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Comparison of the blossom and shoot susceptibility of European and Asian pear cultivars to *Pseudomonas syringae* pv. *syringae*

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Abstract: The susceptibility of 14 pear cultivars to the bacterium *Pseudomonas syringae* pv. *syringae,* the causal agent of bacterial blast, was evaluated using three different methods of *in vivo* inoculation detached shoots inoculation in a growth chamber, and terminal shoot and blossom inoculation of potted trees in a net house in the period 2020–2022. The 20-week assessment of infection symptoms in the net house showed different dynamics of disease development depending on the inoculation method, the weather during the growing season and the susceptibility of the pear cultivars. Most of the cultivars were during the study low susceptible to pathogen and were classified in blossom, terminal as well as detached shoot susceptibility class 2. The European cultivar Kiefer was the least susceptible (susceptibility class 1) to blossom infection, the Asian cultivars Chojuro and Ya Li to terminal shoot infection, and Ya Li to detached shoot infection. The European cultivar William's was the most susceptible to all types of infection, being classified in class 3, moderately susceptible cultivars to infection of terminal shoots, and class 4, highly susceptible cultivars to infection of blossoms and detached shoots. The assessment of susceptibility of pear cultivars to *Pseudomonas syringae* pv. *syringae* in the net house approximated conditions as close as possible to the condition in orchards and should thus be consistent in plantings with similar environmental and weather conditions.

Keywords: bacterial blast; artificial inoculation; growth chamber; net house

The bacterial blast causal agent, bacteria *Pseudomonas syringae* pv. *syringae* (Hall) Winslow et al. (*Pss*) is exceedingly difficult to control and results in significant economic losses in fruit harvest (Kennelly et al. 2007). The production of young trees with systemic infection and the invasion of epiphytic population of *Pss* into wounded plant tissues and scars leads to the development of cork necrosis, girdling and dieback of scaffold limbs and ultimately to the death of a large number of pear trees in orchards. Part of the population of *Pss* support the creation of ice nuclei and exacerbate losses associated with frost damage. Due to climate change, the frequency of the frost periods during the bloom and post bloom stages has recently increased in temperate regions. This has led to a significant reduction in fruit yield (Rodrigo 2000; Chitu, Paltineanu 2020; Tomczyk et al. 2020) and the entry of other pathogens into fruit trees through frost injury. During the cool and wet late summer periods, *Pss* causes the development of huge lesions on the leaves of older or weakened pear trees (Deckers et al. 2008) leading

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to the premature leaf fall and invasion of the pathogen into the intercellular space of parenchyma tissues. The *Pss* affects many species of fruit trees, such as mango (Abdullah et al. 2021), apricot (Parisi et al. 2019), cherry (Oksel et al. 2022), apple (Gašić et al. 2018) and pear (Tabira et al. 2012). On susceptible European pear (*Pyrus communis* L.) cultivars, the pathovar *Pss* exhibited the most frequent and severe pathogenicity (Moragrega et al. 2003; Bultreys, Kaluzna 2010). Bacteria can infect all the ground–parts. The most susceptible are blossoms and young twigs (Gilbert et al. 2010), but all spots and lesions can be a potential source of disseminating causal pathogen in orchards (Bokszczanin et al. 2012). In the orchards of South Korea, the causal bacterium of bacterial shoot blight on Asian pear trees *Pyrus pyrifolia* (Burm.f.) was identified as *Pseudomonas cerasi* (Choi et al. 2020). In Europe, this pathogen has caused blossom dieback and necrotic spots on shoots, leaves and fruits on cherry trees (Kałużna et al. 2016).

Pss has evolved two main virulence strategies, suppression of host immunity and creation of an aqueous apoplast to form its niche in the phyllosphere (Xin et al. 2018). Bacterial protein effectors inhibit the plant immune response and cause virulence in compatible hosts (Macho, Zipfel 2015), and secreted phytotoxins, syringolin A, syringomycin and syringopeptin (Hall et al. 2019) increase pathogenicity on plants by modifying host cell processes and by altering host metabolism (Galán, Waksman 2018). In addition, infection is profoundly influenced by external environmental conditions such as humidity (Velásquez et al. 2018).

Control of bacterial blast is still based on treatments with conventional pesticides with copper compounds (Hafez et al. 2021; Pscheidt, Ocamb 2022) predominantly. Nanopesticides with active silver, copper, titanium or zinc particles display superior properties in the effectiveness and ability to penetrate to the place of action (Tran et al. 2022), but there is still a long way to go to fully utilize nanotechnology in the orchards (Hafez et al. 2021). Disease control with biological compounds such as extracts from plants and microorganisms, antagonistic and plant growth-promoting rhizosphere bacteria and fungi, inducing the systemic resistance of plants (Pieterse et al. 2014; Arora et al. 2022; Scortichini 2022) are limited by weather conditions and the phenological phase of trees (Przybyla et al. 2012). An economical solution in integrated disease control is the planting of resistant pear cultivars (Sundin et al. 2016). Contrary to the causal agent of fire blight disease, *Erwinia amylovora*, less studies were done on the susceptibility of pear cultivars to the causal agent of bacterial blast (Moragrega et al. 2003). To evaluate the susceptibility of pear cultivars and rootstocks to *Pss* strains, a test on detached immature pear fruits, young detached leaves (Yessad et al. 1992; Moragrega et al. 2003; Scortichini et al. 2003), detached blossoms (Latorre et al. 2002), tissue cultures (Scheck et al. 1997), detached shoots and attached shoots (Whitesides, Spotts 1991), and pear seedlings (Yessad-Carreau et al. 1994) is used under controlled conditions. These assays are important in determining how readily infections are initiated and in achieving a more comprehensive susceptibility evaluation of pear cultivars (Hirano, Upper 2000). Under field conditions, the success of artificial inoculation depends on weather patterns to create weather conditions for disease development (Kostick et al. 2019). The results of susceptibility evaluations under field conditions are immediately applicable to agricultural practice.

