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Susceptibility of Japanese plum and pluot cultivars to *Pseudomonas syringae*

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Abstract. The susceptibility of seven cultivars of Japanese plum (Black Star, Gauota, Golden Japan, Crimson Glo, Ozark Premier, Santa Rosa, Vanier) and three cultivars of pluot (Black Gigant®, Dapple Supreme pluot®, Flavorich pluot®) to *Pseudomonas syringae* pathovars *syringae* and *morsprunorum* was evaluated under temperate European climate conditions over a three-year period. The evaluation was carried out in a netted house. The susceptibility of the plants to the causal agents of blossom and terminal shoot infection was calculated on five occasions during the year using a formula summarising all the symptoms that appeared on the plants after artificial inoculation. Most cultivars were classified in BS class 2 as low susceptible to both pathovars of *Pseudomonas syringae*. The Japanese cultivar Santa Rosa was the only cultivar classified in class 4, as highly susceptible to *morsprunorum* pathovar. After terminal shoot inoculation, 60% of the Japanese plum and pluot cultivars were classified as very low susceptible in class 1 and 40% as low susceptible in class 2 to both pathogens tested. Apart from Santa Rosa, the remaining Japanese plums and all plum cultivars showed remarkable vigour and recovery after repeated artificial infection with economically important *Prunus* plant pathogens.

Key words: blossom inoculation, netted house, *Pseudomonas syringae* pv. *morsprunorum, Pseudomonas syringae* pv. *Syringae*.

INTRODUCTION

Plum is a drupe in the genus *Prunus* in the family Rosaceae. Commercially grown genotypes belong to the European plum group (mainly *Prunus domestica* L.) or the Asian plum group (*Prunus salicina* L.) and cherry plums (*Prunus cerasifera* Ehrh.) (Okie & Hancock, 2008). The attractive appearance and flavour of Japanese plum cultivars and pluots, complex *Prunus* hybrid with dominant parentage of plum (*Prunus salicina*) and apricot (*Prunus armeniaca* L.) having fruit resembling plum, are attracting increasing interest from growers. The added value of these cultivars is their nutritional value, particularly their high levels of health benefits anthocyanins (Rampáčková et al., 2021; Tomić et al., 2022). These cultivars are characterised by reduced resistance to cold hardiness and limited adaptability to different soil conditions (Dalbó et al., 2016; Milošević & Milošević, 2018). In temperate regions, climate change is increasingly contributing to pronounced temperature fluctuations during dormancy and spring, which significantly affect the vigour of plum trees and increase their susceptibility to bacterial plant pathogens (Fadón et al., 2020; Seethapathy et al., 2022). Additionally, excessive fertilisation and pesticide application further sensitize plant tissues to pests (Husseini & Akköprü, 2020; Aprile et al., 2021; Tripathi et al., 2022).

In temperate orchards, significant economic losses are associated with bacterial infections caused by members of the *Pseudomonas syringae* (*Ps*) complex (Yang et al., 2023), notably *Ps* pv. *syringae* van Hall 1902 (*Pss*) and *Ps* pv. *morsprunorum* race 1 (Wormald) Young et al. (Lee et al., 2012) (*Psm*). These pathogens severely affect plum production across Central Europe, leading to symptoms such as blossom blast, shoot dieback, and stem cankers. Historical data indicate annual tree mortality rates reaching up to 30% in heavily infected orchards (Sundin, 2007). Recent reports have confirmed that losses of a similar magnitude continue to affect both plum and pluot cultivars in the region (Němcová & Buchtová, 2023).

The pathogens have led to widespread symptoms such as blossom blast, shoot dieback, and canker formation, especially during wet spring seasons. These pathogens contribute to widespread losses through blossom blast, shoot dieback, and canker formation, severely impacting orchard longevity and fruit yield (Konavko et al., 2014). Bacteria are naturally present on plants or plant debris in the agro-ecosystem and are transferred to susceptible plants by wind, rain, birds, insects and technology. The severity of *Ps* infection varies according to annual weather conditions. Outbreaks occur during rainy periods, prolonged high humidity and temperatures below 12 °C (Hunjan & Lore, 2020). Once suitable conditions are established, the initially unculturable *Ps* inoculum reaches a concentration capable of inducing infection within 24 h. Blossoms are most susceptible to *Ps* infection due to the amount of nutrients available (Chan et al., 2021). The changes in the pattern of precipitation along with the warmer weather have increased the incidence of *Ps* diseases in fruit trees in autumns (Roussos, 2024). Through various wounds and frost damage, *Ps* enters the vascular bundles and threatens tree vigour and production (Hulin et al., 2018). The course and severity of infection vary depending on the pathovar or race of the pathogen (Islam et al., 2024).

