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# INFLUENCE OF CULTIVARS AND SEED THERMAL TREATMENT ON THE DEVELOPMENT OF FUNGAL PATHOGENS IN CARROT AND ONION PLANTS

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## Abstract

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Carrot and onion are vegetables representing an important segment of fresh market. They suffer from serious fungal diseases that can inflict great damage on crops, i.e. alternaria leaf blight, peronospora downy mildew, and botrytis neck rot. The resistance of selected carrot and onion cultivars important for the production of vegetables in the Czech Republic was tested by exposure to targeted infection by the above fungal pathogens. The exposure of eleven carrot cultivars to spores of *Alternaria dauci* showed that the most resistant and sensitive cultivars were Katrin, Cortina F1, Afalon F1 and Favorit, Tinga, Berlika F1, respectively. A targeted infection of onion cultivars with *Botrytis aclada* clustered them into three groups: Amfora F1, Bolero, Tosca, Triumf F1 (strong resistance), Avalon, Grenada (medium resistance), Alice, Karmen, Všetana (low resistance). Similar groups were distinguished also after the infection with *Peronospora destructor*: Avalon, Bolero, Tosca (strong resistance), Alice, Amfora F1, Grenada, Karmen, Triumf F1 (medium resistance),Všetana (low resistance). Hot water treatment of carrot seeds applied after the inoculation with *A. dauci* decreased the development of the infection 1.3-2.3-fold, whereas the protective effect observed with onion seeds against the infection by *P. destructor* and *B. aclada* was lower.

Keywords: carrot, onion, plants infection, fungal pathogens, cultivar sensitivity, hot water treatment

## INTRODUCTION

Carrot and onion are important vegetables from the point of view of the size of their production. In the Czech Republic in 2014, the respective sown areas were 1730 and 691 ha, with a total of 9211 ha representing all vegetables (Buchtová, 2014). The above vegetable species suffer from serious diseases, for instance, the leaf blight caused by *Alternaria dauci* that inflicts damage on carrot growth (Farrar et al., 2004). The primary source of the infection are infected seeds used for sowing, but the pathogen can also survive on infected postharvest residues and is spread with the support of rain and wind (Kazda *et al.*, 2003). The dissemination of the pathogenic fungus is promoted by raining weather (Kazda *et al.*, 2003) and temperatures above 24 °C (Rod *et al.*, 2005). Lammerts van Bueren *et al.* (2003) consider the fungus *A. dauci* to be one of plant pathogens whose spreading via seeds has been proven and recommend a chemical treatment of seeds for the conventional system of production and a physical treatment (e.g. hot water treatment, HWT) for the ecological system of production.

Both treatments are thought to be suitable preventative measures that can eliminate this pathogen on seeds and significantly decrease its development, especially in the phase of emergency. Hermansen *et al.* (1999) recommended to eliminate *A. dauci* on seeds by HWT, by applying the temperature of 54 °C for 20 min. Lammerts van Bueren *et al.* (2011) mention the importance of breeding of cultivars resistant to biotic and abiotic stresses as an important purpose of breeding,

together with a good yield and good sensoric quality of the product. In this connection, the tolerance of new cultivars to diseases is thought to be essential for minimization of differences between the yield potential and the actual yield. Cadod (2002) suggests as another argument for using new cultivars in agricultural production a decrease in the amount of pesticides spent and, consequently, minimization of pesticide residues in the vegetables. A good example can be the new cultivars of carrot tolerant to *A. dauci* (Cadod, 2002).

In the case of onion, the downy mildew caused by the fungus *Peronospora destructor* seems to be the most important disease damaging crops (Develash and Sugha, 1997). Characteristically, the plants show yellow-green diffuse patches on leaves and floral stalks or greyish coatings made of sporangiophores and sporangia. The damaged sites are often colonized by a secondary infection of semiparasitic and saprophytic fungi (Ackermann *et al.*, 2004). The fungus is able to survive in seeds, infected bulbs, and plant residues. It is spread by spores whose optimal temperature for germination is 10-12 °C. The germination can occur up to 20 °C but higher temperatures decrease the germination rate. An important condition for the germination is humectation of leaves taking at least 3 h (Kazda *et al.*, 2001; Palti, 1989).