The aim of this three-year study was to compare three inoculation methods under different conditions in order to assess the susceptibility of different European and Asian pear cultivars to *Pseudomonas syringae* pv. *syringae* as effectively as possible.

MATERIAL AND METHODS

Bacterial strains and inoculum preparation. Bacterial strains of *Pss* CPABB 138, 234 and 237 (Collection of Phytopathogenic and Agriculturally Beneficial Bacteria) originated from pear fruit trees with blast disease symptoms were stored in Meat Peptone Broth (Merck, Co., Inc. Kenilworth, New Jersey, USA) at –80 °C in 20% glycerol. All *Pss* strains were cultured on King's B medium (King, 1954) for 24 h at 22 °C. They were washed from the nutrient media in 5 mL of sterile distilled water, adjusted to an optical density at 600 nm (OD_{600}) = 1.0, approximately 10^9 CFU/mL, and diluted to the final concentration of 10^5 CFU/mL. Equal amounts of three *Pss* strain suspensions were mixed, and immediately used for inoculation.

Plant material. A set of 22 trees from each of 4 European pear cultivars (*Pyrus communis* L. – 'Ananaska Česká', 'Clapp´s Favourite', 'Nagevicova' and 'William´s'; interspecific hybrid 'Kiefer') and 10 Asian pear cultivars (*Pyrus bretschneideri* Rehd.

https://doi.org/10.17221/18/2023-HORTSCI

– 'Early Shu', 'Chinese White' and 'Ya Li', *Pyrus pyrifolia* (Burm.f.) – 'Hosui', 'Chojuro', 'Kosui', 'Man San Gi'l, 'Niitaka' and 'Nijissejki') were assessed each year in the period of 2020–2022. Grafted one-year-old pear trees (quince rootstock) were planted into 40-litre pots with horticultural substrate (soil, natural organic matter, leaf and bark moulds, white peat, silica sand, compound mineral fertilizers with trace elements (PG-MIX) and dolomite limestone, pH 5.5–9.0), placed in net houses (two trees that served as negative controls for each cultivar were placed in a separate net house) and irrigated to protect them from drying out in the summer.

Blossom susceptibility. From 2020–2022, one blossom cluster in which all the blossoms were open (phenological stage 61–65 according to the BBCH scale; Martínez-Nicolás et al. 2016), was tagged randomly on one shoot of 10 trees of each pear cultivar to maximize spatial variation in the net house. One ml of mixture of three *Pss* virulent strains was spray-inoculated to the individual cluster of blossoms using a handheld sprayer to ensure local application. Negative controls were inoculated in the same manner with sterile water. The percentage of necrotic blossoms (NB_i) within the cluster (TB_i) was calculated for each tree (*i*) 4–5 days after inoculation. Subsequently, the symptoms of disease progression to other parts of each tree (*i*) – the shoot lesion length (NS_i) in relation to the total shoot length (TS_i), the percentage of leaves with black spots (L_i), fruits with necrosis (F_i), the total tree vitality decline (V_i) and the total number of affected parts (Y_i) of tree were assessed 3, 6, 12 and 20 weeks after inoculation. The mean percentage blossom susceptibility (*BS*) for each pear cultivar (*x*) at each term was calculated using the following formula:

$$BS_x = 10\sum_{i=1}^{10}\left(\frac{NB_i}{TB_i}+\frac{NS_i}{TS_i}+L_i+V_i+F_i\right)/\,Y_i$$

and transferred into a susceptibility score scale according to (Le Lezec et al. 1997) in order of increasing susceptibility: 1 – very low – (0–20%), 2 – low – (> 20–40%), 3 – moderate – (> 40–60%), 4 – high – (> 60–80%), 5 – very high – (> 80–100%). The annual *BS* class of each pear cultivar was determined according to the highest calculated value of BS_x within the 20-week assessment.

Terminal shoot susceptibility. Each year one actively growing shoot (35.0 ± 5.0 cm) in phenological stage 31–35 according to the BBCH scale (shoots about 20–50% of final length; Martínez-Nicolás et al. 2016) was tagged randomly on 10 trees of each pear cultivar in the net house and was inoculated by-cutting off the tip under the first developed leaf using scissors previously immersed in the mixture of three strains of *Pss* (described above). Negative controls were inoculated in the same manner with sterile water. At each term of assessment, 2, 4, 6, 12 and 20 weeks after inoculation, the percentage of the shoot lesion length (NS_i) in relation to the total shoot length (TS_i) was calculated for each tree (*i*). At the same term, the symptoms of disease progression to other parts of tree were assessed – the percentage of leaves with black spots (L_i), fruits with necrosis (F_i) and the total tree vitality decline (V_i) and the total number of affected parts (Y_i) was calculated. The mean percentage terminal shoot susceptibility (TS_x) for each pear cultivar (*x*) at each term was calculated using the following formula:

$$TS_x = 10\sum_{i=1}^{10}\left(\frac{NS_i}{TS_i}+L_i+V_i+F_i\right)/\,Y_i$$

and transferred into a susceptibility score scale according to (Le Lezec et al. 1997) as described above. The annual *TS* class of each pear cultivar was determined according to the highest calculated value of TS_x within the 20-week assessment.

