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ASSESSING RESISTANCE OF NEW APPLE CULTIVARS TO THE FIRE BLIGHT AGENT

SUMMARY

The selection of ten apple cultivars for causal agent of fire blight evaluation was based on their excellent agronomic parameters and strong commercial potential, across various sites. Their susceptibility to the *Erwinia amylovora* was evaluated under net house conditions from 2022 to 2024. Artificial inoculation methods included controlled application of a mixture of bacterial strains to blossoms and terminal shoots. 'Orange Crisp®' was consistently classified as very low susceptible (class 1) to blossom infection. Other cultivars varied, with the most common classification being low susceptibility (class 2), followed by moderate susceptibility (class 3). The positive control 'Gala' varied between class 2 and class 4. For terminal shoot infection, 'Orange Crisp®', 'Ghiva' and 'Lucy' were consistently classified as very low susceptible (class 1). Most new apple cultivars (60%) were low susceptible to blossom infection and very low susceptible to shoot infection. Only 'Allegro' was low susceptible to blossom infection and moderately susceptible to terminal shoot infection. The results obtained under net house conditions closely resemble those in field conditions. These results, combined with the cultivars' promising agronomic traits, strongly support their ongoing commercial potential in global apple-growing regions where *Erwinia amylovora* is a major limiting factor. Their resistance not only enhances marketability but also reduces the need for chemical treatments, promoting more sustainable and eco-friendly apple production.

Keywords: *Erwinia amylovora,* blossom infection, terminal shoot infection, new apple cultivars, net house

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INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora* (*Ea*, Burrill) Winslow *et al*., 1920, is one of the most destructive diseases affecting apple (*Malus* × *domestica*, Borkh.) and other members of the *Rosaceae* family. It induces shoot blight, necrosis, and tree decline, particularly in susceptible cultivars, leading to significant economic losses (Norelli *et al.* 2003; Sălceanu *et al.* 2022). The financial impact of fire blight is immense, with global losses estimated at billions of dollars annually (Norelli et al. 2003). Conventional control methods, such as antibiotic treatments and cultural practices, offer only limited and transient protection (Collinge *et al.* 2022, Li and Ahammed 2023). This highlights the urgent need for long-term, sustainable solutions.

The development of fire blight-resistant apple cultivars through breeding programs has emerged as a pivotal long-term strategy for addressing this issue (Baumgartner *et al*. 2015, Campa *et al.* 2024). Through genetic mapping and molecular marker analysis, researchers have pinpointed specific genomic regions and gene expressions associated with *Ea* resistance traits (Khan *et al.* 2007, Durel *et al.* 2009, Le Roux *et al.* 2010). These findings pave the way for breeding new, resilient apple varieties, ensuring disease-resistant and sustainable apple production (Kairova *et al.* 2023). However, successful new cultivars must also meet market demands, offering fruits that are not only resistant to diseases but also visually attractive, tasty, with excellent storability, and regular bearing (Morariu *et al.* 2025). In addition to these qualities, cold tolerance and adaptability to various soil conditions are crucial for growers (Mei *et al*. 2023, Saini *et al.* 2025). Breeding can enhance the economic traits of apple trees and revolutionize fruit production by developing new cultivars that are resistant to diseases such as fire blight. This would reduce reliance on harmful chemicals, improve environmental sustainability, and benefit both producers and consumers (Kostick *et al*. 2019).

A plethora of methodologies are available for assessing susceptibility *in vivo* (Paraschivu *et al.* 2020, Amashukeli *et al*. 2023). One intermediate approach involves the use of net houses, which offer a semi-controlled environment by providing protection from external infections while allowing exposure to temperature, humidity, and light fluctuations like field conditions (Denancé et al. 2018, Aleksandrova *et al*. 2023, Lienqueo *et al*. 2024). The susceptibility of fruit trees to bacterial infection is assessed by the percentage of necrotic flowers in infected clusters or the length of necrotic lesions in relation to the total length of the shoots (Paraschivu *et al.* 2020, Amashukeli *et al*. 2023). Some studies further investigate the secondary effects of infection on tree vigour and fruit production. The effectiveness of artificial inoculation depends on several critical factors, including bacterial strain virulence, inoculum concentration, temperature, humidity, and plant developmental stage (Amashukeli *et al.* 2023, Azarbad and Junke 2024). In the temperate zone, climate change is increasingly causing significant temperature fluctuations during dormancy and spring, affecting the vigour and susceptibility of fruit trees to pathogens (Fadón *et al*. 2020).