Another important disease of onion is the neck rot caused by the fungus *Botrytis aclada* (syn. *Botrytis allii*). The disease rarely appears to the end of the harvest but more often during the storage and results in softening and rotting of bulb necks. The source of infection are contaminated seeds from which the infection spreads to attack seedlings on whose leaves conidias are formed that, subsequently, infect surrounding plants. Another source of infection are postharvest residues, set onions or seed-production plants, and sclerotia in soil (Rod *et al.*, 2005).

The purpose of the study was to compare the resistance of cultivars of carrot and onion currently used in the production of vegetables for the fresh market to major fungal pathogens *A. dauci*, *P. destructor* and *B. aclada* causing great commercial losses. The fungal strains used were obtained from acknowledged collections of microorganisms or, in the case of *P. destructor*, where such strains could not be purchased, from leaf biomass collected in a heavy contaminated onion field. The inoculation method used a direct infection of seedlings with a spore suspension prepared in vitro or, in the case of *P. destructor*, with a buffer extract prepared from an infected leaf biomass. The sensitivity to the infection was measured against non-treated controls. Also, the efficiency of HWT applied after the inoculation of seeds with the fungal pathogens to decrease the development of the corresponding infections was investigated.

## MATERIAL AND METHODS

### Plant and fungal material

*Alternaria dauci* CBS 117098 originating from New Zealand was purchased from Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands and *Botrytis aclada* DSM 62081 from Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany. The fungus *Perenospora destructor* could not be obtained from acknowledged collections. The inoculum was therefore prepared directly from plant biomass collected in a field from onion growth massively affected by this phytopathogenic fungus. (cf. Abd-ElRazik et Lorbeer, 1980).

For testing the resistance of carrot cultivars to the alternaria infection, the cultivars (in parentheses – year of variety permission) Afalon F1 (2009), Aneta F1 (2009), Berlika F1 (2011), Cortina F1 (2007), Darina (2000), Favorit (2005), Francis (2009), Katrin (2006), Marion F1 (2008), Naomi (2009), and Tinga (1998) were used. The onion cultivars included in the study were Alice (1970), Amfora F1 (2013), Avalon (2012), Bolero (2012), Grenada (2007), Karmen (1967), Tosca (2008), Triumf F1 (2010) and Všetana (1946). Some of the cultivars were also used to determine the effect of HWT of seeds on the development of fungal pathogens. All carrot and onion cultivars were obtained from Moravoseed a.s., Czech Republic.

### Inoculum preparation and infection

Fungal strains were maintained on Potato Dextrose Agar (PDA, Oxoid, UK) (39 g per litre). Spore inoculum of *A. dauci* was prepared according to a modified method of Strandberg (1977). The fungus was grown on the vegetable juice V8 agar medium (10 % V/V, vegetable juice, 2 % W/W agar, pH 6) in Petri dishes inoculated with 0.5x0.5 cm agar blocs covered with fungal mycelium from 2-week-old PDA agar cultures. The Petri dishes were incubated for two weeks at 24 °C under an exposure to long-wave UV irradiation (Blacklight Blue LT-T5 13 W, 12 h light/12 h dark regime) at high humidity to ensure sporulation. Then a spore suspension was prepared using sterile distilled water containing 0.01 % (V/V) Triton X-100 and adjusted to a concentration of $5 \times 10^5$–$1 \times 10^6$ spores per ml that was used for inoculation of plants by atomizer at a rate of 5 ml per plant (Shahin and Shepard, 1979; Strandberg, 1987). The V8 vegetable juice was obtained from Campbell Foods, Belgium. After the application of the spore suspension, the inoculated plants in the period of primary leaves were kept under a propylene textile cover for 48 h to maintain a high humidity. Similar method of inoculation was also used when carrot seeds were infected. Before and after the inoculation, the plants were further incubated in a Binder KBW 400 phytotron chamber under conditions of a 12-h photoperiod (10 kLx) with day/night temperatures of 20/18 °C.

The inoculum of *B. aclada* was prepared and applied using modified methods of Köhl *et al.* (1997) and Presly (1985), respectively. First, a static, liquid-medium pre-culture in Malt Extract Broth (Oxoid, UK) was grown for two weeks at 24 °C. This culture was gently homogenized (Ultraturrax mixer, Germany) at the room temperature and used for inoculation of Petri dishes with solid Oatmeal Agar medium pH 6 (Difco, USA) containing 2 % (W/W) agar. The fungus was grown for two weeks in the dark and then a spore suspension was prepared and used for inoculation of plants in the period of primary leaves as mentioned above.