Detached shoot susceptibility. Each year of the study, 12 shoots of each pear cultivar with a diameter of 0.7–0.9 cm, a length of 35.0 ± 5.0 cm, and at phenological growth stage 31–35 (shoots about 20–50% of final length; Martínez-Nicolás et al. 2016) were taken, two shoots were always cut from individual untreated trees. One ml of mixture of three virulent strains of *Pss* and sterile distilled water, serving as a negative control, were applied by a handheld sprayer on leaves of 10 or two detached shoots, respectively. Shoots were placed in an individual container with sterile distilled water in the growth chamber at a temperature regime of 15/10 °C (day/night), relative humidity of 80–90%, a photoperiod regime of 12/12 hours (day/night). On the 3rd, 6th and 10th day after inoculation, the percentage of leaves with superficial necrosis on each shoot was assessed. The mean detached shoot susceptibility DS_x for each pear cultivar (*x*) was calculated and transformed into the ranking scale of Le Lezec et al. (1997) as described above. The *DS* class of each pear cultivar was determined according to the highest value of DS_x within a 10-day assessment.

Statistical analysis. The effect of the term of disease symptom assessment within the vegetation

https://doi.org/10.17221/18/2023-HORTSCI

season on the calculated BS_x and TS_x and estimated DS_x of each pear cultivars to *Pss* was analysed by analysis of variance (ANOVA) and the post hoc Tukey's test. Mean percentage susceptibility values that were not statistically significantly different were assigned the same letters in Tukey's test. The influence of the growing season on the annual susceptibility class of the pear genotypes over the course of three years was evaluated for each inoculation method separately using the two-way ANOVA. $P < 0.05$ was considered as the threshold for significance ($^*P < 0.05$). Differences among the three methods of the artificial inoculation of pear cultivars were analysed using Fisher's Least Significant Difference (LSD) test in the R software version 4.1.2. (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The susceptibility of European and Asian pear cultivars against the bacterial blast pathogen *Pseudomonas syringae* pv. *syringae*, was assessed in three assays between 2020 and 2022 by inoculation of randomly targeted cluster of blossoms and terminal shoots on individual trees in a net house and on detached shoots in a growth chamber.

After application of the *Pss* mixture to the blossom cluster, 28.5–100% of the pear cultivars, depending on the year, showed the first symptoms of blossom blast within 7 days (Figure 1). After 3–6 weeks, the first necrotic spots appeared on the shoot and leaves around the inoculation site. In all pear cultivars, the increase in disease rates on the 6th, 12th, and 20th week of observation (Table 1) continued. Depending on the cultivar and term assessment, *Pss* expanded through the vascular system and expressed blast symptoms on the shoots and leaves near or at a distance from the place of inoculation. In the cultivars Kieffer, Man San Gil and Nijissejkii, lesions on the fruits were developed in the 6th week after inoculation (Figure 2). For all pear cultivars, the most severe symptoms of infection after blossom inoculation in the net house were recorded in 2022. According to the Turkey's test, the first statistically significant change in the mean *BS* values for the very low and low susceptible pear cultivars was recorded at the 12th week of the assessment and for the moderate and high susceptible cultivars at the 6th week after inoculation. In very low susceptible cultivars, the BS score at week 20 was not statistically significant. For more susceptible pear cultivars, statistically significant differences were also found between BS values at week 12 and week 20. During the study, pear cultivars were most often classified as susceptibility class 2, meaning they had low susceptibility to *Pss* infection of blossoms. The European cultivar Kiefer was during the study the least susceptible (susceptibility class 1) to blossom infection. The European cultivar William's was the most susceptible to blossom infection. In 2022 was classified as very high susceptible in susceptibility class 5. The two-way ANOVA for the *BS* showed a significant effect of cultivar, term of assessment as well as year. How-

Figure 1. The first symptoms of blossom blast on the Asian pear cultivar 'Niitaca' were observed within 7 days after spray inoculation of a randomly targeted blossom cluster with a bacterial mixture of *Pseudomonas syringae* pv. *Syringae*

Figure 2. In the Asian pear cultivar 'Nijissejkii', lesions were observed on fruit and leaves 6 weeks after inoculation of blossoms with a mixed culture of virulent *Pseudomonas syringae* pv. *syringae* strains

https://doi.org/10.17221/18/2023-HORTSCI

Table 1. Blossom susceptibility of Asian and European pear cultivars to *Pseudomonas syringae* pv. *syringae* strains in the net house in 2020–2022