Additionally, plant tissues are more susceptible to pests when subjected to fertilisation and pesticide application (Husseini and Akköprü 2020, Tripathi *et al.* 2022; Aprile *et al.* 2021, Ahmed *et al*. 2024).

Several global apple breeding programs concentrate on developing apple cultivars resistant to fire blight (Peil *et al. 2*009, Emeriewen *et al.* 2017) however, only a few also address polygenic resistance to scab (Baumgartner *et al.* 2015). The aim of this study was to evaluate the resistance of advanced apple cultivars/new selections with market potential to fire blight, *Ea*, with a focus on both blossom and terminal shoot infections.

MATERIAL AND METHODS

The cultivars and new selections (Table 1) were propagated and closely observed during all evaluation periods at various stages of testing across Europe, South Africa and the United States in parallel. Dozens of agronomic parameters were assessed to support their practical application in both intensive plantings and the hobby market. The screening of resistance to *Ea* was conducted over four consecutive growing seasons (2021-2024). All cultivars were chip-grafted on virus-free M.9 T337 rootstocks. The saplings were planted in the autumn in 40-litre containers and placed in net house with drip irrigation.

Suspensions of virulent *Ea* strains CPABB 138, CPABB 234 and CPABB 237 (Collection of Phytopathogenic and Agriculturally Beneficial Bacteria, Czech Republic) in tap water at a concentration of 10^5 CFU ml^{-1} were mixed in equal ration and used for inoculation (Pánková *et al.* 2023).

One randomly selected open-flowered cluster at phenological stage 61-65 in 5 apple plants was sprayed with 1 ml of the mixed *Ea* suspension. In the other 5 experimental plants, the top of a randomly selected shoot at phenological stage 35-39 was cut below the first undeveloped leaf with scissors infected with the same *Ea* suspension. Control plants of 'Gala' cultivar were inoculated in the same manner. Blossom blight, shoot blight and other symptoms of progressive infection on other parts of the plants were assessed four times during the growing season. For each plant (i) the total number of plant organs with symptoms of *Ea* infection (Y_i), percentage of necrotic blossoms (NB_i) in the inoculated cluster (NB_i/TB_i) or the percentage of necrotic lesion (NS_i) on the inoculated terminal shoot (NS_i/TS_i) were calculated, the percentage of leaves with necrotic lesions (L_i), damaged fruit (F_i) and the decrease in total tree vigour (V_i) were determined (Pánková et al., 2023). For each term of assessment, the mean value of blossom susceptibility (BS_x) and terminal shoot susceptibility (TS_x) to *Ea* were calculated for each cultivar (x):

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

The highest calculated values BS_x and TS_x for *Ea* were transformed into 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 %) and the class of blossom and terminal shoot susceptibility to *Ea* for each cultivar in a given year was determined.

The effects of evaluation term, growing season and method of inoculation were assessed by analysis of variance (ANOVA) followed by post hoc Tukey test and Fisher's LSD test using TIBCO® Data Science / Statistica™ software, version 14.0.0.15 (StatSoft Inc., USA).

Table 1 Selected apple cultivars with a potential genetic predisposition to resistance against *Erwinia amylovora* in comparison with a positive control

Apple cultivar designation	Parent 1	Parent 2	Ancestor with resistance	Presumed quantitative trait locus	Reference
'UEB 0648/1' 'Orange Crisp®'	'Fuji'	'Heliodor'	'Delicious' s.l.	FLO5 FLO10	Le Roux et al., 2010
'UEB 4536/1' 'Camelot'	'UEB 3317/1'*	'Mira'	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB 4702/1' 'Ellipso®'	'UEB 3317/1'*	'Mira'	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB 5060/1' 'Lyra'	'Barby'	'SQ 159'	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB I-20/1' 'Rubelit'	'Topaz'	'UEB 2732/2'**	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB 3802/6' 'Allegro'	'Julia'	'Ametyst'	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB I-406/1' 'Bonita'	'Topaz'	'Cripps Pink'	?		
'UEB I-647/1' 'Ghiva'	'Fuji'	'Heliodor'	'Delicious' s.l.	FLO5 FLO10	Le Roux et al., 2010
'UEB I-18/1' 'Telse'	'Topaz'	'Elstar'	'Cox's Orange Pippin'	FB_F7	Khan et al., 2007
'UEB I-181/3' 'Lucy'	'Topaz'	'Fuji'	'Delicious' s.l.	FLO5 FLO10	Le Roux et al., 2010
'Gala'	'Golden Delicious'	'Kidd's Orange Red'	'Delicious' s.l. 'Cox's Orange Pippin'	FLO5 FLO10 FB_F7	Le Roux et al., 2010; Khan et al., 2007