In the case of *P. destructor*, plant leaves collected from an onion field massively affected by a spontaneous *P. destructor* infection were homogenized in a mixer at the room temperature and extracted by 0.2 M phosphate buffer pH 7 (250 g plant biomass, 2000 ml phosphate buffer) at 4 °C for 30 min. After decantation, the infusion was used for inoculation of plants in the period of primary leaves with an atomizer as described above (Abd-ElRazik et Lorbeer, 1980; Buloviene et Surviliene, 2006). The incubation of onion plants after inoculation was similar as in the case of carrot seedlings.

### Tests of variety resistance

The seeds of the cultivars of carrot and onion tested were sown in plastic dishes containing moist PR33 sand (Provodínské písky). For each cultivar an amount of 4 × 50 seeds was used that and the sprouted plants were subsequently inoculated with the fungal pathogens, namely, *A. dauci* in the case of carrot and *B. aclada* or *P. destructor* in the case of onion. Consequently, an amount of 200 plants per cultivar was tested in one experiment. The controls were not inoculated but the other treatments were similar. The subsequent incubation of the plants is described above.

### HWT treatment

In the experiments where the seeds were thermally treated with hot water (HWT) the following variants were used: infected seeds, non-treated seeds (control), infected seeds subsequently HWT treated, non-infected seeds subsequently HWT treated (control). Each of the variants represented a sowing of 4 × 50 seeds, including the controls. An amount of 200 plants per cultivar was tested in one experiment. The standard HWT protocol was the following (Moravoseed, 2012): The seeds were put in a sac from Monofil PAD material, the sac was immersed in water (37 °C) for 2 min to pre-warm the seeds, than the sac was immersed in hot water (50 °C) for 20 min. The air bubbles were removed manually at the beginning of the treatment, the sac was placed so that it did not touch the bottom of the bath, and the water bath was stirred continuously during the incubation. Then the seeds were cooled down by inserting in cold water for 5 min and, subsequently, they were spread on a screen and dried by air current (28 °C) blowing from the bottom.

The experiments testing the cultivar resistance and HWT effect were realized in the period of 2012-2014: *A. dauci* (sowing 27/11 2012, inoculation 06/12/2012, evaluation 11/01 2013), *B. aclada* (sowing 04/08 2014, inoculation 14/08 2014, evaluation 15/09 2014), *P. destructor* (sowing 06/10 2014, inoculation 16/10 2014, evaluation 18/11 2014). In the HWT experiments, the seeds were sown immediately after the cultivar resistance experiments were terminated and the time span of the experiments was similar.

#### *Disease assessment*

The plant infection was visually assessed using a modified method of Pawelec *et al.* (2006) when the relative number of leaves affected by the infection per plant was determined. The arbitrary 0-9 scale included the following symptoms: 0 points – no leaves affected, 1 point - <5 % leaves affected per plant, 3 points – 5–30 % leaves affected per plant, 5 points – 30–60 % leaves affected per plant, 7 points – 60–90 % leaves affected per plant, and 9 points - >90 % leaves affected per plant or severe defoliation occurred.

#### *Statistical evaluation*

Statistical analysis was carried out using the STATISTICA 12.0 software system (Stat Soft). The data measuring the infections by fungal pathogens were analyzed by 'ANOVA' statistical program with the subsequent application of Fisher's LSD test ($p \leq 0,05$).