Week of assessment	Blossom susceptibility (%)					Susceptibility class**	Blossom susceptibility (%)					Susceptibility class	Blossom susceptibility (%)					Susceptibility class
	1st	3rd	6th	12th	20th		1st	3rd	6th	12th	20th		1st	3rd	6th	12th	20th	
Pear cultivar	2020						2021						2022					
‘Ananaska Česká’	13.3^{e-k*}	13.4^{e-k}	19.5^{d-g}	20.0^{d-g}	25.0^{cd}	2	5.0^{i-p}	13.3^{e-k}	16.0^{d-h}	18.3^{d-g}	20.8^{d-g}	2	13.3^{m-q}	8.4^{q-u}	8.0^{h-m}	18.3^{h-m}	30.3^{bc}	3
‘Clapp´s Favourite’	0.0^{p}	4.5j–p	22.5^{d-f}	4.0^{ab}	40.0^{ab}	3	0.0^{p}	0.0^{p}	4.8^{i-p}	6.3^{i-p}	13.3^{e-k}	2	4.5^{t-y}	8.6^{q-u}	24.3^{d-g}	28.2^{cd}	42.9^{a}	4
‘Early Shu’	10.0^{g-p}	10.4^{g-o}	12.0^{g-m}	$20.^{0d-g}$	20.0^{d-g}	2	5.0^{i-p}	10.0^{g-p}	10.0^{g-p}	15.6^{d-i}	20.0^{d-g}	2	20.6^{e-j}	6.8^{r-x}	17.0^{j-n}	23.1^{d-h}	22.8^{d-i}	2
‘Hosui’	2.5^{m-p}	0.0^{p}	0.0^{p}	10.5^{g-o}	10.5^{g-o}	1	0.0^{p}	2.5^{m-p}	2.5^{m-p}	2.5^{m-p}	11.4^{g-n}	1	20.5^{e-k}	15.8^{j-o}	20.1^{f-l}	25.6^{c-f}	30.8^{bc}	3
‘Chojuro’	0.0^{p}	0.0^{p}	1.0^{n-p}	13.0^{g-m}	13.0^{g-m}	1	0.0^{p}	0.0^{p}	0.0^{p}	8.7^{g-p}	15.8^{d-h}	2	13.1^{m-q}	14.9^{l-p}	15.3^{j-p}	17.5^{i-n}	18.1^{h-m}	2
‘Chinese White’	3.2^{l-p}	0.7^{op}	4.0^{j-p}	10.0^{g-p}	20.0^{d-g}	2	0.0^{p}	3.2^{l-p}	5.7^{i-p}	25.0cd	26.7cd	2	4.0^{u-y}	10.0^{p-t}	10.0^{p-t}	15.0^{k-p}	10.2^{p-s}	2
‘Kieffer’	3.3^{l-p}	1.3^{n-p}	6.2^{j-p}	10.0^{g-p}	10.0^{g-p}	1	0.0^{p}	3.3^{l-p}	10.3^{g-p}	11.1^{g-n}	13.3^{e-k}	1	6.2^{s-y}	1.4^{xy}	4.4^{u-y}	7.2^{r-w}	10.2^{p-s}	2
‘Kosuji’	10.0^{g-p}	3.3^{l-p}	13.7^{e-j}	16.3^{d-h}	15.0^{d-i}	2	1.7^{n-p}	10.5^{g-o}	23.8^{de}	27.2^{cd}	31.1^{a}	3	13.7^{m-q}	1.1^{y}	1.8^{w-y}	10.2^{p-s}	14.6^{l-p}	2
‘Man San Gil’	0.0^{p}	0.0^{p}	6.9^{h-p}	13.0^{g-m}	20.0^{d-g}	2	0.0^{p}	0.0^{p}	17.5^{d-h}	17.8^{d-h}	26.5^{cd}	2	6.9^{r-x}	6.1^{s-y}	20.0^{g-l}	26.0^{c-e}	35.3^{b}	3
‘Nagevicova’	7.5^{h-p}	2.0^{m-p}	6.0^{j-p}	23.4^{de}	47.5^{a}	3	6.6^{i-p}	7.5^{h-p}	8.3^{h-p}	10.3^{g-p}	15.0^{d-i}	2	5.6^{s-y}	1.7^{w-y}	8.2^{q-u}	13.5^{m-q}	10.4^{o-s}	2
‘Nijisseikii’	5.0^{i-p}	5.0^{i-p}	20.0^{d-g}	20.0^{d-g}	25.0^{cd}	2	3.3^{l-p}	4.3^{j-p}	25.5^{cd}	30.8^{ab}	31.1^{a}	3	19.6^{g-l}	2.3^{v-y}	8.3^{q-u}	16.2^{j-n}	23.5^{d-h}	2
‘Niitaca’	15.0^{d-i}	1.8^{n-p}	2.5^{m-p}	15.0^{d-i}	20.0^{d-g}	2	0.0^{p}	2.5^{m-p}	4.3^{j-p}	5.0^{i-p}	11.6^{g-m}	2	15.9^{j-o}	1.8^{w-y}	7.2^{r-w}	12.2^{n-r}	13.0^{m-q}	3
‘William´s’	3.3^{l-p}	2.3^{m-p}	24.5^{d}	35.0^{bc}	40.0^{ab}	3	0.0^{p}	3.3^{l-p}	10.0^{g-p}	20.0^{d-g}	30.0^{bc}	3	2.3^{v-y}	5.2^{s-y}	7.2^{r-w}	24.3^{d-g}	43.9^{a}	5
‘Ya Li’	0.0^{p}	3.6^{k-p}	3.7^{j-p}	11.0^{g-n}	10.9^{g-n}	1	0.0^{p}	0.0^{p}	1.5^{n-p}	7.9^{h-p}	10.0^{g-p}	1	6.9^{r-w}	3.2^{u-y}	7.4^{r-v}	8.7^{q-u}	23.3^{d-h}	3

*mean followed by the same letters within each year are not significantly different ($P < 0.05$) according to the post hoc Tukey’s test; **annual blossom susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest percentage of bacterial blast disease symptoms on the trees during the 20-week assessment

https://doi.org/10.17221/18/2023-HORTSCI

ever, when only two consecutive years were compared, differences were observed between 2021 and 2022, no significant differences were found between year 2020 and 2021.

First blight symptoms on inoculated terminal shoots in the net house were observed within 2–6 weeks depending on the year and pear cultivar (Table 2). *Pss* caused shoot necrosis progressing from the inoculation point from the surface and through the veins to other twigs, leaves and fruits of trees and expressed as spots and lesions of various sizes. In 'Kiefer', 'Man San Gil' and 'Nijissejkii' pear cultivars, the infection on fruit appeared as lesions on the skin. According to the Turkey's test, the first statistically significant change in mean *TS* values was recorded between weeks 6 and 12 of the assessment, depending on the cultivar (Figure 3). The statistically significant differences in *TS* values increased throughout the season regardless of cultivar and year. In the study, pear cultivars were most often classified as low susceptible in susceptibility class 2 and very low susceptible in susceptibility class 1 to *Pss* infection of terminal shoots in the net house. The European cultivars 'Clapp's Favourite' and 'William's' were the most susceptible in this inoculation assay and were classified as moderately susceptible in susceptibility class 3. The two-way ANOVA for the *TS* showed a significant effect of cultivar and term of assessment and no effect of the year.