* 'UEB 3317/1' = ('Vanda' x 'Bohemia'); ** 'UEB 2732/2' = ['UEB 1477/1' ('Jolana' x 'Rubín') x 'UEB 1052/5' ('Linda' x 'Kidd's Orange Red')], s.l. = *sensu lato*

For testing purposes, alongside the widely commercialized 'Gala' cultivar as a positive control, 10 new apple cultivars resistant to scab were selected from

the apple breeding program of the Institute of Experimental Botany of the Czech Academy of Sciences, v.v.i. (IEB). These cultivars are in various stages of advanced testing or are often already being commercially implemented in the Czech Republic and abroad.

'Bonita'

A cultivar introduced for intensive apple orchards in 2015, 'Bonita' has quickly gained popularity. Its bright red fruits are not only attractive but also characterized by high and regular yields. Over 1.5 million trees have already been planted, especially in Europe, South Africa, and the USA. The assessment of resistance or susceptibility to fire blight can significantly impact the cultivation of 'Bonita' in areas with a high risk of fire blight outbreaks (e.g., Germany, Switzerland, South Tyrol, USA).

'Rubelit'

This cultivar is easy to grow and produces delicious, well-stored fruit. 'Rubelit' has been established in Austria, Italy, Switzerland, and the Czech Republic, where several tens of thousands of trees have been planted so far.

'Ghiva'

The 'Ghiva' cultivar is notable for its exceptional flesh firmness, high sugar content, and long storability. Its fruits are ideal for transport as they are resistant to bruising. 'Ghiva's' potential lies in Poland, where the planting of approximately 0.5 million trees began in 2024. Its highly transportable fruits, resistant to bruising, have a promising future in the southeastern global markets, where sweet-tasting fruit is significantly preferred.

'Orange Crisp®'

The cultivar 'Orange Crisp®' is marketed under the brand name 'Orange Crisp®'. Its orange-red fruits are characterized by crispy flesh, excellent taste, and good storability – qualities particularly demanded in Western markets.

'Lucy'

An alternative to the globally successful 'Fuji' cultivar, 'Lucy' offers earlier harvest maturity, a balanced sugar and acid content, and monogenic resistance to scab. It has so far found use mainly in Switzerland and the Czech Republic, where several tens of thousands of trees have been planted.

'Ellipso®' ('UEB 47021'), 'Camelot', and 'Lyra'

The newest cultivars from the apple breeding program. 'Ellipso®' ('UEB 47021') produces dark red fruits, 'Camelot' offers bicolored fruits, and 'Lyra' produces yellow fruits. All of them are characterized by polygenic resistance to scab and are particularly suitable for organic farming. Their market potential is especially expected in Central Europe, both in intensive orchards and domestic gardens.

'Allegro'

A summer cultivar with polygenic resistance to scab. 'Allegro' has smaller to medium-sized bicolored fruits, a sweet taste, and relatively good storability for a summer apple. It is mainly grown in Central Europe (Czech Republic, Poland, Slovakia, Hungary, Switzerland, and Germany).

'Telse'

Telse offers a resistant alternative to the Elstar cultivar, which is especially popular in Benelux countries and Germany. Its fully aromatic taste makes it attractive, but it requires regular thinning of fruits to achieve consistent yields. Its fruits are not intended for long storage, which currently limits its wider application.

RESULTS AND DISCUSSION

The results of the evaluation of the susceptibility of ten apple cultivars to blossom infection of *Ea* in year 2022-2024 are summarised in Table 2.

Table 2 Blossom susceptibility of apple cultivars to *Erwinia amylovora* in a net house (2022-2024)