## RESULTS AND DISCUSSION

### Resistance of carrot and onion cultivars to fungal pathogens

When the resistance of eleven selected cultivars of carrot to the infection with *A. dauci* was tested, significant differences in the sensitivity of the cultivars were observed (Table I). The fungal infection was measured on the background of a spontaneous infection detected in the non-inoculated controls that was in the range of 0.52 to 3.75 points of the arbitrary, infection-rate, 9-point scale. This scale was also used for the evaluation of the targeted fungal infection that developed during the experiment. The cultivars with the lowest and highest spontaneous infection were Berlika F1 and Katrin, respectively. The difference of the infection rates of the non-infected plant controls and the infected plants demonstrated the efficiency of the infection by *A. dauci*. The increase of the infection evaluated according to the arbitrary 9-point scale was in the range of 1 to 5.4-fold (Table I). The lowest increase of the infection (1 to1.1-fold) after inoculation with *A. dauci* was found in the cultivars Katrin, Cortina F1 and Afalon F1 showing that these varieties most resisted the fungal infection. The

I: *Infection rates measured in carrot cultivars after inoculation with Alternaria dauci.*

| Carrot | | | |
|---|---|---|---|
| **Cultivar** | **Treatment** | **Degree of infection (arbitrary points)** | **Relative change of infection after inoculation** |
| Darina | Control | 2,25 ± 0,48$^{cde}$ | 2.0x |
| | Infected | 4,50 ± 0,83$^{g}$ | |
| Favorit | Control | 1,35 ± 0,28$^{b}$ | 2.8x |
| | Infected | 3,79 ± 0,72$^{g}$ | |
| Berlika F1 | Control | 0,52 ± 0,14$^{a}$ | 5.4x |
| | Infected | 2,83 ± 0,54$^{e}$ | |
| Naomi | Control | 3,71 ± 0,33$^{g}$ | 1.5x |
| | Infected | 5,38 ± 0,62$^{h}$ | |
| Tinga | Control | 1,39 ± 0,24$^{b}$ | 2.8x |
| | Infected | 3,88 ± 0,66$^{g}$ | |
| Katrin | Control | 3,75 ± 0,59$^{g}$ | 1.1x |
| | Infected | 4,17 ± 0,66$^{g}$ | |
| Francis | Control | 1,50 ± 0,27$^{bc}$ | 1.3x |
| | Infected | 2,00 ± 0,25$^{bcd}$ | |
| Cortina F1 | Control | 1,33 ± 0,51$^{b}$ | 1.1x |
| | Infected | 1,42 ± 0,36$^{b}$ | |
| Afalon F1 | Control | 2,63 ± 0,52$^{de}$ | 1.0x |
| | Infected | 2,63 ± 0,35$^{de}$ | |
| Aneta F1 | Control | 2,50 ± 0,34$^{de}$ | 1.8x |
| | Infected | 4,50 ± 0,61$^{g}$ | |
| Marion F1 | Control | 1,38 ± 0,34$^{b}$ | 1.4x |
| | Infected | 1,96 ± 0,44$^{bcd}$ | |

Note: The values of degree of infection marked by different letters (a-h) were significantly different.

highest increase of the infection after inoculation (2.8-5.4-fold) was observed in the Favorit, Tinga and Berlika F1 cultivars suggesting that they were the most sensitive to the infection by *A. dauci*. The other cultivars exhibiting the increase of infection of 1.3–2-fold had a medium resistance to the *A.dauci* infection. Differences in the resistance to the *A. dauci* infection between individual cultivars of carrot were reported by Carvalho *et al.* (2005). The sensitivity to the infection in resistant cultivars can be lower by about 25 % compared to that of the sensitive cultivars (Gugino *et al.*, 2007).

A similar experiment testing the resistance of nine cultivars of onion to the fungus *P. destructor* also showed significant differences between the cultivars with respect to their sensitivity to the fungal pathogen. Here, the inoculation was not with a spore suspension as with the other pathogens in this study, but with a homogenized, plant leaves biomass containing the fungus that was collected in the field. In this case the background of the spontaneous infection was higher, 1.81 to 4.50 points (Table II). Some cultivars did not show an increase of infection after the inoculation of plants with the fungus, namely Avalon, Bolero and Tosca, suggesting their resistance (1 to1.1-fold increase). The highest increase of the infection after inoculation with *P. destructor* was detected in the Všetana plants (3.5-fold). This cultivar was evidently the most sensitive to the *P. destructor* infection. The cultivars Alice, Amfora F1, Grenada, Karmen and Triumf F1 exhibited a medium sensitivity to the infection (1.2-1.5-fold increase). The application of the plant biomass extract may have a protective effect that could contribute to the lower infection values found after inoculation of some cultivars (Table II). Leaf extracts and leachates were found inhibitory to sporangial germination and germ tube growth of *P. destructor*, probably due to substances toxic to the pathogen (Yarwood, 1943; Dewelash et Sugha, 1996). Recently, geneticists breeding new cultivars of onion have intensely worked on the production of new cultivars resistant to the fungus by crossing the onion species *Allium cepa* and *Allium roylei*, the latter carrying a resistance gene to this fungal pathogen (Scholten *et al.*, 2007).