Depending on the pear cultivar and the year of assessment the symptoms of *Pss* infection on detached shoots in the growth chamber were observed 2–5 days after inoculation (Table 3). In most cultivars in response to infection, black lesions were developed and progressed during the 10-day assessment. In the Asian cultivars 'Early Shu', 'Man San Gil' and 'Niitaca', mainly wilting, and discoloration were observed. No blight symptoms were observed in the Asian cultivar 'Ya Li' in 2021. According to the Turkey's test, the statistically significant differences in *DS* values increased throughout ten days assessment regardless of cultivar and year. The differences among *DS* values were very similar in all years. Pear cultivars were most often classified as low susceptible in susceptibility class 2 and very low susceptible in susceptibility class 1 to *Pss* infection of detached shoots in the growth chamber. The European cultivar 'William's' as was the most susceptible in this inoculation assay (Figure 4) and was classified as highly susceptible in susceptibility class 4. The two-way ANOVA for the *DS* showed a significant effect of cultivar and term of assessment and no effect of the year.

Figure 3. After inoculation of a randomly selected terminal shoot of the European cultivar 'Clapp's Favourite' with a mixed inoculum of *Pseudomonas syringae* pv. *syringae* strains, progression of infection from the inoculation site to other branches and leaves was observed between 6 and 12 weeks, manifested by lesions of varying sizes

Figure 4. The symptoms of *Pseudomonas syringae* pv. *syringae* infection on detached shoots of the European cultivar 'William's' in the growth chamber were observed 2–5 days after inoculation

https://doi.org/10.17221/18/2023-HORTSCI

Table 2. Terminal shoot susceptibility of Asian and European pear cultivars to *Pseudomonas syringae* pv. *syringae* strains in the net house in 2020–2022

Week of assessment	Terminal shoot susceptibility (%)					Susceptibility class**	Terminal shoot susceptibility (%)					Susceptibility class	Terminal shoot susceptibility (%)					Susceptibility class
	2^{nd}	4^{th}	6^{th}	12^{th}	20^{th}		2^{nd}	4^{th}	6^{th}	12^{th}	20^{th}		2^{nd}	4^{th}	6^{th}	12^{th}	20^{th}	
Pear cultivar	2020						2021						2022					
'Ananaska Česká'	0.0^{j*}	1.3^{j}	1.9^{ij}	10.0^{e-g}	24.0^{bc}	2	0.0^{h}	0.0^{h}	5.0^{f-h}	8.1^{d-h}	17.3^{a-d}	2	5.8^{l-s}	0.6^{v}	4.5^{m-v}	30.2^{c}	46.8^{a}	3
'Clapp´s Favourite'	0.0^{j}	0.0^{j}	4.5^{g-j}	5.0^{g-j}	5.0^{g-j}	1	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	1	0.0^{v}	23.2^{d-f}	4.6^{m-v}	13.3^{hi}	25.1^{de}	2
'Early Shu'	0.0^{j}	1.1^{j}	2.3^{ij}	15.8^{de}	14.0^{d-f}	2	0.0^{h}	5.0^{f-h}	5.0^{f-h}	0.5^{h}	16.2^{a-e}	2	1.4^{s-v}	0.0^{v}	18.3^{g}	22.8^{d-g}	23.3^{d-f}	2
'Hosui'	0.0^{j}	0.0^{j}	0.0^{j}	0.0^{j}	3.0^{ij}	1	0.0^{h}	0.0^{h}	0.0^{h}	0.5^{h}	0.0^{h}	2	7.2^{j-p}	11.6^{ij}	10.0^{i-l}	10.9^{i-k}	27.5^{cd}	2
'Chojuro'	0.0^{j}	5.3^{g-j}	6.3^{g-j}	4.5^{g-j}	4.5^{g-j}	1	0.0^{h}	0.0^{h}	0.5^{h}	8.3^{d-h}	17.0^{a-e}	1	3.1^{n-v}	7.8^{j-n}	6.7^{k-r}	6.8^{j-r}	10.0^{i-l}	1
'Chinese White'	0.0^{j}	1.0^{j}	3.5^{h-j}	10.0^{e-g}	10.0^{e-g}	1	0.0^{h}	2.0^{h}	3.5^{gh}	6.7^{e-h}	13.3^{b-g}	2	0.0^{v}	0.0^{v}	5.0^{m-u}	5.0^{m-u}	10.0^{i-l}	1
'Kieffer'	0.0^{j}	2.2^{ij}	10.0^{e-g}	20.0^{cd}	20.0^{cd}	2	0.0^{h}	1.0^{h}	10.0^{c-h}	10.0^{c-h}	14.2^{b-f}	2	1.6^{s-v}	1.0^{s-v}	3.0^{o-v}	7.0^{j-q}	24.8^{d-f}	2
'Kosuji'	0.0^{j}	0.9^{j}	3.5^{h-j}	2.0^{ij}	2.0^{ij}	1	0.0^{h}	1.2^{h}	8.3^{d-h}	12.6^{c-g}	18.3^{a-d}	2	0.0^{v}	2.2^{q-v}	1.1^{s-v}	1.8^{s-v}	20.0^{fg}	1
'Man San Gil'	0.0^{j}	0.8j	4.3^{g-j}	15.7^{de}	15.0^{de}	2	0.0^{h}	1.7^{h}	10.0^{c-h}	15.3^{a-f}	16.7^{a-e}	2	0.0^{v}	0.9^{t-v}	2.0^{r-v}	18.0^{gh}	30.4^{c}	2
'Nagevicova'	0.0^{j}	0.0^{j}	4.8^{g-j}	8.0^{f-i}	9.6^{e-h}	1	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	3.3^{gh}	1	0.0^{v}	0.8^{t-v}	4.0^{n-v}	4.4^{m-v}	20.0^{fg}	2
'Nijisseikii'	3.0^{ij}	4.1^{g-j}	4.5^{g-j}	20.3^{cd}	23.0^{c}	2	3.3^{gh}	4.8^{f-h}	9.2^{d-h}	20.0^{a-c}	23.3^{ab}	3	0.0^{v}	0.0^{v}	7.7^{j-o}	8.9^{i-m}	20.0^{fg}	2
'Niitaca'	0.0^{j}	0.0^{j}	4.0^{g-j}	15.0^{de}	15.0^{de}	1	0.0^{h}	0.0^{h}	0.0h	0.0^{h}	0.0^{h}	1	0.0^{v}	1.1^{s-v}	2.5^{p-v}	21.0^{e-g}	40.1^{b}	3
'William´s'	0.0^{j}	0.9^{j}	2.5^{ij}	30.0^{b}	49.0^{a}	3	0.0^{h}	1.9^{h}	2.9 gh	17.8^{a-d}	25.0a	3	0.0^{v}	0.0^{v}	4.3^{m-v}	5.5^{l-t}	45.6^{a}	3
'Ya Li'	0.0^{j}	0.0^{j}	3.8^{g-j}	8.0^{f-i}	7.9^{f-i}	1	0.0^{h}	0.0^{h}	0.0^{h}	0.0^{h}	10.0^{c-h}	1	0.0^{v}	5.3^{l-u}	0.7^{t-v}	1.0^{s-v}	3.3^{n-v}	1

