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Comparison of the shoot and blossom susceptibility of European and Asian pear cultivars to fire blight across different conditions

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Abstract: The susceptibility of 14 pear cultivars to the bacterium *Erwinia amylovora*, the causal agent of fire blight disease, was evaluated using three different methods of *in vivo* inoculation – the leaf inoculation method on detached shoots of pear cultivars in a climate chamber, and the terminal shoot and blossom inoculation methods on potted trees of the same pear cultivars in a net house. The results of the artificial inoculations of the European and Asian pear cultivars were compared using the susceptibility score scale, as well as the ANOVA and post hoc methods. The differences among the pear cultivar susceptibility increased during the evaluation periods in the given year, but there were no significant differences between the annual evaluation in 2020 and 2021. The results of the pear cultivar susceptibility after inoculation of the detached shoots differed significantly from the results of the terminal shoot and blossom inoculation. The detached shoot susceptibility under pathogen-favourable conditions in the climate chamber was lower than the terminal shoot and blossom susceptibility in the net house for most pear cultivars. In both years, the highest values of the terminal shoot and blossom susceptibility were coincidently calculated for the Ananaska česká European old cultivar and the Nijissejkii Asian cultivar.

Keywords: *Erwinia amylovora*; artificial inoculation; climate chamber; net house

The fire blight causal agent, the bacteria *Erwinia amylovora* (Burrill) Winslow et al., (EA) still affects many species of the rosaceous family, including pome fruit trees, such as pears, *Pyrus* L. (*P.*), and apples *Malus* Mill. in most temperate regions of the world (Vanneste 2000). From the surfaces, EA moves through the epidermis into the intercellular space of parenchyma tissues or enters the host through wounds and scars, migrates rapidly through the whole plant via the xylem vessels, and can result in significant yield reduction and/or tree death (Taylor et al. 2003). All the ground- and underground parts of the hosts can be infected by EA and can be a potential source of disseminating this pathogen except for the seeds (Bokszczanin et al. 2012). Entry through blossoms is the predominant infection pathway of EA leading to the natural manifestation of the fire blight disease (Sobiczewski et al. 2015). The pathogen can survive in an uncultured size of inoculum for months or years until the conditions for fire blight outbreaks are met (Taylor et al. 2003). EA injects several types of pro-

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tein effectors into the host plant cell to suppress or bypass host plant defence mechanisms, causes hypersensitive cell death, and can subsequently cause the outbreak of fire blight in susceptible genotypes (Emeriewen et al. 2019; Cui et al. 2021).

Protection of trees against fire blight is still very difficult. Integrated control programmes in orchards include the reduction of abiotic stress during the transportation and planting of young trees, regular irrigation and nutrition, gentle and reasonable cuttings, regular inspection of trees and the application of chemical and biological agents. The effectivity of applied chemical agents based on copper compounds and biological agents is strongly influenced by the weather conditions and the phenotypic phage of the trees (Przybyla et al. 2012). In the long-term, most of them are not efficient enough to ensure safe pear production if we continue to plant susceptible cultivars. Therefore, especially in localities where symptoms of fire blight disease have been observed, there is an urgent need for resistant pear cultivars (Gusberti et al. 2015). For this purpose, studies on the development of disease-resistant genotypes have gained importance (Evrenosoğlu & Mertoğlu 2018; Pândaru et al. 2019; Zurn et al. 2020).

The European cultivars of *P. communis* that predominate in fruit production are classified as moderately or highly susceptible to fire blight (Przybyla et al. 2012). The Asian pear cultivars have displayed high genetic diversity and resistance to EA in the areas of origin. Genetic sources of fire blight resistance have been found in European cultivars and local varieties (Przybyla et al. 2012; Sehic et al. 2012; Urrestarazu et al. 2019) as well as in the cultivated or wild Asian *Pyrus* species, *P. bretschneideri*, *P. sinkiangensis*, *P. ussuriensis* (Bell 2019). Most of the "Nashi" cultivars (*P. pyrifolia*) were more susceptible than the "Li" cultivars (*P. ussuriensis* and *P. bretschneideri*) (Quinet & Wesel 2019). Fire blight resistance in pears shows polygenic inheritance and is affected by multiple factors (Evrenosoğlu et al. 2019).