Significant differences in the infection rates between the onion cultivars were also detected when the onion plants were inoculated with a spore suspension of *B. aclada* (Table II). Even here the background of the spontaneous infection was rather high, 3.06 to 5.5 points. Four cultivars (Amfora F1, Bolero, Tosca, Triumf F1) showed a low or non-existent increase of the infection after the inoculation of plants with the fungus (1 to1.1-fold increase) suggesting a resistance to the infection,

II: *Infection rates measured in onion cultivars after inoculation with Peronospora destructor and Botrytis aclada.*

| Onion | | | | | |
|---|---|---|---|---|---|
| **Cultivar** | **Treatment** | **Degree of infection by *Peronospora destructor* (arbitrary points)** | **Relative change of infection after inoculation** | **Degree of infection by *Botrytis aclada* (arbitrary points)** | **Relative change of infection after inoculation** |
| Alice | Control | $3{,}31 \pm 0{,}90^{bcd}$ | 1.3x | $3{,}88 \pm 0{,}89^{bcd}$ | 1.4x |
| | Infected | $4{,}38 \pm 0{,}90^{efg}$ | | $5{,}50 \pm 0{,}65^{gh}$ | |
| Amfora F1 | Control | $4{,}50 \pm 0{,}7^{6fg}$ | 1.4x | $5{,}25 \pm 0{,}70^{g}$ | 1.1x |
| | Infected | $6{,}25 \pm 0{,}5^{1i}$ | | $6{,}00 \pm 0{,}58^{h}$ | |
| Avalon | Control | $3{,}19 \pm 0{,}91^{bcd}$ | 1.0x | $4{,}19 \pm 0{,}83^{de}$ | 1.2x |
| | Infected | $3{,}06 \pm 0{,}94^{bc}$ | | $5{,}00 \pm 0{,}683^{fg}$ | |
| Bolero | Control | $4{,}50 \pm 0{,}7^{6fg}$ | 0.7x | $3{,}94 \pm 0{,}73^{cde}$ | 1.1x |
| | Infected | $3{,}31 \pm 0{,}89^{bcd}$ | | $4{,}25 \pm 0{,}66^{de}$ | |
| Grenada | Control | $4{,}25 \pm 0{,}79^{ef}$ | 1.2x | $4{,}50 \pm 0{,}76^{ef}$ | 1.2x |
| | Infected | $5{,}13 \pm 0{,}52^{gh}$ | | $5{,}25 \pm 0{,}70^{g}$ | |
| Karmen | Control | $3{,}63 \pm 0{,}81^{cde}$ | 1.5x | $3{,}31 \pm 0{,}81^{ab}$ | 1.4x |
| | Infected | $5{,}50 \pm 0{,}70^{hi}$ | | $4{,}50 \pm 0{,}76^{ef}$ | |
| Tosca | Control | $4{,}19 \pm 0{,}81^{ef}$ | 0.9x | $5{,}50 \pm 0{,}62^{gh}$ | 1.0x |
| | Infected | $3{,}94 \pm 0{,}87^{def}$ | | $5{,}50 \pm 0{,}62^{gh}$ | |
| Triumf F1 | Control | $2{,}63 \pm 0{,}72^{a}$ | 1.4x | $3{,}06 \pm 0{,}79^{a}$ | 1.1x |
| | Infected | $3{,}81 \pm 0{,}75^{cdef}$ | | $3{,}50 \pm 0{,}85^{abc}$ | |
| Všetana | Control | $1{,}81 \pm 0{,}62^{a}$ | 3.5x | $3{,}13 \pm 0{,}72^{a}$ | 1.4x |
| | Infected | $6{,}25 \pm 0{,}63^{i}$ | | $4{,}50 \pm 0{,}76^{ef}$ | |

Note: The values of degree of infection marked by different letters (a-h) were significantly different.