*mean followed by the same letters within each year are not significantly different ($P < 0.05$) according to the post hoc Tukey's test; **annual terminal shoot susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest percentage of bacterial blast disease symptoms on the trees during the 20–week assessment

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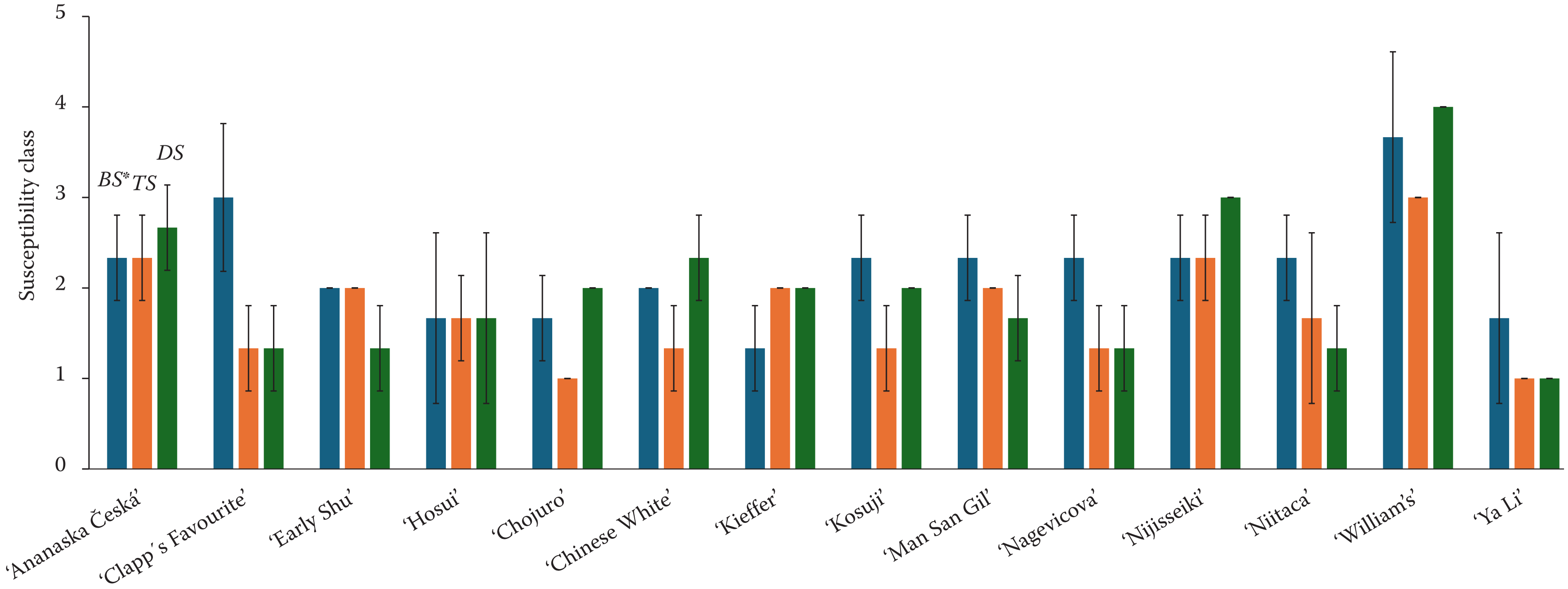

Figure 5. Comparison of the blossom, terminal, and detached shoot susceptibility class to *Pseudomonas syringae* pv. *syringae* strains in Asian and European pear cultivars for the period 2020–2022

*the represents significant differences at 0.05 level according to Fisher's LSD test, each bar is the mean ± SE of susceptibility class for the period 2020–2022; *BS* – blossom susceptibility; *TS* – terminal shoot susceptibility; *DS* – detached shoot susceptibility