	Blossom susceptibility in apple cultivars											
Evaluation term	Week / 2022				Week / 2023				Week / 2024			
Apple cultivar	2.[c*]	4.[b]	8.[b]	12.[a]	2.[c]	4.[bc]	8.[ab]	12.[a]	2.[c]	4.[bc]	8.[ab]	12.[a]
'Orange Crisp®'	1.6 ± 1.4	6.9 ± 7.7	6.3 ± 7.6	12.2 ± 10.1	1.3 ± 4.0	4.0 ± 3.5	5.0 ± 10.0	10.0 ± 10.0	2.3 ± 1.0	3.0 ± 3.0	3.0 ± 3.0	10.0 ± 3.0
'Camelot'	0.0 ± 0.0	3.0 ± 9.0	4.9 ± 5.0	5.8 ± 5.7	1.0 ± 1.9	1.0 ± 2.0	3.0 ± 2.0	24.0 ± 3.0	10.5 ± 9.4	10.0 ± 10.0	10.0 ± 3.0	25.0 ± 20.0
'Ellipso®'	0.0 ± 0.0	2.5 ± 3.8	5.4 ± 9.1	16.5 ± 7.9	2.6 ± 1.8	15.0 ± 5.0	25.3 ± 5.7	46.8 ± 1.5	11.9 ± 9.7	27.0 ± 20.0	10.0 ± 10.0	8.0 ± 3.0
'Lyra'	0.5 ± 1.5	0.9 ± 2.5	1.0 ± 2.0	4.3 ± 6.0	3.3 ± 3.0	6.9 ± 5.0	7.0 ± 5.0	10.0 ± 10.0	17.1 ± 3.4	20.0 ± 10.0	20.0 ± 10.0	30.0 ± 10.0
'Rubelit'	0.5 ± 0.5	9.5 ± 1.5	20.0 ± 2.0	48.3 ± 6.0	5.8 ± 0.6	10.0 ± 10.0	20.0 ± 15.0	30.0 ± 20.0	12.5 ± 5.5	17.0 ± 3.0	20.0 ± 13.0	30.0 ± 30.0
'Allegro'	2.0 ± 4.0	9.6 ± 2.6	25.5 ± 9.6	26.2 ± 23.9	5.9 ± 4.0	17.8 ± 2.0	28.0 ± 2.0	50.0 ± 2.0	6.8 ± 8.0	31.8 ± 14.8	40.0 ± 40.0	50.0 ± 30.0
'Bonita'	3.1 ± 1.9	3.8 ± 3.0	11.0 ± 7.0	12.1 ± 10.0	10.0 ± 5.0	17.0 ± 3.0	21.0 ± 16.0	25. ± 10.0	10.0 ± 2.3	9.0 ± 1.0	20.0 ± 10.0	21.0 ± 10.0
'Ghiva'	0.5 ± 0.5	6.9 ± 6.4	7.2 ± 6.4	7.3 ± 7.1	3.0 ± 2.0	4.0 ± 3.0	18.0 ± 10.0	30.0 ± 20.0	4.0 ± 1.2	5.0 ± 1.0	10.0 ± 10.0	10.0 ± 10.0
'Telse'	0.8 ± 0.5	8.2 ± 5.6	8.7 ± 6.1	15.5 ± 9.5	5.0 ± 5.0	5.0 ± 5.0	18.0 ± 10.0	25.0 ± 20.0	9.1 ± 7.8	34.3 ± 30.6	40.0 ± 30.0	50.0 ± 30.0
'Lucy'	0.0 ± 0.0	0.0 ± 0.0	9.5 ± 8.5	9.6 ± 1.2	10.0 ± 5.0	10.0 ± 5.0	17.0 ± 3.0	20.0 ± 15.0	7.9 ± 0.2	21.5 ± 3.1	30.0 ± 30.0	45.0 ± 30.0
'Gala'	2.5 ± 2.1	7.6 ± 3.5	10.2 ± 7.8	18.9 ± 11.8	5.5 ± 2.1	27.0 ± 3.0	38.2 ± 2.8	65.0 ± 15.0	6.7 ± 1.1	6.0 ± 5.0	16.0 ± 3.0	22.0 ± 13.0

***** Weeks followed by the same letters within each year are not significantly different ($P < 0.05$) according to the post hoc Tukey's test

During the three-year trial, the first symptoms of blossom blight were observed 5-7 days after artificial inoculation in almost all apple cultivars tested. *BS* values at week 2 of the trial ranged from 0 ('Camelot', 'Ellipso®', 'Lucy') to 17.1 for 'Lyra' in 2024. The first symptoms of infection, which progress to shoots, leaves and fruits around the inoculated flower clusters, were observed 3-8 weeks after inoculation, depending on the year of the trial and the apple cultivar (Fig. 1a-d).