The susceptibility of Japanese plum and pluot cultivars grown in temperate climates to any bacterial pathogens is a topic that has yet to be extensively researched. The most common method for evaluating the susceptibility of plants to Ps bacteria is through inoculation of detached flowers, leaves, or shoots. This approach provides valuable insights into the interaction between the pathogen and the plant organ (Aleksandrova et al., 2023; Lienqueo et al., 2024). The assessment of fruit trees in a greenhouse setting by inoculation of blossoms or terminal shoots is a more compact approach, but the degree of agreement with the experience of agricultural practice is variable (Bophela et al., 2020). While cultivar susceptibility evaluations under different weather and soil conditions in field trials are less consistent, they are better transferable to practice, thereby reducing economic losses due to inappropriate variety selection (Carvalho et al., 2021; Burbank et al., 2023).

This study aimed to evaluate the susceptibility of blossom and terminal shoots to *Pss* and *Psm* in selected Japanese plum and pluot cultivars under netted house conditions simulating a temperate climate.

MATERIALS AND METHODS

Plant material

The level of susceptibility of 7 Japanese plum (Black Star, Gauota, Golden Japan, Crimson Glo, Ozark Premier, Santa Rosa, Vanier) and 3 pluot (Black Gigant®, Dapple Supreme pluot®, Flavorich pluot®) cultivars to *Pss* and *Psm* was evaluated for three consecutive growing seasons (2021–2023). All cultivars were grafted by chip-budding on GF 305 peach rootstocks. Twenty-four saplings of each Japanese plum and pluot cultivar were planted in the autumn in 40-litre containers, placed in two netted houses, and winterized. The potted trees were maintained under a uniform care regimen within the netted houses, which were designed to simulate field-like conditions. Trials were conducted at an altitude of 250 m a.s.l. in a major fruit-growing region of Central Europe. To mitigate environmental stress, trees were irrigated during summer to prevent drought and covered with coco mats in winter to protect against frost damage.

Bacterial strains and inoculum preparation

Suspensions of virulent *Pss* strains CPABB 138, CPABB 234 and CPABB 237 (Collection of Phytopathogenic and Agriculturally Beneficial Bacteria, Czech Republic) in sterile water at a concentration of 10^5 CFU mL^{-1} were mixed in equal ration and used for inoculation. Mixed suspension of *Psm* strains CPABB 23 and CPABB 24 was prepared and applied in the same way.

Inoculation of Japanese Plum and Pluot Cultivars

Potted trees of Japanese plum and pluot cultivars were inoculated at two distinct phenological stages following the BBCH scale (Meier et al., 1994). Five trees per cultivar were treated at blossom stage BBCH 61-65, when all flowers within a cluster were open. A single randomly selected flower cluster on each plant was sprayed with 1 mL of a mixed *Pss* suspension. In a separate set of five trees per cultivar, inoculation was performed at shoot stage BBCH 35-39, corresponding to shoots reaching 50–90% of their final length. On each plant, a randomly chosen shoot was cut below the first undeveloped leaf using scissors dipped in the same mixed *Pss* suspension.

In the same way, the same number of plants were inoculated with a mixed suspension of *Psm*. Four trees of each plum and pluot cultivar were used as negative controls. Two were sprayed with 1 mL of sterile water on one randomly selected blossom cluster, and in the other two, one randomly selected terminal shoot was cut below the first undeveloped leaf using scissors dipped in sterile water.

Evaluation of Cultivar Susceptibility

Blossom blight, shoot blight, and other symptoms of progressive pseudomonad infection affecting various plant organs were evaluated five times (at 1, 3, 6, 12, and 20 weeks after inoculation) during each growing season from 2021 to 2023.