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Table 3. Detached shoot susceptibility of Asian and European pear cultivars to *Pseudomonas syringae* pv. *syringae* strains in the growth chamber in 2020–2022

Day of assessment	Necrosis on leaves (%) 3rd	6th	10th	Susceptibility class**	Necrosis on leaves (%) 3rd	6th	10th	Susceptibility class	Necrosis on leaves (%) 3rd	6th	10th	Susceptibility class
Pear cultivar		2020				2021				2022		
'Ananaska Česká'	5.0^{de*}	5.0^{de}	50.0^{a}	3	5.0^{ef}	15.0$^{c–f}$	20.0$^{c–e}$	2	15.0$^{c–e}$	20.0$^{b–e}$	45.0^{ab}	3
'Clapp´s Favourite'	5.0^{de}	15.0$^{c–e}$	15.0$^{c–e}$	1	2.0^{ef}	5.0^{ef}	15.0$^{c–f}$	1	2.0^{e}	5.0^{de}	20.0$^{b–e}$	2
'Early Shu'	1.0^{e}	5.0^{de}	5.0^{de}	1	0.0^{f}	5.0^{ef}	5.0^{ef}	1	5.0^{de}	5.0^{de}	20.0$^{b–e}$	2
'Hosui'	5.0^{de}	1.0^{e}	5.0^{de}	1	0.0^{f}	10.0$^{d–f}$	15.0$^{c–f}$	1	10.0^{de}	25.0$^{b–e}$	40.0$^{a–c}$	3
'Chojuro'	25.0^{bc}	15.0$^{c–e}$	25.0^{bc}	2	5.0^{ef}	25.0$^{b–d}$	25.0$^{b–d}$	2	25.0$^{b–e}$	25.0$^{b–e}$	30.0$^{b–d}$	2
'Chinese White'	0.0^{e}	5.0^{de}	25.0^{bc}	2	5.0^{ef}	20.0$^{c–e}$	40.0^{b}	3	10.0^{de}	10.0^{de}	20.0$^{b–e}$	2
'Kieffer'	5.0^{de}	15.0$^{c–e}$	20.0^{cd}	2	5.0^{ef}	10.0$^{d–f}$	25.0$^{b–d}$	2	10.0^{de}	10.0^{de}	20.0$^{b–e}$	2
'Kosuji'	5.0^{de}	25.0^{bc}	25.0^{bc}	2	5.0^{ef}	5.0^{ef}	25.0$^{b–d}$	2	5.0^{de}	15.0$^{c–e}$	20.0$^{b–e}$	2
'Man San Gil'	5.0^{de}	20.0^{cd}	25.0^{bc}	2	0.0^{f}	10.0$^{d–f}$	10.0$^{d–f}$	1	10.0^{de}	20.0$^{b–e}$	25.0$^{b–e}$	2
'Nagevicova'	2.0^{e}	10.0$^{c–e}$	20.0^{cd}	1	0.0^{f}	5.0^{ef}	10.0$^{d–f}$	1	5.0^{de}	10.0^{de}	20.0$^{b–e}$	2
'Nijisseikii'	10.0$^{c–e}$	5.0^{de}	50.0^{a}	3	20.0$^{c–e}$	30.0^{bc}	40.0^{b}	3	30.0$^{b–d}$	40.0$^{a–c}$	45.0^{ab}	3
'Niitaca'	1.0^{e}	10.0$^{c–e}$	15.0$^{c–e}$	1	0.0^{f}	15.0$^{c–f}$	15.0$^{c–f}$	1	5.0^{de}	10.0^{de}	20.0$^{b–e}$	2
'William´s'	1.0^{e}	10.0$^{c–e}$	60.0^{a}	4	10.0$^{d–f}$	10.0$^{d–f}$	60.0^{a}	4	5.0^{de}	15.0$^{c–e}$	65.0^{a}	4
'Ya Li'	0.0^{e}	10.0$^{c–e}$	15.0$^{c–e}$	1	0.0^{f}	0.0^{f}	0.0^{f}	1	5.0^{de}	10.0^{de}	15.0$^{c–e}$	1

*mean followed by the same letters within each year are not significantly different (P < 0.05) according to the post hoc Tukey's test; **annual detached shoot susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest percentage of bacterial blast necrosis on leaves during the 10-day assessment

The differences in the susceptibility class of pear cultivars to *Pss* (Figure 5) determined by three different assays of inoculation were according to Fisher's LSD test at the threshold of significance (P = 0.05). The European cultivar 'Kiefer' was the least susceptible (susceptibility class 1) to blossom infection, the Asian cultivars 'Chojuro' and 'Ya Li' to terminal shoot infection, and 'Ya Li' to detached shoot infection. The European cultivar 'William's' was the most susceptible to all types of infection, being classified in class 3, moderately susceptible cultivars to infection of terminal shoots, and class 4, highly susceptible cultivars to infection of blossoms and detached shoots.

DISCUSSION

The assessment of susceptibility to bacterial blast is important for pear breeding and for combating disease by integrated management strategies that include low risk pest management practices in orchards and copper or agriculturally beneficial bacteria applications during the whole year (Wenneker et al. 2012; Keswani et al. 2020; Schaeffer et al. 2021).

