each of 100 seeds) were germinated, using the Italian rule DM 22 /12/ 1992 for official methods for seed analyses (https://www.gazzettaufficiale.it/eli/gu/1993/01/04/2/so/1/sg/pdf) and according to the International Seed Testing Association (ISTA 2020).

Statistical analyses

Statistical analyses were carried out using software R version 4.1.1 (R Core Team, 2021). Dry heat-treated and untreated germination rates for each lot were compared by Chi-square tests. Germination data from dry heat-treated, water heat-treated and untreated groups on five carrot seed lots (# 5E, 6F, 38, 205, and 942) and two parsley seed lots (# P2 and P3) were compared by Chi-square tests; when significant, *post-hoc* multiple comparison tests were carried out to establish statistical significance of differences between each pair of groups.

Lso-free/heat-treated, Lso-free/untreated, Lso-infected/heat-treated and Lso-infected/untreated groups were analysed by Chi-square tests, followed by *post-hoc* multiple comparisons. This analysis was carried out for carrot and parsley seed lots whose germinability was above the limit for marketable seeds (Council Directive 2002/55/EC). For fennel and celery, this comparison was not feasible because no infected lots were available.

Table 1b. *Post-hoc* multiple comparison test results following Chi-square test with more than two treatments, when significant difference among groups was indicated in the first test (Table 1a).

#	Multiple comparison test			
	Comparison	Chi-square	*P* value	Significance
Carrot 205	n.t. vs dry	0	1	ns
	n.t. vs water	48.65	<0.00001	*****
	dry vs water	48.65	<0.00001	*****
Carrot 942	n.t. vs dry	0.19	0.666219	ns
	n.t. vs water	65.12	<0.00001	*****
	dry vs water	71.45	<0.00001	*****
Parsley P2	n.t. vs dry	6.16	0.013091	*
	n.t. vs water	21.11	<0.00001	*****
	dry vs water	5.07	0.024339	*

RESULTS

High levels of Lso infection were observed in carrot and parsley seeds, with 15 of 24 carrot seed lots positive for Lso, and six of ten parsley seed lots positive for Lso. All the fennel and celery lots were Lso-free (Table 1a). Table 1a reports germination rates of all the tested seed lots after treatments, as well as Chi-square test results (Chi-square value, *P* value, and statistical significance). When more than two treatments were compared (*i.e.,* no treatment, dry treatment and water treatment), and a Chi-square test indicated a statistically significant difference among them, *post-hoc* multiple comparison test results are reported (Table 1b).

Six Lso-free carrot seed lots showed low germination rate (i.e., # 31, 196, 198, 199, 201 and 202), and five of them were blind samples provided by SF-ER. They were