The phenotypic scoring of resistance is mainly undertaken by *in vivo* shoot inoculation tests on grafted trees by hypodermic needle (Bell 2019) or by bisecting the two youngest leaves with infected scissors under quarantine conditions in greenhouses (Ruz et al. 2008; Sobiczewski et al. 2015). Blossom inoculation tests in a greenhouse have been performed in a few studies. They may be important in determining how readily infections are initiated in an orchard and in achieving a more comprehensive susceptibility evaluation of pear cultivars (Bell 2019). In the field, the success of this method is highly dependent on the occurrence of the susceptible blossom tissues and weather conditions suitable for sufficient disease pressure (Kostick et al. 2019). The inoculation of detached shoots as a cheap fast-phenotyping method for pear susceptibility to bacterial pathogens rather than whole trees would speed up this process (Araujo et al. 2021). Differences in the virulence of EA strains are common, but a strain is expected to have the same level of virulence in all pear cultivars. The use of a mixture of different highly virulent EA strains chosen based on a previous successful infection should ensure a consistent strain × pear genotype interaction (Aleksandrova & Dimitrova 2021).

The aim of this two-year study was to compare three inoculation methods under different conditions in order to evaluate the susceptibility of different European and Asian pear cultivars to the causal agent of fire blight disease as effectively as possible.

MATERIAL AND METHODS

Bacterial strains and inoculum preparation. Bacterial strains of EA strains 138, 234 and 237 originating from pome fruit trees with fire blight disease symptoms were stored in Meat Peptone Broth (Merck & Co., Inc., USA) at –80 °C in 20% glycerol. All the EA strains were cultured on Nutrient Agar (Merck Millipore, USA) for 24 h at 28 °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 a final concentration of 1 × 10^5 CFU/mL. Equal amounts of three EA strain suspensions were mixed together, and immediately used for inoculation.

Plant material. Grafted one-year-old pear trees of 14 European and Asian commercial and old pear cultivars (Table 1) were planted into 40-litre pots containing a mix (1 : 1) of two different horticultural substrates (ZS I and ZS II; SOL, Czech Republic) which consisted of a different ratio of soil, natural organic matter and other substrates (leaf and bark moulds, white peat, silica sand, compound mineral fertilisers with trace elements (PG-MIX) and dolomite limestone, pH 5.5–9.0). The pear trees were transferred to a net house and winterised. All

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Table 1. List of the pear cultivars

Pear cultivar	*Pyrus* species	Origin	Susceptibility class[A]
Ananaska česká	*communis*	Czech Republic, old variety (1754)	1–2
Clapp's favourite	*communis*	USA, Massachusetts (1860)	3–4
Nagevicova	*communis*	Italy (France), old variety	2–3
William's	*communis*	England (1765)	4
Kieffer	interspecific hybrid *P. pyrifolia* × Williams Bartlett.	USA, Philadelphia (1863)	2
Early Shu	*bretschneideri*	Japan	2–3
Chinese White (Nashi)	*bretschneideri*	China	3
Ya Li	*P. × bretschneideri*	China, old variety	1
Hosui	*pyrifolia*	Japan (1972)	3–4
Chojuro	*pyrifolia*	Japan (1889)	2–3
Kosui	*pyrifolia*	Japan (1959)	3–4
Man Sun Gil	*pyrifolia*	Japan (1939)	1–2
Niitaka	*pyrifolia*	Japan (1915)	1–2
Nijissejkii	*pyrifolia*	Japan (1898)	2–3

[A]Susceptibility according to the information provided by the pear cultivar suppliers

the potted trees were kept under the same maintenance programme. They were irrigated to protect them from drying out in the summer and covered by a coco mat to protect them from frost injuries in winter.

Detached shoot susceptibility. Altogether, ten shoots 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 the final length; Martínez-Nicolás et al. 2016) were cut from ten untreated pear trees (one shoot per tree) of each cultivar (Table 1) in 2020. Each shoot was placed in an individual container with sterile distilled water in the climate chamber. A 5 mL mixture of the three virulent EA strains was applied by a handheld sprayer on the leaves of ten shoots per pear cultivar, and sterile distilled water, serving as a negative control was applied on the leaves of two shoots per pear cultivar. The detached shoots were incubated at a temperature regime of 25/17 °C (day/night), relative humidity of 80–90%, a photoperiod regime of 12/12 h (day/night). Water was changed and 0.3 cm of the basal part of the shoots was cut daily. At each assessment date (4, 7, 10 and 14 days after inoculation), the percentage of leaves with superficial necrosis on each shoot was evaluated, the mean percentage of the necrotic leaves for each pear cultivar was calculated and transformed into the fire blight susceptibility score according to the ranking scale of Le Lezec et al. (1997) and Sobiczewski et al. (2015) 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 detached shoot susceptibility (DS) class of each pear cultivar was determined according to the highest mean value of the percentage of leaves with necrosis on a shoot within a 14-day evaluation period.