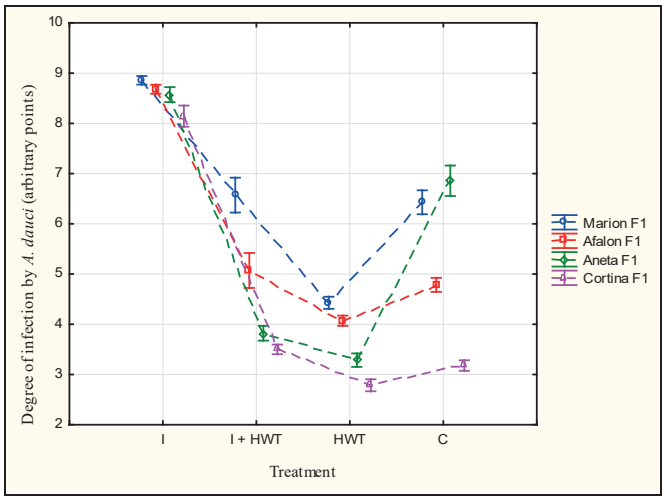
whereas the cultivars Alice, Karmen and Všetana exhibited a high, 1.4-fold increase of the infection after inoculation with the spore suspension. The medium increase of infection, 1.2-fold was observed with the Avalon and Grenada cultivars. Evidently, the Všetana cultivar was the most sensitive to the two fungal infections.

The above results are in agreement with those published by other authors, for instance, the specific intervariety resistance to *A. dauci* described by Rod *et al.* (2005) or a higher resistance of new cultivars of carrot bred to obtain a higher resistance to this fungal pathogen (Farrar *et al.*, 2004). Differences in the resistance of various cultivars of vegetable species to phytopathogenic fungi were also described by Koike et al. (2007) (cabbage, *A. dauci*) and Simko et al. (2014) (lettuce, *Golovinomyces cichoraceum*).

## Protective effect of HWT acting against fungal infection

The thermal treatment of seeds with hot water is a standard procedure recommended to reduce the infection by seed surface-borne pathogens such as *Alternaria dauci*. Farrar et al. (2004) discussed the importance of the temperature used and the period of its application since high temperatures and long treatments can result in a decreased seed germination capacity. A thermal treatment of carrot seeds after the inoculation significantly decreased the development of *A. dauci* in all the cultivars tested. The decrease was in the range of 1.3 to 2.3-fold (Fig. 1, Table III). HWT also decreased the development of a spontaneous infection by pathogens present on the seeds surface; the range of decrease ranged from 1.1 to 2.1-fold (Table III). The observations are in accordance with the results published by Nega et al. (2003) who referred to a decrease of infection by seed-borne pathogens present on the seeds of vegetables of up to 90 % using a maximal temperature of 50 °C applied for not more than 25 min. Similarly, Mancini and Romanazzi (2013) described a more than 95 % reduction of the occurrence of *A. dauci* infection after a treatment of carrot seeds at 50–53 °C for 10–30 min. The HWT treatment was also efficient against *A. brassicicola* where the use of temperatures of 50 or 53 °C applied for 25–30 and 10 min, respectively, was recommended for the treatment of cabbage seeds (Mancini and Romanazzi, 2013).

In the case of onion seeds the effect of HWT on the infection by *P. destructor* and *B. aclada* was lower and often the highest degree of infection was found in the non-treated controls of both cultivars (Table 4). As to the pathogenic fungus *P. destructor* a transfer by infected seeds has so far not been clearly specified in the literature, however, some authors assume such a way of pathogen dissemination (Rod et al., 2005). Neither the inoculation of Alice cultivar seeds with the homogenized-plant-leaves biomass




1: *Effect of HWT of carrot seeds on the development of targeted infection by Alternaria dauci and of a spontaneous infection of control plants.*
Note: Variant inoculated with *A. dauci*, I; variant inoculated with *A. dauci* subsequently exposed to HWT, I+HWT; control variant without inoculation and HWT, C; control variant without inoculation subsequently exposed to HWT, HWT. The carrot cultivars tested: Marion F1, Afalon F1, Aneta F1 and Cortina F1.

containing *P. destructor* collected in the field nor the infection with a spore suspension of *B. aclada* resulted in a higher degree of infection of sprouted plants compared to the infection of the control, non-infected seeds and of the inoculated seeds where HWT was subsequently applied. Consequently, the effect of HWT could not be measured (Table IV). In this case, only a weak protective effect of HWT against the spontaneous infection was detectable with *B. aclada*. In the case of Amfora F1 cultivar, a protective effect of HWT was observed with respect to both the *B. aclada* infection and the spontaneous infection (Table IV).