For each plant (i), the following parameters were determined: the total number of infected plant organs (Yi), the percentage of necrotic blossoms in the inoculated cluster (NBi/TBi), the percentage of necrotic lesions on the inoculated terminal shoot (NSi/TSi), the percentage of leaves with necrotic lesions (Li), the proportion of damaged fruit (Fi), and the decline in overall tree vigour (Vi) in comparison to negative control. For each evaluation date, the mean blossom susceptibility (*BSx*) and terminal shoot susceptibility (*TSx*) to *Pss* and *Psm* were calculated for each cultivar (*x*) using the formula:

$$BS_x\ (TS_x) = 1/5 \sum_{i=1}^{5} \left(\frac{NBi}{TBi} * 100 + \frac{NSi}{TSi} * 100 + L_i + V_i + F_i \right) / Y_i. \qquad (1)$$

The highest calculated values of BS_x and TS_x for each pathovar were than transformed into the international scale of susceptibility (Le Lezec et al., 1997): 1 – very low (0–20.0%); 2 – low (> 20.0–40.0%); 3 – moderate (> 40.0–60.0%); 4 – high (> 60.0–80.0%); 5 – very high (> 80.0–100%). Based on this scale, each Japanese plum and pluot cultivar was assigned a susceptibility class for blossom and terminal shoot infection by *Pss* and *Psm* for each year of evaluation.

The effects of place of infection (blossom, terminal shoot), evaluation term and year on the calculated *BS* and *TS* to *Pss* and *Psm* for Japanese plum and pluot 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). $P < 0.05$ was considered as the threshold for significance.

RESULTS AND DISCUSSION

For three consecutive growing seasons (2021–2023), a total of five calculated *BS* and *TS* values to the pathogens *Pss* and *Psm* for each plum and pluot cultivar tested are summarized in supplementary tables A–D (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx).

Blossom susceptibility

The findings revealed that, irrespective of the cultivar and *Ps* pathovar tested, *BS* values recorded at week 3 were lower than those calculated 7 days after blossom inoculation. From week 6 through week 20, a consistent trend was observed, characterized by a gradual increase in BS values, reflecting progressive symptom development as shown in Fig. 1 (A–C).

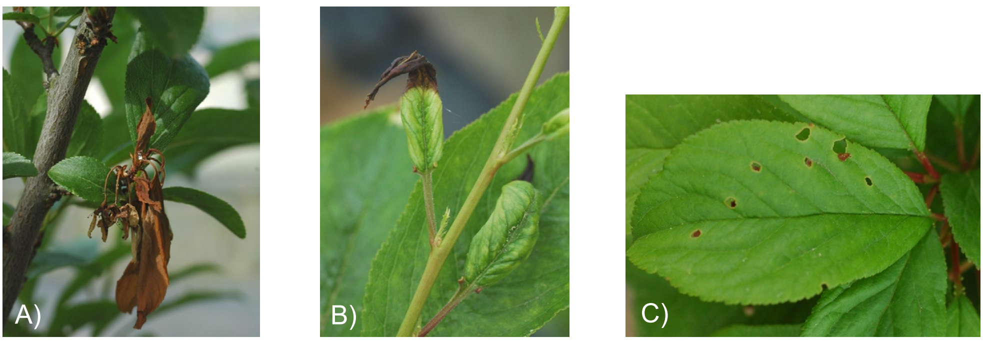

Figure 1. Symptoms of pseudomonas blight on Japanese plum and pluot cultivars observed during the 2022 growing season in a netted house, following artificial inoculation of randomly selected blossom clusters with a bacterial mixture of *Pseudomonas syringae* pv. *Morsprunorum*. A – blossom blight on Japanese plum cultivar Black Star, observed during the first evaluation, one week after artificial inoculation; B – necrotic tips observed on pluot cultivar Black Gigant®, observed at the third evaluation term, 6 weeks after inoculation; C – necrotic leaf spots on Japanese plum cultivar Golden Japan, observed during the fourth evaluation, 12 weeks after inoculation.

A comparison of the annual *BS* classes of the tested cultivars, determined according to the highest *BS* values, revealed statistically significant differences ($P < 0.05$) in their susceptibility to *Pss* and *Psm* pathogens are summarized in supplementary tables E–F (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx) and Fig. 2. Most of the cultivars exhibited low susceptibility to blossom infection, categorized in class 2. Two exceptions were identified, Flavourish pluot® was classified into class 1 as being very low susceptible to *Pss* infection, and Santa Rosa was classified into class 4 as being highly susceptible to *Psm* infection. Six of the ten cultivars evaluated, namely Black Star, Crimson Glo, Santa Rosa and three pluot cultivars, were classified in the higher susceptibility category regarding infection by *Psm*. Two cultivars were assigned to the same susceptibility class for both pathogens. Only Vanier was assigned to a higher susceptibility class for *Pss*.