Terminal shoot susceptibility. Potted trees of each pear cultivar were shoot-inoculated in phenological stage 35–39 according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale (shoots about 50–90% of the final length; Martínez-Nicolás et al. 2016) in a net house for two successive years (2020 and 2021). Each year, one actively growing shoot (35.0 ± 5.0 cm) was tagged randomly on ten trees of each pear cultivar (Table 1), and was inoculated by cutting the tip off under the first developed leaf using scissors previously immersed in the mixture of the three EA strains (described above). At each assessment date (2, 4, 6, and 8 weeks after inoculation), the percentage of the shoot lesion length in relation to the total shoot length for each tree was calculated, the mean percentage value of the portion of shoot lesions for each cultivar was calculated (Sobiczewski et al. 2015), and transformed into the fire blight susceptibility score according to the

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ranking scale of Le Lezec et al. (1997) as described above. The annual terminal shoot susceptibility (TS) class of each pear cultivar was determined according to the highest percentage of the shoot lesion length in relation to the total shoot length within the 8-week evaluation period. After the last assessment, the inoculated shoots were cut off.

Blossom susceptibility. 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 ten trees of each pear cultivar (Table 1) to maximise the spatial variation in the net house for two successive years (2020 and 2021). A mix of three EA virulent strains was spray-inoculated to the individual cluster of blossoms using a handheld sprayer to ensure local application. At each assessment date (2, 4, 6, 8, 12 and 20 weeks after inoculation), the percentage of necrotic blossoms (NB) within the cluster (TB), the percentage of the shoot lesion length (NS) in relation to the total shoot length (TS), and the estimated percentage of leaves with fire blight symptoms (L) on the shoot were scored for each tree (i). The mean percentage blossom susceptibility (BS) for each pear cultivar (x) was calculated using the following formula and transferred into the susceptibility score scale according to (Le Lezec et al. 1997) as described above.

$$BS_x = 10\sum_{i=1}^{10}\left(\frac{NB_i}{TB_i}+\frac{NS_i}{TS_i}+L_i\right) \quad (1)$$

The annual BS class of each pear cultivar was determined according to the highest calculated value of BS within the 20-week evaluation period. After the last assessment, the shoots were cut off.

Statistical analysis. The effect of the date of the disease symptom assessment on the estimated DS, and calculated TS and BS of the tested pear cultivars to EA across one (DS) or two years was analysed using an analysis of variance (ANOVA) and Tukey's post hoc test. A two-way ANOVA was carried out for the three methods used for the artificial inoculation of the pear cultivars in combination with the cultivar susceptibility-date of assessment and cultivar susceptibility-year. $P < 0.05$ was considered as the threshold for significance ($^*P < 0.05$). The differences among the three methods of the artificial inoculation of the pear cultivars were analysed using Fisher's Least Significant Difference (LSD) test. TIBCO® Data Science/Statistica™ software (version 14.0.0.15; StatSoft Inc.) was used for the statistical analysis.

RESULTS

The study evaluated and compared three *in vivo* methods of artificial inoculation on a spectrum of 14 European and Asian pear cultivars. According to the ANOVA results and Tukey's post hoc test, regardless of the method of inoculation, there were significant differences in the severity of the fire blight symptoms on the different pear cultivars on the different assessment dates ($P < 0.05$; Tables 2–5). The DS of the pear cultivars to EA in the climate chamber differed significantly according to Fisher's LSD test ($P < 0.05$) from the TS and BS in the net house (Figure 1). The largest differences between the climate chamber and the net house methods were revealed in the third susceptibility class: the number of moderately susceptible pear cultivars differed by 28.6% between the detached shoot and terminal shoot inoculation methods. The TS and BS of pear cultivars susceptible to EA did not differ significantly according to Fisher's LSD test ($P < 0.05$). The results were the same for the highly susceptible (4) and very susceptible (5) pear cultivars in both years, and for the very low susceptible and low susceptible pear cultivars in the second year of evaluation.

Detached shoot susceptibility. Depending on the cultivar, the first black lesions appeared on the leaves of the detached shoots within 4–6 days, progressed up to 10–12 days, and then all the shoots including the control wilted and the assessment was ended. The results of the DS to EA in the climate chamber are summarised in Table 2. According to the highest percentage values, most of the pear cultivars in the pathogenicity test on the detached shoots were classified as low susceptible (35.7%) or high susceptible (28.6%) to EA. The William's European pear cultivar and the Chojuro Asian cultivar showed very low susceptibility according to the susceptibility scale. No pear cultivar was very susceptible to EA.