III: *Effect of HWT of carrot seeds on the development of targeted infections by fungal pathogens and of a spontaneous infection of control plants.*

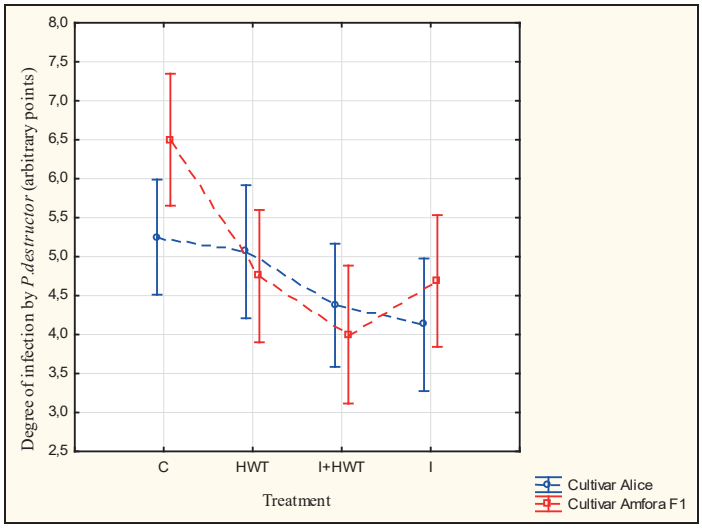
| Carrot | | | |
|---|---|---|---|
| **Cultivar** | **Treatment** | **Degree of infection by *Alternaria dauci* (arbitrary points)** | **Relative change of infection rate due to HWT[1,2]** |
| Marion F1 | Infection | 8,86 ± 0,09$^{j}$ | 1.3x$^{1}$ |
| | Infection + HWT | 6,57 ± 0,35$^{h}$ | |
| | Control + HWT | 4,43 ± 0,12$^{ef}$ | 1.5x$^{2}$ |
| | Control | 6,43 ± 0,24$^{h}$ | |
| Afalon F1 | Infection | 8,68 ± 0,90$^{j}$ | 1.7x$^{1}$ |
| | Infection + HWT | 5,07 ± 0,35$^{g}$ | |
| | Control + HWT | 4,07 ± 0,10$^{de}$ | 1.2x$^{2}$ |
| | Control | 4,79 ± 0,14$^{fg}$ | |
| Aneta F1 | Infection | 8,57 ± 0,15$^{ij}$ | 2.2x$^{1}$ |
| | Infection + HWT | 3,82 ± 0,15$^{cd}$ | |
| | Control + HWT | 3,29 ± 0,14$^{ab}$ | 2.1x$^{2}$ |
| | Control | 6,86 ± 0,30$^{h}$ | |
| Cortina F1 | Infection | 8,14 ± 0,21$^{i}$ | 2.3x$^{1}$ |
| | Infection + HWT | 3,50 ± 0,10$^{bc}$ | |
| | Control + HWT | 2,79 ± 0,12$^{a}$ | 1.1x$^{2}$ |
| | | 3,18 ± 0,10$^{ab}$ | |

Note: The values of infection degree marked with different letters (a-j) were significantly different. Relative decrease of the infection rate due to HWT applied to the seeds after the inoculation [1]; relative decrease of the infection rate due to HWT applied to non-inoculated seeds [2].