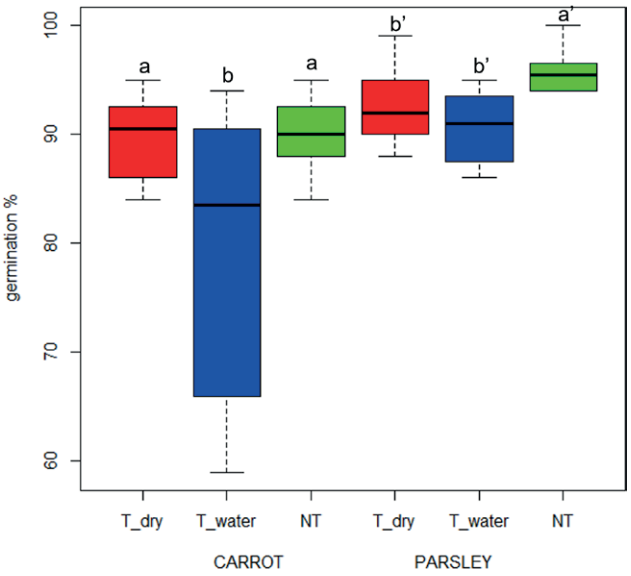

Figure 1. Mean germination rates (%) for carrot and parsley seeds after different treatments. Left: rates for dry heat-treated (T_dry), water heat-treated (T_water) and untreated (NT) carrot seed lots. Means and standard deviations were calculated for n = 2000 seeds from five seed lots. Right: germination rates for dry heat-treated (T_dry), water heat-treated (T_water) and untreated (NT) groups of parsley seed lots. Means and standard deviations were calculated for n = 800 seeds from two seed lots. Statistical significance of differences was determined using Chi-square *post-hoc* multiple comparison tests: different letters indicate significantly different treatments.

official samples whose storing conditions and expiry date were unknown.

The germination rates of carrot seeds decreased after dry heat treatment in four of 24 seed lots, of at least six cultivars and two 2 hybrids, while germination increased in the treated hybrid Rainbow F1 (Table 1a). For the other *Apiaceae* seed lots, reductions of germination were detected for four of ten parsley seed lots from five cultivars, three of five fennel seed lots of three cultivars, and one hybrid and one of two celery seed lots of two cultivars (Table 1a).

The experiment using five carrot seed lots (# 5E, 6F, 38, 205, and 942) and two parsley seed lots (# P2 and P3), which were selected among those having germination rates greater than the limit of marketable seeds (Council Directive 2002/55/EC), was carried out applying dry and water treatments. For the carrot seed lots # 205 and 942, dry heat treated and untreated seeds had similar germination rates while the water treatment reduced germination. The germination rates for the other three carrot seed lots were not significantly different for all the treatments (Table 1, a and b). For parsley, the seed lot P2 gave differences between each pair of treatments, with greatest germination (96.5%) for the untreated seeds, and the least (87.8%) for water treated seeds (Table 1, a and b). Overall, for carrot, the comparison of treatments showed decreases ($P < 0.00001$) of germinability for water treated seeds compared to dry treated or untreated seeds, which were comparable to each other (Figure 1, Table S1). The same experiment for parsley seed (Figure 1, Table S1) showed that germinability decreased after both types of treatment ($P < 0.001$).

The effects of heat treatments were also evaluated by comparing the germination rates for Lso-infected and Lso-free seed lots of carrot and parsley. The seed lots were chosen among those with germination rates above the limit for marketable seeds (Council Directive 2002/55/EC). For carrot seed, heat treatment decreased the germinability of both Lso-infected and Lso-free seed groups compared to their untreated controls (Figure 2 A, Table S2); for parsley, the heat treatment only decreased germinability of Lso-infected seeds (Figure 2B, Table S2). Overall, Lso-infected seeds had greater germinability than Lso-free seeds, either with or without treatments.

Similar comparisons were not carried out for celery and fennel seeds, since no Lso-infected seed lots were available.

DISCUSSION

Carrot is one of the most important root vegetables and it is cultivated in many countries (Que *et al.*, 2019). Carrot seeds and phloem sieve tubes within the seed coats are known to transmit several pathogens (Kuan *et al.*, 1985; Zhang *et al.*, 2020), including Lso (Bertolini *et al.*, 2015), which has been detected in infected seed lots to produce approx. 12-24% of infected seedlings. Two other distinct experimental replications did not show any Lso seed transmission (Loiseau *et al.* 2017a; 2017b; Carminati *et al.,* 2019), and further studies did not find evidence of Lso seed transmission in carrot (Haapalainen *et al.* 2017, Oishi *et al.*, 2017; Mawassi *et al.*, 2018; Fujikawa *et al.*, 2020b; Nissinen *et al.*, 2021). Based on these results, some seed import restrictions have been eased.

As Lso has been widespread in commercial *Apiaceae* seed lots (Ilardi *et al.*, 2016; Monger and Jeffries, 2016), heat treatment is the only procedure for ensuring seed importation.