Terminal shoot susceptibility. Depending on the year and pear cultivar, limited black lesions appeared on the inoculated terminal shoots within 7–10 days, followed by the progression of disease symptoms backwards from the shoot tip. In general, the reaction of the shoots to the EA infection de-

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Table 2. Detached shoot susceptibility of the pear cultivars to fire blight after artificial inoculation with a mixture of *Erwinia amylovora* strains in the climate chamber in 2020

Pear cultivar	Percentage of leaves with necrosis on the day of assessment				Susceptibility class
	4^{a}	7^{ab}	10^{b}	14^{c}	
Ananaska česká	5.0 ± 5.9	35.0 ± 15.0	40.0 ± 10.3	40.0 ± 15.1	2
Clapp's favourite	0.0 ± 0.0	20.0 ± 8.8	30.0 ± 14.1	30.0 ± 12.6	2
Nagevicova	0.0 ± 0.0	20.0 ± 10.2	30.0 ± 14.1	35.0 ± 15.9	2
William's	0.0 ± 0.0	10.0 ± 0.5	20.0 ± 8.4	20.0 ± 6.8	1
Kieffer	10.0 ± 8.3	40.0 ± 17.4	50.0 ± 30.0	70.0 ± 21.7	4
Early Shu	10.0 ± 7.8	40.0 ± 17.0	50.0 ± 24.1	65.0 ± 35.9	4
Chinese White (Nashi)	0.0 ± 0.0	0.0 ± 0.0	20.0 ± 10.4	25.0 ± 11.2	2
Ya Li	0.0 ± 0.0	20.0 ± 8.4	40.0 ± 15.6	40.0 ± 10.3	2
Hosui	10.0 ± 6.2	50.0 ± 22.0	60.0 ± 20.1	60.0 ± 18.7	3
Chojuro	0.0 ± 0.0	15.0 ± 6.4	15.0 ± 6.1	19.0 ± 3.5	1
Kosui	10.0 ± 8.2	40.0 ± 20.3	40.0 ± 17.3	45.0 ± 17.5	3
Man San Gil	20.0 ± 8.8	40.0 ± 10.8	70.0 ± 10.2	70.0 ± 2.3	4
Niitaka	20.0 ± 10.2	45.0 ± 19.6	60.0 ± 20.4	60.0 ± 10.4	3
Nijissejkii	20.0 ± 8.4	50.0 ± 5.2	70.0 ± 2.1	80.0 ± 4.5	4

[a–c]The different letters indicate significant differences among the assessments 4, 7, 10 and 14 days after inoculation at a 0.05 significance level according to Tukey's post hoc test
The detached shoot susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest mean percentage of the leaves with necrosis during the 14-day assessment period; each value is the mean ± SD of ten replicates

veloped slowly during the first 2–4 weeks after inoculation, then accelerated, and the measurements made at six and eight weeks after infection revealed the highest TS values. The results of the evaluation of the TS to EA in the net house in 2020 and 2021 are summarised in Table 3. According to the susceptibility scale, most of the pear cultivars were moderately susceptible, 50.0% in 2020 and 42.8% in 2021. In both vegetation seasons, the Ananaska česká European cultivar and the Nijissejkii Asian cultivar showed very high susceptibility to EA. The Chinese White Asian cultivar expressed very low susceptibility to the fire blight causal agent in 2020 and low susceptibility in 2021. Also, the William's European cultivar and the Chojuro Asian cultivar expressed low susceptibility to EA according to Le Lezec's scale in both years.

Blossom susceptibility. The dynamics of the fire blight symptoms' development in the individual cultivars after the blossom infection varied over 20 weeks (Table 4). Generally, the first blossom blight symptoms were observed seven days after inoculation in all the cultivars and in both years. Depending on the year and pear cultivar, the first shoot lesions and leaf necrosis under the inoculated blossom cluster were observed 3–5 weeks after inoculation, followed by the progression of these disease symptoms up to 13–16 weeks. After that, the dynamics of the disease symptoms slowed down or stopped, and a partial revitalisation in some cultivars was observed between 16–20 weeks. The Chojuro and Chinese White Asian cultivars showed very low susceptibility to EA according to the susceptibility scale during the first year of the assessment and low susceptibility during the second year. Most of the pear cultivars expressed low susceptibility, 36.0% in 2020 and moderate susceptibility, 42.8% in 2021. Altogether, 43% of the pear cultivars showed higher susceptibility to EA in the second year of the assessment. In total, 36% of the cultivars were ranked one class higher, and the Kosui cultivar was classified two classes higher on the susceptibility scale in the second year of the assessment. The results obtained in the net house were similar in the cases of the very highly susceptible cultivars, the Ananaska česká European cultivar and the Nijissejkii Asian cultivar, in both years.