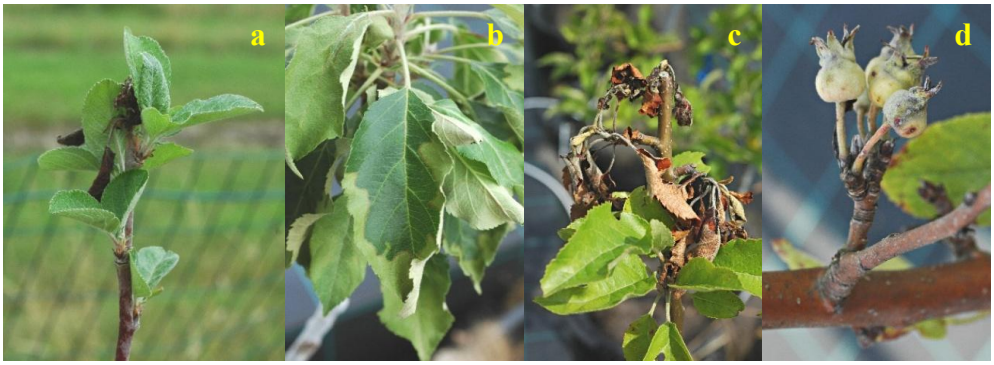

Fig. 1 Symptoms of fire blight on apple cultivars after artificial inoculation of randomly selected blossom clusters with a mixture of *Erwinia amylovora* bacterial strains

(**a**) Blossom blight on the apple cultivar 'Bonita' 10 days after artificial inoculation. (**b**) Discoloured leaf margins on the apple cultivar 'Bonita' at week 4. (**c**) Necrotic terminal shoot on the apple cultivar 'Telse' at week 8. (**d**) Dwarfed infected fruits on the apple cultivar Bonita cultivar 8 weeks after inoculation

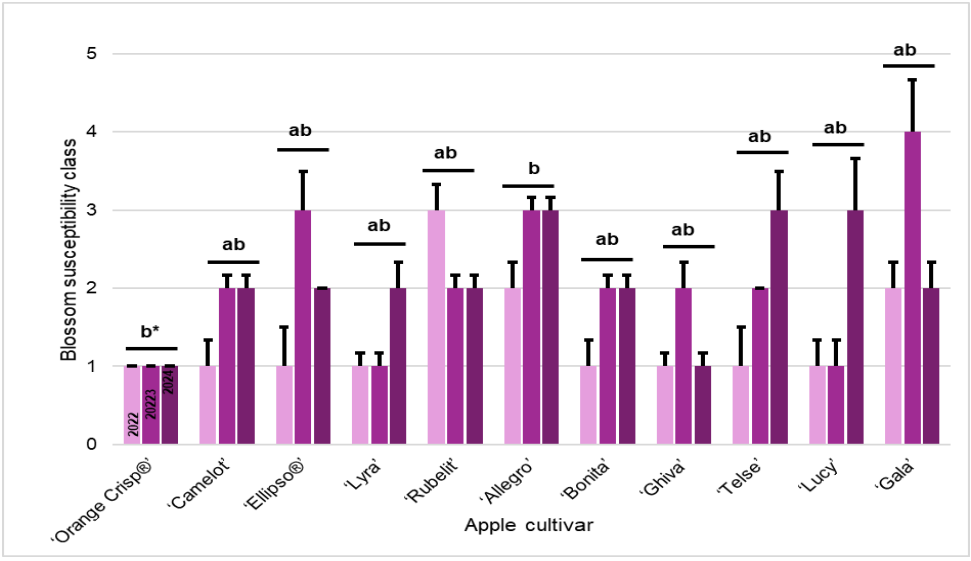

Fig. 2 Comparison of blossom susceptibility (*BS*) classes in 10 apple cultivars to the causal agent of fire blight, *Erwinia amylovora*, evaluated on potted trees in a net house from 2022 to 2024

Each bar represents annual *BS* class ± SD, calculated from the mean of three replicates determined within the 2022-2024 study; * groups of bars labelled with the same letters are not significantly different at the 0.05 level according to Fisher's LSD test

The development of disease symptoms in 80 % of apple cultivar and positive control continued until week 12, according to *BS* values (Table 2). At week 12, the *BS* values in these cultivars ranged from 5.5 to 50.0, while the

positive control ranged from 18.9 to 65.0. Exceptionally, in 'Ghiva', the *BS* value remained unchanged from week 8, and in 'Ellipso®', the *BS* value decreased at week 12 due to the observed revitalisation process. According to the determined susceptibility class to *Ea,* 'Orange Crisp®' was classified as very low susceptible (class 1) to blossom infection throughout the trial (Fig. 2). The other cultivars were classified into different susceptibility classes during the study. The most common classification was low susceptibility in class 2 (11 times), followed by class 1 and moderate susceptibility in class 3 (6 times). The positive control varied between class 2 and class 4 throughout the study. The effect of the vegetation season was statistically significant ($F = 0.43$, $n = 3$, $P < 0.05$).