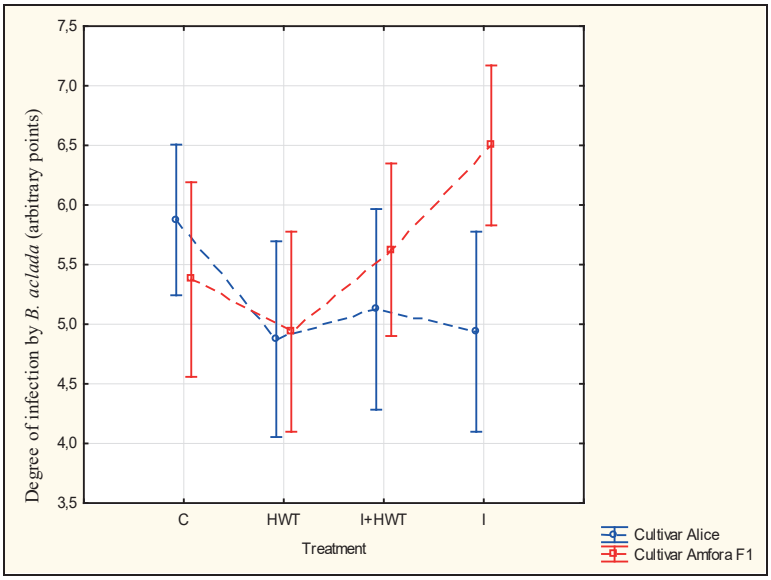
IV: *Effect of HWT of onion seeds on the development of the targeted infections by fungal pathogens and of the spontaneous infection of control plants.*

| Onion | | | | | |
|---|---|---|---|---|---|
| **Cultivar** | **Treatment** | **Degree of infection by *Peronospora destructor* (arbitrary points)** | **Relative change of infection rate due to HWT1,2** | **Degree of infection by *Botrytis aclada* (arbitrary points)** | **Relative change of infection rate due to HWT1,2** |
| Alice | Infection | 4,13±0,85$^{a}$ | 0.9x$^{1}$ | 4,94±0,84$^{ab}$ | 1.0x$^{1}$ |
| | Infection+HWT | 4,38±0,79$^{ab}$ | | 5,13±0,84$^{ab}$ | |
| | Control + HWT | 5,06±0,85$^{cd}$ | 1.0x$^{2}$ | 4,88±0,82$^{a}$ | 1.2x$^{2}$ |
| | Control | 5,25±0,74$^{d}$ | | 5,88±0,63$^{d}$ | |
| Amfora F1 | Infection | 4,69±0,85$^{bc}$ | 1.2x$^{1}$ | 6,50±0,67$^{e}$ | 1.2x$^{1}$ |
| | Infection+HWT | 4,00±0,89$^{a}$ | | 5,63±0,72$^{cd}$ | |
| | Control + HWT | 4,75±0,85$^{bcd}$ | 1.4x$^{2}$ | 4,94±0,84$^{ab}$ | 1.1x$^{2}$ |
| | Control | 6,50±0,85$^{e}$ | | 5,38±0,82$^{bc}$ | |

Note: The values of degree of infection marked by different letters (a-e) are significantly different. Relative change of the infection rate due to HWT applied to the seeds after the inoculation [1]; relative change of the infection rate due to HWT applied to non-inoculated seeds [2].




2: *Effect of HWT of onion seeds on the development of Peronospora destructor infection.*
Note: Variant inoculated with *P. destructor*, I; variant inoculated with *P. destructor* subsequently exposed to HWT, I+HWT; control variant without inoculation and HWT, C; variant without inoculation subsequently exposed to HWT, HWT. The cultivars tested: Alice and Amfora F1.




3: *Effect of HWT of onion seeds on the development of Botrytis aclada infection.*
Note: Variant inoculated with *B. aclada*, I; variant inoculated with *B. aclada* subsequently exposed to HWT, I+HWT; control variant without inoculation and HWT, C; variant without inoculation subsequently exposed to HWT, HWT. The cultivars tested: Alice and Amfora F1.

## CONCLUSION

The resistance of selected carrot and onion cultivars important for the vegetable production in the Czech Republic was investigated by exposure to targeted infections by *A. dauci*, *P. destructor* and *B. aclada* causing carrot leaf blight, onion downy mildew and onion neck rot. The carrot cultivars most resistant to *A. dauci* were Katrin, Cortina F1, and Afalon F1. The respective onion cultivars showing strong resistance to *B. aclada* and *P. destructor* were Amfora F1, Bolero, Tosca, Triumf F1 and Avalon, Bolero, Tosca. The most sensitive onion cultivar to both infections was Všetana.
HWT of carrot seeds applied after the inoculation with *A. dauci* decreased the development of the infection 1.3-2.3-fold, whereas the protective effects observed with onion seeds against *P. destructor* and *B. aclada* were weaker.

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