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Table 3. Terminal shoot susceptibility of the pear cultivars to fire blight after artificial inoculation with a mixture of *Erwinia amylovora* strains in the net house in 2020 and 2021

Pear cultivar	2020					2021				
	percentage of shoot lesions				susceptibility class	percentage of shoot lesions				susceptibility class
	week 2[c]	week 4[bc]	week 6[ab]	week 8[ab]		week 2[c]	week 4[bc]	week 6[abc]	week 8[a]	
Ananaska česká	30.0 ± 20.0	49.5 ± 20.0	80.0 ± 20.0	90.0 ± 10.0	5	20.0 ± 24.5	58.0 ± 24.0	86.0 ± 8.9	90.0 ± 30.9	5
Clapp's favourite	9.8 ± 7.2	22.9 ± 14.9	49.9 ± 35.0	51.9 ± 38.0	3	18.8 ± 25.7	19.0 ± 22.1	25.6 ± 9.6	46.4 ± 18.7	3
Nagevicova	20.0 ± 10.3	23.0 ± 20.0	40.0 ± 10.0	50.3 ± 30.0	3	20.0 ± 17.3	22.5 ± 8.3	28.5 ± 14.3	42.5 ± 4.3	3
William's	8.5 ± 8.7	20.0 ± 13.0	20.0 ± 9.0	23.4 ± 10.0	2	5.3 ± 4.7	11.2 ± 4.7	21.2 ± 4.0	26.7 ± 9.6	2
Kieffer	10.8 ± 10.0	14.2 ± 10.5	48.2 ± 20.3	44.0 ± 20.5	3	20.8 ± 16.8	27.5 ± 13.5	38.7 ± 4.6	55.5 ± 15.5	3
Early Shu	15.0 ± 10.9	20.0 ± 10.2	32.0 ± 10.8	23.6 ± 19.8	3	35.0 ± 19.0	34.2 ± 22.0	36.4 ± 20.5	63.8 ± 19.9	4
Chinese White (Nashi)	5.0 ± 0.0	5.0 ± 5.0	10.0 ± 0.0	15.9 ± 10.0	1	4.2 ± 10.5	10.0 ± 10.0	19.0 ± 12.3	25.0 ± 10.0	2
Ya Li	25.5 ± 1.2	25.0 ± 10.0	45.0 ± 1.4	42.5 ± 25.0	3	20.0 ± 10.0	60.0 ± 40.0	65.0 ± 15.0	65.0 ± 25.0	4
Hosui	35.0 ± 20.8	43.4 ± 29.0	50.0 ± 33.4	51.7 ± 34.6	3	35.0 ± 20.8	42.4 ± 33.3	48.8 ± 2.2	53.2 ± 25.2	3
Chojuro	29.0 ± 12.2	29.7 ± 5.5	38.3 ± 20.4	24.0 ± 20.0	2	29.0 ± 12.2	35.9 ± 17.4	35.0 ± 28.0	38.4 ± 19.3	2
Kosui	40.0 ± 10.0	50.6 ± 10.0	60.3 ± 20.0	65.0 ± 20.0	4	20.0 ± 28.3	26.7 ± 18.9	26.0 ± 4.7	50.0 ± 18.5	3
Man San Gil	50.0 ± 2.0	51.3 ± 10.0	60.0 ± 30.0	60.0 ± 20.0	4	30.0 ± 12.0	30.0 ± 14.1	33.3 ± 15.0	78.0 ± 31.2	4
Niitaka	21.2 ± 10.7	20.7 ± 20.0	40.1 ± 24.6	55.4 ± 10.2	3	15.0 ± 11.0	16.0 ± 4.9	26.0 ± 14.9	48.0 ± 8.9	3
Nijissejkii	30.0 ± 20.5	60.4 ± 19.2	83.3 ± 10.0	80.0 ± 15.0	5	35.0 ± 11.2	38.0 ± 10.1	38.3 ± 12.1	85.8 ± 8.2	5

[a–c]The different letters indicate significant differences among the assessments 2, 4, 6 and 8 weeks after inoculation at a 0.05 significance level according to Tukey's post hoc test

The annual terminal shoot susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest percentage of the terminal shoot lesions during the 8-week assessment period; each value is the mean ± SD of ten shoots

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Table 4. Pear blossom susceptibility to fire blight after artificial inoculation with a mixture of *Erwinia amylovora* strains in the net house in 2020 and 2021