The cumulative results of the three-year study show that for all three inoculation methods, most pear cultivars were classified in the same susceptibility class or the resulting *Pss* susceptibility scores differed by one class. The difference of two classes between *BS* and identical *TS* and *DS* was only found for the European cultivar 'Clapp's Favourite'. The shift in terminal shoot symptom development is influenced by the later phenological stage of the pear trees at inoculation. The dry late spring and early summer months, followed by a month of excessive rainfall, resulted in late growth of young shoots and rapid development of disease symptoms on shoots and leaves, which were evident when assessed at 20 weeks. High susceptibility of actively growing shoots, even in late summer, agrees with the authors of the studies Decker et al. (2008) and Spotts and Cervantes (1995). Despite differences in weather conditions, the progress of infection after inoculation in the net house did not differ sig-

https://doi.org/10.17221/18/2023-HORTSCI

nificantly between growing seasons when comparing annual *BS* and *TS* pear cultivars. A total of five assessment spread over the growing season made it possible to follow the progression of infection from the site of inoculation to surrounding leaves, shoots and fruit. After blossom inoculation, infection development was most dynamic between weeks 6 and 12 of assessment. After terminal shoot inoculation, the Turkey's test showed that especially the more susceptible cultivars showed significant differences in *TS* even between weeks 12 and 20 of the assessment. The climate changes lead to the first blossoming in a short warm period followed by wet and cold early spring weather favouring multiplication of *Pss* in the infected blossom cluster and its penetration into the inner tissues as well as its transmission and survival on the fruiting branches (Decker et al. 2008). In accordance with inoculation studies performed under field conditions (Bell 2019; Kostick et al. 2019; Cui et al. 2021), the length and frequency of these cold, wet spring periods determined the conditions for further *Pss* spread, inoculum size, rate of spread to other plant tissues, symptom severity, and ultimately annual susceptibility ($P < 0.05$).

Low temperature and high humidity were also set in the growth chamber. Leaves on detached shoots were inoculated with the same inoculum concentration and at the same phenological stage (31–35) as the terminal shoots of pear cultivars in the net house. The *DS* of the pear cultivars in the growth chamber was more similar to their *TS* than the *BS* in the net house. For a total of five pear cultivars, the mean values of *DS* and *TS* were even the same over the three-year study. In the accordance with Spotts and Cervantes (1995) and (Kennelly et al. 2007), favourable temperature and high humidity and a pathogen concentration of 10^5 CFU/shoot are the main conditions for the rapid development of *Pss* infection symptoms in the growth chamber. According to same authors, infection of terminal shoots in the field was observed only when the concentration of *Pss* inoculum exceeded 10^6 CFU/shoot, in addition to high humidity. Based on the similarity of *DS* and *TS* results of individual cultivars, this study also shows the great importance of the same phenological phase of pear cultivars. Spotts and Cervantes (1995) also observed in orchard experiments that current-season shoots were most susceptible to infection during active growth.

For the most objective evaluation of the selected inoculation methods, both European and Asian cultivars were included in the range of cultivars evaluated. Most cultivars, regardless of origin, had low susceptibility to *Pss* and were classified in *BS, TS,* and *DS* class 2. Of the spectrum evaluated, the European pear cultivar William's was most susceptible to infection of flowers, terminal shoots and detached shoots, classified as highly susceptible in class 4. On the other hand, Ya Li was the least susceptible in the terminal shoot and detached shoot inoculation tests and the European pear cultivar Kiefer in the blossom inoculation test. The cultivars were classified as very low susceptible in susceptibility class 1. Thus, no major differences in susceptibility between European and Asian cultivars were observed in this study. The Asian cultivars tested in the net house blossomed earlier and fruited profusely, unlike the European varieties. Contrary to some studies (Decker and Schoofs, 2001), infection symptoms on fruit after blossom inoculation were only observed on the Asian cultivars Man San Gil and Chojuro and the European cultivar Kiefer. In most cases, infected and severely damaged blossoms failed to develop fruit after *Pss* inoculation., and in accordance with Decker and Schoofs (2001), small cracks on the bark and later canker oozing lesions were observed on fruiting branches. *Pss* infection during the bloom can significantly reduce a crop even in well treated pear plantings.

The study highlighted the importance of 20-week assessment of the dynamics of bacterial blast symptoms development in individual pear cultivars to accurately determine the late summer infection on regrowth shoots and fruits. The frequency and severity of outbreaks has increased due to a different course of weather in spring and early autumn and the need for resistant pear cultivars is urgent (Gilbert et al. 2010). In young pear trees, *Pss* can completely destroy a crop and in the next year can cause the premature death of trees (Montesinos, Vilardell 1988). The assessment of susceptibility of pear cultivars to *Pss* in the net house approximated conditions as close as possible to the condition in orchards (Shwartz et al. 2003) and should thus be consistent in plantings with similar environmental and weather conditions. As pointed out (Decker et al. 2008; van der Zwet et al. 2012), the conditions for the development of bacterial blast disease symptoms in the net house are very close to field conditions, depending on the genetic origin of the pear cultivars, the phenological stage of the trees at the time of infection and weather during the growing season. As in the net house, trees in the orchard are

https://doi.org/10.17221/18/2023-HORTSCI

protected from water stress by regular irrigation and from the adverse effects of extreme weather events by protective nets. According to susceptibility classification, pear cultivars were found to be less susceptible to *Pss* than to the causal agent of fire blight, *Erwinia amylovora* (Pánková et al. 2023). The results of this study provided important insights for ongoing trials using a range of agriculturally beneficial bacteria for biological control in pear orchards.

CONCLUSION

The study provides the evidence that the susceptibility of the spectrum of European and Asian pear cultivars to causal agent of bacterial blast was similar under growth chamber and net house conditions in the period 2020–2022. By extending the observation period of trees in the net house to 20 weeks, symptoms on emerging shoots and fruits in late summer could be recorded and included in the overall assessment of susceptibility of pear cultivars to *Pss*. Most of pear cultivars were classified in class 2 with low susceptibility to *Pseudomonas syringae* pv. *syringae*.

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