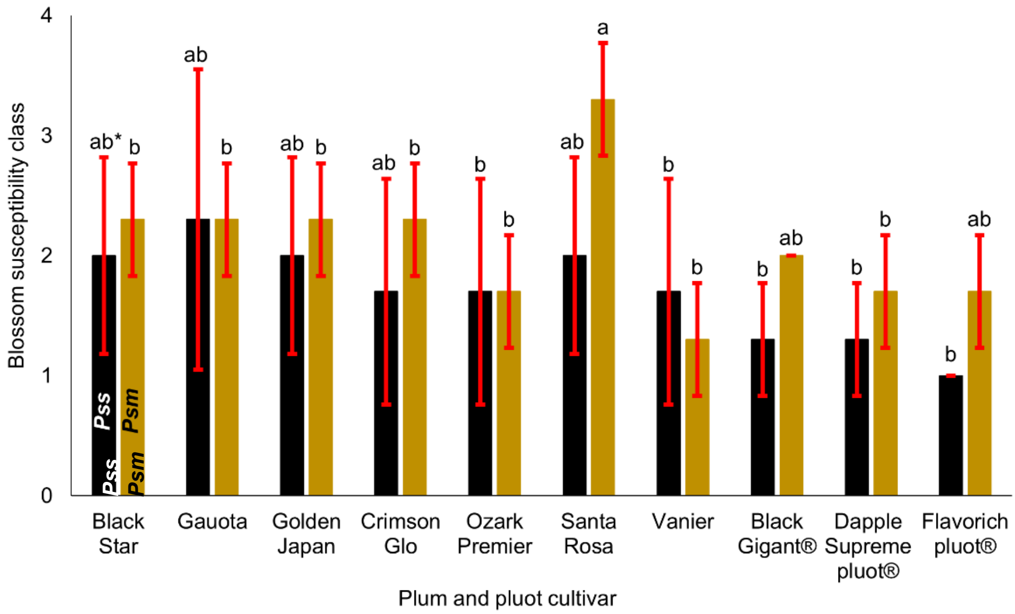

Figure 2. Comparison of the blossom susceptibility classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the blossom susceptibility classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility; 4 – high susceptibility) to *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum*, evaluated in five trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Terminal shoot susceptibility

Following the infection of the terminal shoots, the initial disease symptoms, manifesting as black water-soaked lesions progressing from the site of inoculation along the shoots (Fig. 3, A and B), were observed at week 3, regardless of the *Ps* pathovar used. As the infection progressed to other parts of the inoculated trees, *TS* values increased at varying rates throughout the remainder of the growing season, depending on the specific plum or pluot cultivar. These data are summarized in supplementary tables C and D (Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx).

Figure 3. Symptoms of pseudomonas blight on Japanese plum and pluot cultivars observed during the 2022 growing season in a netted house, following artificial inoculation of randomly selected terminal shoots with a bacterial mixture of *Pseudomonas syringae* pv. *Morsprunorum*.

A – symptoms on Japanese plum cultivar Crimson Glo, observed during the fourth evaluation, 12 weeks after inoculation; B – necrotic terminal shoot on Japanese plum cultivar Santa Rosa, observed during the fifth evaluation, 20 weeks after inoculation.

A comparison of the annual *TS* classes of the tested cultivars revealed statistically significant differences ($P < 0.05$) in susceptibility to the pathogens *Pss* and *Psm* (supplementary tables E–F; Susceptibility of Japanese plum and pluot cultivars to Pseudomonas syringae-Supplementary Tables.xlsx) at the three cultivars tested (Fig. 4). Two of these, Black Star and Dapple Supreme pluot®, demonstrated heightened susceptibility to *Pss*. In contrast, the cultivar Santa Rosa was found to be the most susceptible cultivar to *Psm* infection on terminal shoots. Seven cultivars demonstrated comparable susceptibility to both pathogens. The plum cultivars Gauota, Ozark Premier, Vanier and the pluot cultivars Black Gigant® and Flavorich pluot® were classified as very low susceptibility in class 1 throughout the study. The plum cultivars Golden Japan, Crimson Glo and the pluot cultivar Dapple Supreme® were equally susceptible to both pathogens and were placed in Class 1 in 2021 and Class 2 in 2022 and 2023.