Pear cultivar	2020							2021						
	percentage of fire blight disease symptoms						SC	percentage of fire blight disease symptoms						SC
	week 2[c]	week 4[c]	week 6[bc]	week 8[abc]	week 12[ab]	week 20[abc]		week 2[bc]	week 4[bc]	week 6[bc]	week 8[a]	week 12[a]	week 20[abc]	
Ananaska česká	20.0 ± 24.5	38.0 ± 2.4	40.0 ± 9.0	80.0 ± 5.9	85.0 ± 10.0	79.5 ± 29.6	5	30.0 ± 24.5	28.0 ± 24.0	46.0 ± 8.9	90.0 ± 30.9	90.0 ± 11.0	35.7 ± 12.9	5
Clapp's favourite	10.1 ± 2.7	10.9 ± 8.1	25.6 ± 2.4	38.7 ± 1.7	40.3 ± 20.0	39.1 ± 19.4	3	18.8 ± 25.7	19.0 ± 22.1	15.6 ± 9.6	36.4 ± 18.7	47.3 ± 29.9	31.0 ± 30.8	3
Nagevicova	18.6 ± 17.3	25.5 ± 3.8	28.0 ± 10.4	40.1 ± 8.2	45.5 ± 14.3	43.0 ± 13.0	3	20.0 ± 17.3	22.5 ± 8.3	28.5 ± 14.3	42.5 ± 4.3	47.5 ± 4.3	46.3 ± 33.7	3
William's	10.3 ± 5.0	13.3 ± 6.4	20.0 ± 14.0	20.7 ± 19.0	28.0 ± 6.4	26.0 ± 12.3	2	5.3 ± 4.7	11.2 ± 4.7	21.2 ± 4.0	26.7 ± 9.6	25.0 ± 9.6	25.0 ± 13.4	2
Kieffer	20.8 ± 16.8	17.5 ± 14.2	28.0 ± 14.6	35.5 ± 5.0	38.1 ± 10.4	24.2 ± 2.5	2	40.8 ± 16.8	27.5 ± 13.5	18.7 ± 4.6	55.5 ± 15.5	53.8 ± 14.3	24.3 ± 24.5	3
Early Shu	35.0 ± 10.9	42.0 ± 30.2	39.4 ± 28.0	53.0 ± 41.2	63.0 ± 29.0	63.6 ± 4.2	4	35.8 ± 19.0	34.2 ± 22.0	33.4 ± 20.5	63.8 ± 19.9	60.4 ± 20.4	63.6 ± 19.8	4
Chinese White (Nashi)	4.0 ± 2.5	10.0 ± 10.0	10.0 ± 10.0	15.0 ± 13.2	10.0 ± 9.4	5.6 ± 4.4	1	4.2 ± 10.5	20.0 ± 10.0	19.0 ± 12.3	25.0 ± 10.0	15.0 ± 11.5	5.9 ± 4.3	2
Ya Li	20.0 ± 10.0	20.0 ± 17.4	35.2 ± 12.5	50.5 ± 8,9	58.5 ± 10.2	49.8 ± 10.6	3	40.0 ± 10.0	40.0 ± 20.0	45.0 ± 15.0	55.0 ± 25.0	55.0 ± 15.0	22.5 ± 17.5	3
Hosui	35.0 ± 20.8	30.4 ± 13.7	42.8 ± 20.5	50.3 ± 21.7	55.0 ± 23.0	43.4 ± 29.0	3	55.0 ± 20.8	42.4 ± 33.3	28.8 ± 22.2	63.2 ± 25.2	60.0 ± 33.4	51.7 ± 34.6	3
Chojuro	9.0 ± 2.1	15.0 ± 7.0	13.5 ± 8.0	13.4 ± 10.0	15.3 ± 2.0	9.7 ± 5.5	1	29.0 ± 12.2	25.9 ± 17.4	35.0 ± 28.0	33.4 ± 19.3	38.3 ± 20.4	36.4 ± 21.3	2
Kosui	20.0 ± 28.3	20.1 ± 10.9	20.0 ± 14.0	20.0 ± 18.5	33.7 ± 12.2	26.5 ± 2.9	2	40.0 ± 28.3	26.7 ± 18.9	26.0 ± 4.7	70.0 ± 18.5	63.3 ± 4.7	70.0 ± 25.2	4
Man San Gil	30.0 ± 12.0	38.1 ± 13.4	43.0 ± 18.7	47.0 ± 35.6	58.0 ± 13.8	41.3 ± 4.7	3	50.0 ± 12.0	30.0 ± 14.1	33.3 ± 15.0	78.0 ± 31.2	78.0 ± 31.2	78.3 ± 25.0	4
Niitaka	15.0 ± 5.6	15.0 ± 9.8	15.0 ± 15.0	24.9 ± 19.8	33.4 ± 15.1	22.0 ± 13.7	2	20.0 ± 11.0	16.0 ± 4.9	16.0 ± 4.9	40.0 ± 8.9	45.3 ± 6.9	33.2 ± 15.2	3
Nijissejkii	33.5 ± 21.0	40.0 ± 10.0	63.2 ± 10.0	60.8 ± 10.0	80.9 ± 11.8	84.2 ± 20.9	5	35.0 ± 11.2	38.0 ± 10.1	38.3 ± 12.1	85.8 ± 8.2	85.3 ± 8.2	85.0 ± 24.5	5