In the present study experiments, six Lso-free carrot seed lots had low germination rates, and five of them were official samples whose storage conditions and expiry dates were unknown. The age of these lots and/or storage conditions may be a reason for such reduced germinability. The presence of other pathogens in the samples may have also caused low germinability. These

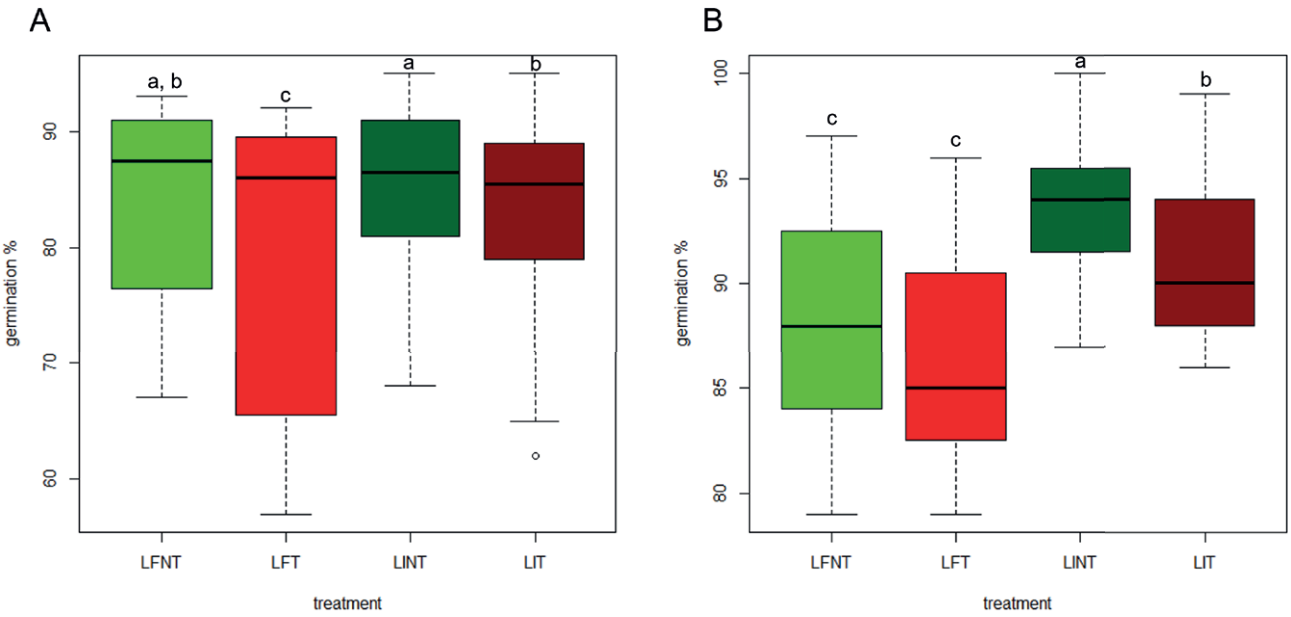

Figure 2. Mean germination rates (%) of Lso-free untreated (LFNT), Lso-free dry treated (LFT), Lso-infected untreated (LINT) and Lso-infected dry treated (LIT) seeds of carrot (A) or parsley (B). In boxplot A, the values are each expressed as mean ± standard deviation for n = 6000 Lso-infected seeds from 15 seed lots, and n = 1200 Lso-free seeds from three lots. In boxplot B, n = 1600 seeds from four seed lots. Different letters accompanying each treatment indicate statistically significant differences between treatments (Chi-square *post-hoc* multiple comparison tests).

factors were not assessed, but, during the germination experiments, no fungal contamination was observed when the germination dishes were inspected.