Susceptibility to *Pseudomonas syringae*

Across all three growing seasons, the *BS* class ratings for *Ps* pathovars were consistently higher than *TS* class ratings. Plum and pluot cultivars were low susceptible to *Ps* blossom infection, typically falling into *BS* class 2. Regarding terminal shoot infection, 50% of the tested cultivars were classified as very low susceptible (class 1), while 40% were considered low susceptible (class 2). The only exception was the cultivar Santa Rosa, which was moderately susceptible to blossom infection (class 3) and low susceptible to terminal shoot infection (class 2). Over the years, greater variability was observed in BS class ratings for individual cultivars. Over the years, greater variability was observed in BS class ratings for individual cultivars. Statistical analysis using the *LSD* test ($P < 0.05$) revealed that the differences between *BS* and *TS* class ratings to *Ps* became more pronounced and statistically significant, particularly when comparing low and moderately susceptible Japanese plum and pluot cultivars (Fig. 5).

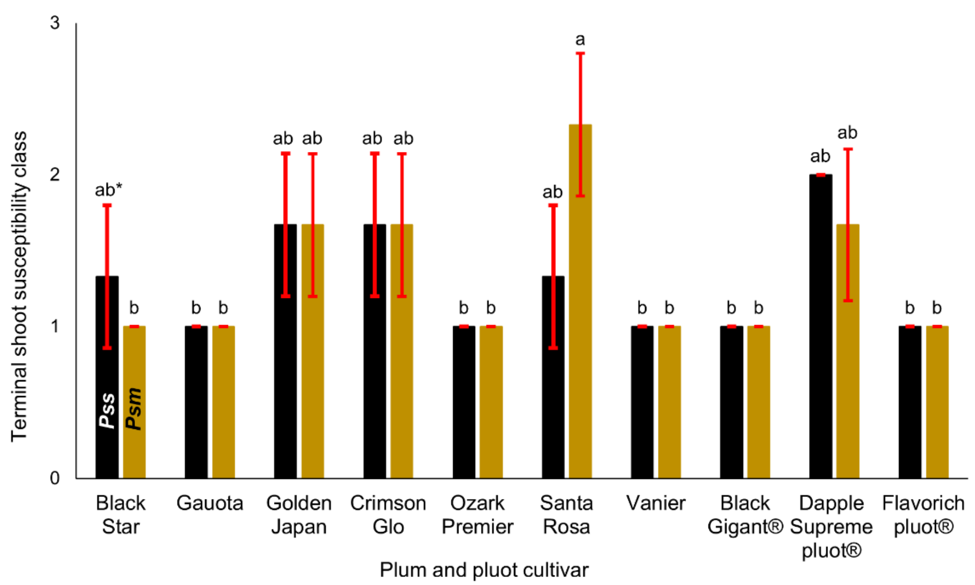

Figure 4. Comparison of the terminal shoot susceptibility classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* pv. *syringae* (*Pss*) and *Pseudomonas syringae* pv. *morsprunorum* (*Psm*) determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the terminal shoot classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility) to *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringa*e pv. *morsprunorum*, evaluated in five trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Throughout the three-year study, both *Ps* pathovars exhibited a similar infection progression in inoculated plants during the growing season, inducing comparable symptoms such as desiccation and necrosis, despite belonging to distinct phylogroups within the *Ps* complex. Specifically, *Pss* belongs to PG2, while *Psm* is classified as PG3 for race 1 and PG1 for race 2 (Chen et al., 2022; He et al., 2023). *Pss* and *Psm* share key mechanisms of pathogenesis, notably the production of phytotoxins and deployment of the type III secretion system (T3SS), which delivers effector proteins into host cells to suppress plant immune responses (Hulin et al., 2018). This mechanism was evident during the inoculation of blossom clusters, which triggered a near-identical hypersensitive reaction across pathovars. Following this initial reaction, a secondary phase referred to as the 'symptom-free period' was observed. Consistent with previous findings (Yang et al., 2023), the duration of this phase and the subsequent timing and severity of disease symptoms varied depending on the susceptibility of the plum or pluot cultivar, the inoculation method, the specific pathovar involved, and the prevailing weather conditions during each evaluation period. To assess the implications of pathogenic mechanisms for orchard management, alongside the progression of *Pss* and *Psm* infections from the inoculation site to other plant organs, the vigour of inoculated trees was also evaluated in comparison to the negative control. These vigour assessments were incorporated into the mean *BS* and *TS* values calculated for each evaluation period.