SC – susceptibility class

[a–c]The different letters indicate significant differences among the assessments 2, 4, 6, 8, 12 and 20 weeks after inoculation at 0.05 significance level according to Tukey's post hoc test

The annual blossom susceptibility class (Le Lezec et al. 1997) of each pear cultivar was determined according to the highest percentage of fire blight disease symptoms on the trees during the 20-week assessment period; each value is the mean ± SD of ten replicates

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Table 5. ANOVA analysis of the susceptibility of the pear cultivars to fire blight disease observed across different dates of assessment, inoculation method and two years

Source of variability	Sum of squares	Difference	Mean squares	*F*-ratio (*P* = 0.05)	*P*-value
Detached shoots					
Cultivar (C)	11 595.21	13	891.94	2.46	< 0.05
Date of assessment (D)	13 163.71	3	4 387.90	16.55	< 0.05
C × D	1 988.79	39	51.00	–	–
Terminal shoots					
Cultivar	18 568.22	13	1 428.32	5.23	< 0.05
Date of assessment	14 382.28	7	2 054.61	6.85	< 0.05
C × D	31 196.88	104	299.97	–	–
Year (Y)	67.04	1	67.04	–	–
C × Y	25.55	13	1.97	–	–
Blossoms					
Cultivar	20 080.97	13	1 544.69	4.51	< 0.05
Date of assessment	19 651.43	11	1 786.49	6.09	< 0.05
C × D	45 752.04	156	293.28	–	–
Year	41.63	1	41.63	–	–
C × Y	1 006.83	13	77.45	–	–

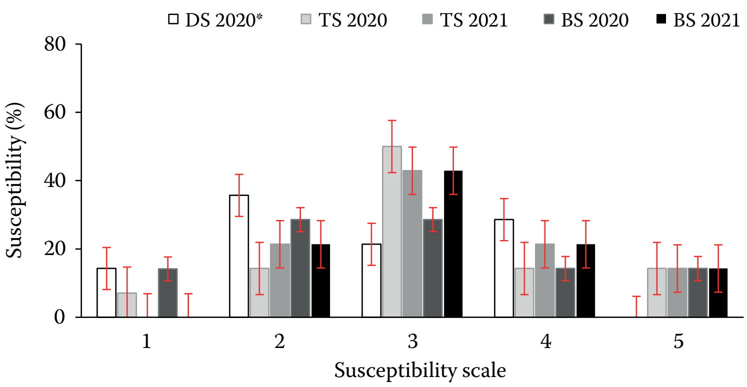

Figure 1. Susceptibility score scale for 14 pear cultivars after artificial inoculation with a mixture of *Erwinia amylovora* strains in a pathogenicity test on the detached shoots (DS) in the climate chamber, and on the terminal shoots (TS) and blossom clusters (BS) randomly tagged on potted pear trees in the net house in 2020 and 2021

*Significant differences at a 0.05 significance level according to Fisher's LSD test

Each bar is the mean ± SE of ten replicates

DISCUSSION

For comparison of the three inoculation methods, 14 commercial and old European and Asian pear cultivars with known varying susceptibility to the fire blight causal agent of the Rosaceae family were selected. Our evaluation of the susceptibility of pear cultivars, such as Ananaska česká and Nijissejkii, differed significantly from fruit nurseries and producers' information. Indeed, (Sobiczewski et al. 2008), our study confirmed the strain-dependent susceptibility to EA, after artificial infection with selected highly virulent strains of EA, the pear susceptibility can differ significantly from their susceptibility to the natural EA infection. Moreover, the susceptibility of pear cultivars to EA has proven to be less consistent after planting in different areas with different environmental and weather conditions (Peil et al. 2009; Sobiczewski et al. 2015).