Dry heat treatment of seeds has been commonly described for disinfection from many pathogens (fungi, bacteria). Exposure to temperatures between 55 and 75°C was reported to be lethal for seeds of conifer (Baker, 1929), bunch grass (Wright, 1970; Koppenaal and Colombo, 1988) and bottle gourd (Nakamura, 1982). More recently, a comprehensive study on 66 wild species from 22 plant families (but not *Apiaceae*) reported that dry heat treatment at 60°C for 1 h did not cause significant decreases in seed germinability, and germination was increased for ten species (Godefroid *et al*., 2017). Grondeau *et al*. (1992) suggested that rehydration of seeds after dry treatments could be necessary to achieve germination. These reports are therefore contradictory, showing lethal and no effects of heat treatments on seed germination, depending on the species, the thermal protocol and rehydration procedures.

The present study showed that germination rates decreased after dry heat treatments for four of 24 carrot seed lots. The effect was even more evident for parsley and fennel, where germination was reduced for four of ten parsley seed lots and three of five fennel seed lots. These data indicate slight to moderate impacts of dry heat treatments on *Apiaceae* seed germinability, depending on the species. In carrot, three of the four varieties showing germination reductions (*i.e*., Flakkée, Nantese, Berlicum) are extensively used by commercial growers, and represent a major share of the carrot market. Therefore, the impacts of heat treatments on seed producers and importers are likely to be relevant. Only for the carrot hybrid Rainbow F1 did heat treatment improve the germinability of seeds. This could be related to the low germination percentage of the untreated seed lot, which was below the minimum germination rate standards required for commercialization (65% for carrot seed, Council Directive 2002/55/EC). However, other carrot seed lots with germination percentages below the minimum standards (*i.e*., lots 196, 198, 199, 201 and 202) did not show significant differences in germinability between treated and untreated samples (Table 1 a).

Hot water treatment is the oldest type of heat treatment for seeds. For carrot seeds, it is used for disinfection from *Xanthomonas campestris* pv. *carotae* (Ark and Gardner, 1944). Ten min at 52°C are sufficient to inactivate the bacterium. Studies on germination of seeds after hot water disinfection indicate variability among plant species from temperate ecosystems, and but this topic has not been widely studied. Some authors have indicated that carrot seed disinfection procedures using hot water, as well as hypochlorite treatment, did not affect germinability, but chemical treatments with ethanol or

oxytetracycline greatly decreased germination (Fujikawa *et al*., 2020a). Other studies have reported decreased germination of carrot seeds after hot water treatments, indicating the thermal treatment limit was 50°C for 40 min. For higher temperatures or longer exposure times, germinability decrease has been observed (Merfield, 2005). Strandberg and White (1989) reported that ten of 25 carrot hybrids had reduced seedling emergence in greenhouse experiments after hot water treatments of seeds at 50°C for 20 min. Fourteen hybrids were not affected, and one had increased seedling emergence after treatment. Injurious effects on germination induced by hot water seed treatments were also reported by Grondeau *et al.* (1994), depending on exposure time. Bolton *et al.* (2019) reported that heat water treatment at 35°C applied to 293 carrot accessions reduced germination from a mean of 63.8% to 33.0%. Data from the present study showed that germination of two of five carrot varieties and one of two parsley varieties decreased after hot water treatments, confirming previous literature, and supporting dependence of this effect on plant variety. In carrot, the hot water treatment reduced germination more than dry treatment, whereas in parsley, germination decreased after both treatments compared to no treatment. As reported above, use of water for heat treatment is not considered suitable by stakeholders. For carrot seed, the results achieved support the use of dry heat rather than water.

The decrease in germination rates after heat treatments, which involved Lso-free and infected seeds of carrot and only Lso-infected parsley seeds, further confirms that impacts of heat treatments of seeds is strongly dependent on species. The data obtained in the present study indicate that heat treatments of seeds can have economic impacts, from the direct costs of the procedures, and from resulting decreases in seed germinability of some cultivars of *Apiaceae* species. Carrot seed germinability decrease after heat treatment in some economically important cultivars, and the same trend was found in other *Apiaceae* species.

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