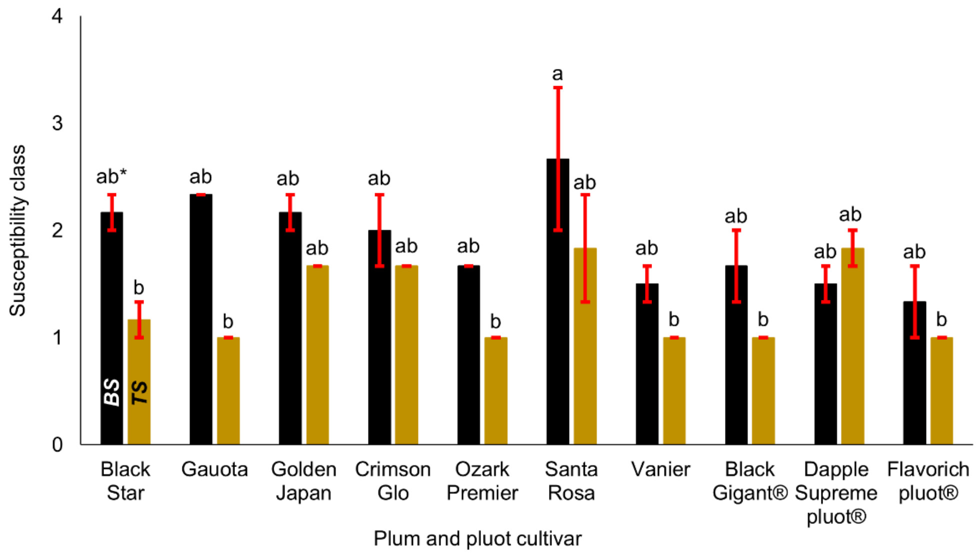

Figure 5. Comparison of the blossom (*BS*) and terminal shoot susceptibility (*TS*) classes in Japanese plum and pluot cultivars to *Pseudomonas syringae* determined in 2021–2023 in a netted house.

Each bar represents the mean ± SD of the susceptibility classes (1 – very low susceptibility; 2 – low susceptibility; 3 – moderate susceptibility; 4 – high susceptibility) to *Pseudomonas syringae*, evaluated in ten trees of each cultivar over the period 2021–2023; * indicates a statistically significant difference at the 0.05 level according to Fisher's *LSD* test.

Following blossom infection with *Pss*, a 'symptom-free period' persisted until the third evaluation date in week 6 for the low susceptible cultivars Golden Japan, Black Star, and Santa Rosa, when necrotic spots were observed on leaves and shoots near the inoculation site. In moderately susceptible plum varieties Black Star, Golden Japan, Santa Rosa, as well as in the almost moderately susceptible plum variety Gauota, the infection had spread to shoots near the inoculation site by the same evaluation date, which manifested itself in their gradual wilting. Following the application of the *Psm* inoculum to the blossom clusters, the interval between the onset of the hypersensitive reaction and the subsequent appearance of symptoms on the shoots was reduced. In the low susceptible plum cultivars Black Star, Gauota, Golden Japan, Crimson Glo and Santa Rosa, water-soaked lesions manifested immediately on the shoots below the cluster of inoculated blossoms. Subsequently, they appeared on the leaves from week 6 onwards, gradually drying up and the necrotic tissue falling off. In the moderately susceptible plum cultivar Santa Rosa, from week 12 onwards, the entire branches exhibited sudden desiccation, and the experimental plants demonstrated heightened susceptibility to water stress. The divergent post-hypersensitivity strategies exhibited by *Pss* and *Psm* in plant tissues, manifesting as differential rates of spread and intensity of infection symptoms on plant organs, are attributable to the presence of distinct effectors specific to individual pathovars (Hulin et al., 2018; Ruinelli et al., 2019; Santos et al., 2024). In accordance with the findings of several studies (Xin et al., 2018; Ruinelli et al., 2019; Chai et al., 2023), which highlight the critical role of high humidity in sustaining elevated epiphytic populations of *Ps* on plant surfaces and enhancing bacterial survival and infectivity in aerosols, the frequency and severity of *Pss* and *Psm* infection symptoms increased in 2022 due to springtime precipitation. Across most of the evaluated cultivars, a statistically significant correlation was observed between *BS* values and the year of evaluation, regardless of the pathovar involved.