The inoculation of the detached shoots was included in the study as the possibility for the rapid and cheap assessment of the susceptibility of pear cultivars to EA. Leaf wilting and foliar necrosis developed rapidly within 10 days, but the progression of the disease symptoms did not continue in the following days. Thus, despite the conditions in the climate chamber being favourable for disease expression, the estimated DS for the given pear cultivar was finally different and predominantly lower than the TS and BS in the net house. Our results supported previous observations that shoots which stopped growing were more resistant to infection (Ozrenk et al. 2012). Inoculation of the detached shoots was recently successfully used for evaluating bacterial canker resistance of pear

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genotypes in Brazil (Araujo et al. 2021), and boxwood blight susceptibility (Guo & Olsen 2015). According to our assessment, the results of the DS must be considered with caution over the whole susceptibility scale and, when compared to the results of the TS and BS, supported the opinion that shoot growth is an important factor determining the host plant susceptibility to fire blight (Sobiczewski et al. 2015).

Inoculating EA at a natural pathogen concentration of 10^5 CFU/mL and incubating without the use of plastic bags to increase the humidity delayed the development of the first fire blight symptoms in the net house by several days compared to the nearly 100% humidity in greenhouse studies (Ruz et al. 2008; Erfani-Moghadam & Zarei 2022), but approximated the conditions as close as possible to the conditions in orchards during the spring. In general, the permanently higher humidity in the net house compared to the field was of high importance for the development of the disease symptoms (Shwartz et al. 2003).

Virtually all the blossom clusters and terminal shoots exposed to the virulent EA strains developed a local plant defence reaction in the first phase, but the speed and sensitivity of induced response to EA in the following phase was very individual in each pear cultivar, reflecting a common observation that a pathogen burden is not always predictive of the disease development and the susceptibility level (Przybyla et al. 2012; Cui et al. 2021). In agreement with the results of other ratings of fire blight susceptibility (Emeriewen et al. 2019; Kostick et al. 2019), the ANOVA analysis and Tukey's post hoc test showed, with all the EA inoculation methods, significant differences in the percentage of disease symptoms at the different assessment dates. Decreasing BS values 20 weeks after inoculation at up to 85.7% of the European and Asian pear cultivars from the first, second and third susceptibility class, indicated an induced defence response, maintaining and, at least, the local overcoming of the EA infection. The late date of assessment enabled observing a decrease in the EA activity during the late summer of the temperate climate zone, due to the decreasing temperatures, humidity, and the availability of nutritional resources for the multiplying and spreading of the pathogen in the plant tissues in the presence of other bacterial species competing for the same space and nutrients.

Surprisingly, the weather conditions were not so important according to the ANOVA analysis of the annual ratings of the fire blight TS and BS under the net house conditions in comparison with inoculation studies performed under field conditions (Bell 2019; Kostick et al. 2019; Cui et al. 2021). The steel net house probably provided a suitable environment for the further multiplication of the pathogen and penetration into plant tissues even at the lower than optimal temperatures after artificial inoculation, due to the prolonged wetness in comparison to the field conditions (Shwartz et al. 2003).

No significant differences were found between the TS and BS ratings of the potted pear cultivars to EA during the study. Susceptible pear cultivars expressed the same disease progress and displayed severe symptoms, such as oozing shoot blight and foliar necrosis, approximately 6–12 weeks regardless of the inoculation method and year of assessment. TS and BS class 5 identically included the very susceptible Nijissejkii Asian pear cultivar and the Ananaska European old cultivar, while TS and BS classes 1 and 2 identically included the very low and low susceptible Chinese White and Chojuro Asian cultivars. In accordance with other studies, the screening of susceptibility to EA via artificial inoculation is most efficient in evaluating highly susceptible and highly resistant apple and pear cultivars (Ruz et al. 2008; Sobiczewski et al. 2015). The biggest differences in the TS and BS pear cultivar ratings were found in the middle of the susceptibility scale. TS to EA is not necessarily correlated with BS even under greenhouse conditions, therefore, cultivar characterisations should be determined by their susceptibility in both the blossoms and shoots (Shwartz et al. 2003; Peil et al. 2009; Erfani-Moghadam & Zarei 2022). The environmental conditions, predominantly the diurnally fluctuating interacting effects of the temperature and wetness duration after the artificial inoculation of the terminal shoots and blossoms were crucial for the development of fire blight symptoms and decided whether the pear cultivar would be assigned as more or less susceptible to Ea in the annual classification. For testing these varieties, the net house is the optimal choice and the results could improve the fire blight warning system.

CONCLUSION

Our data provide the evidence that the fire blight susceptibility of the spectrum of European and Asian

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pear cultivars to EA under climate chamber conditions differed significantly from their susceptibility under net house conditions. During the two-year study, significant differences between the terminal shoot and blossom susceptibility rating of the pear cultivars in the net house were not found. As with the natural infection in the field, the blossom artificial inoculation provided the opportunity to observe all the phases of the plant response to the EA infection, pathogen recognition, maintaining, and overcoming pathogen attack at very low, low and moderately susceptible European and Asian pear cultivars.

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