The physiological response of plum and pluot trees to terminal shoot infection was slower than their response to blossom infection. This difference in response speed and intensity can be explained by the fact that blossoms, being more succulent and nutrient-rich, offer a high-moisture environment with elevated metabolic activity, which promotes rapid bacterial multiplication. (O'Malley & Anderson, 2021). Two-way ANOVA revealed that, for both *Ps* pathovars, there were no statistically significant differences in the *TS* classification of individual plum and pluot cultivars across the 2021 to 2023 growing seasons. The weaker response to varying seasonal conditions can be attributed to differences in tissue physiology compared to blossoms. Actively growing terminal shoots are highly susceptible to *Ps* infection, particularly under conditions of high humidity and bacterial concentrations exceeding 10^5 CFU/ shoot (Hulin et al., 2018; Mustafa et al., 2021; Broniarek-Niemiec et al., 2023). However, the gradual maturation of shoots, accompanied by increasing lignification and structural integrity, contributes to a more robust defense mechanism that slows the spread of *Ps* pathogens. Mature tissues often have lower metabolic activity and fewer nutrient resources, making them less favourable for bacterial proliferation (Xin et al., 2018; Huang et al., 2022. The differing mechanisms of infection dissemination, regardless of *Ps* pathovar resulted in statistically significant discrepancies between blossom susceptibility class and terminal shoot susceptibility class in LDS tests across Japanese plum and pluot cultivars.

The first clearly visible symptoms of infection, including shoot tip desiccation and the development of small necrotic lesions on leaves and terminal shoots, were observed on Santa Rosa trees six weeks after inoculation with *Psm* bacteria. This cultivar was classified as low susceptible (*TS* class 2), or as moderately susceptible (*TS* class 3) in 2023. Santa Rosa was the only cultivar that showed a clearly observable progression of infection throughout the 20-week evaluation period. By week 20, premature yellowing, leaf drop, and complete desiccation of the inoculated terminal shoots were recorded in its trees. Plum and plum cultivars Golden Japan, Crimson Glo and Dapple Supreme pluot® were predominantly classified in *TS* class 2, regardless of the *Ps* pathovar or year of evaluation. These cultivars consistently exhibited visible symptoms of infection, including dark necrotic spots on the inoculated terminal shoots and, later, on surrounding shoots, typically observed in week 12 or 20. Disease progression in these cultivars was slow and often subtle, remaining nearly unnoticed throughout the growing season. Such mild disease symptoms were detectable primarily due to the extended 20-week evaluation period and could easily go unnoticed under typical commercial orchard conditions. The delayed onset of symptoms, sometimes appearing up to 20 weeks after inoculation, suggests that although certain cultivars may initially appear resistant to severe disease, they gradually express susceptibility if environmental conditions continue to support pathogen proliferation (Popović et al., 2021; Jin et al., 2023). The extended evaluation period allowed for observations during late summer in a temperate climate, when decreasing temperatures created more favourable conditions for the multiplication and spread of *Ps* pathogens. The increasing concentration of *Ps* inoculum toward the end of the growing season may enhance its survival in subcortical tissues during dormancy and increase the risk of disease outbreaks in susceptible cultivars during the spring (Kostick et al., 2019).

This comprehensive three-year study, conducted under netted house conditions that closely simulated field environments, evaluated the vigour and susceptibility of promising Japanese plum and pluot cultivars to major bacterial pathogens in temperate climates (Kostick et al., 2019; Cui et al., 2021). These findings are essential for developing effective orchard management strategies to mitigate bacterial infections in stone fruit production.

CONCLUSIONS

- The plum and pluot cultivars tested showed higher susceptibility to blossom infection compared to terminal shoot infection and were more susceptible to bacterial strains of *Pseudomonas syringae* pv. *morsprunorum* than to *Pseudomonas syringae* pv. *syringae*.
- Notably, the plum cultivars Vanier and Ozark Premier, along with the pluot cultivar Flavorich pluot®, exhibited very low susceptibility to *Pseudomonas syringae* infection.
- Fruit cultivars resistant to *Pseudomonas syringae* pathogens are crucial for maintaining orchard health in temperate regions, due to the significant financial losses caused by the premature death of infected trees.

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