First symptoms of artificial infection on terminal shoots were observed as brown or black lesions around the place of inoculation in week 4 in all apple cultivar tested in year 2022 and 2024. In year 2023 first symptoms were observed in week 2. The *TS* values ranged from 0 in 'Camelot', 'Allegro', 'Ghiva' to 10 in cultivar 'Bonita', 'Telse', 'Lucy' (Table 3).

Table 3 Terminal shoot susceptibility of apple cultivars to *Erwinia amylovora* in a net house (2022-2024)

Terminal shoot susceptibility in apple cultivars												
Evaluation term	Week / 2022				Week / 2023				Week / 2024			
Apple cultivar	2.^{c*}	4.^{b}	8.^{b}	12.^{a}	2.^{c}	4.^{bc}	8.^{b}	12.^{a}	2.^{c}	4.^{b}	8.^{b}	12.^{a}
'Orange Crisp®'	0.0 ± 0.0	5.4 ± 7.9	10.8 ± 4.0	18.0 ± 17.8	1.5 ± 3.2	5.8 ± 17.3	10.0 ± 0.0	10.0 ± 0.0	0.0 ± 0.0	3.0 ± 6.4	4.1 ± 2.5	9.1 ± 8.5
'Camelot'	0.0 ± 0.0	1.3 ± 4.2	3.7 ± 6.3	4.7 ± 5.4	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 3.7	25.0 ± 4.5	0.0 ± 0.0	9.0 ± 8.9	9.1 ± 8.5	9.1 ± 8.5
'Ellipso®'	0.0 ± 0.0	0.2 ± 0.6	1.7 ± 3.2	2.0 ± 3.9	1.0 ± 3.0	1.0 ± 3.0	14.5 ± 2.7	25.0 ± 4.5	0.0 ± 0.0	3.0 ± 6.4	5.1 ± 2.5	5.6 ± 5.0
'Lyra'	0.0 ± 0.0	0.6 ± 1.5	3.0 ± 2.3	7.0 ± 6.7	3.0 ± 4.6	8.3 ± 4.2	15.3 ± 3.2	15.5 ± 15.0	0.0 ± 0.0	19.0 ± 18.7	29.1 ± 8.5	29.1 ± 18.5
'Rubelit'	0.0 ± 0.0	1.8 ± 1.5	10.0 ± 5.4	13.0 ± 4.5	2.5 ± 2.5	16.3 ± 8.3	5.5 ± 5.0	35.0 ± 34.0	0.0 ± 0.0	3.0 ± 3.0	3.1 ± 2.5	3.1 ± 2.5
'Allegro'	0.0 ± 0.0	2.2 ± 2.0	4.9 ± 4.0	7.0 ± 6.4	0.0 ± 0.0	10.0 ± 9.0	25.3 ± 4.6	45.0 ± 5.0	0.0 ± 0.0	0.0 ± 0.0	10.0 ± 10.0	10.1 ± 8.5
'Bonita'	0.0 ± 0.0	3.5 ± 3.5	4.2 ± 4.0	6.0 ± 5.0	10.0 ± 9.0	23.0 ± 9.0	28.5 ± 7.6	50.0 ± 28.0	0.0 ± 0.0	12.0 ± 12.0	19.1 ± 8.5	21.1 ± 8.5
'Ghiva'	0.0 ± 0.0	3.0 ± 3.0	3.9 ± 3.6	5.0 ± 3.9	0.0 ± 0.0	4.5 ± 4.5	8.0 ± 5.0	15.0 ± 2.0	0.0 ± 0.0	3.0 ± 2.4	3.1 ± 2.5	3.1 ± 2.5
'Telse'	0.0 ± 0.0	4.9 ± 2.1	9.9 ± 4.3	19.0 ± 8.3	10.0 ± 9.0	10.0 ± 9.0	14.5 ± 8.9	30.0 ± 10.0	0.0 ± 0.0	1.0 ± 1.0	22.1 ± 17.5	10.1 ± 8.5
'Lucy'	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.5	3.0 ± 1.3	10.0 ± 3.0	11.0 ± 2.9	12.0 ± 2.9	15.0 ± 2.4	0.0 ± 0.0	0.0 ± 0.0	12.0 ± 10.0	9.1 ± 2.5
'Gala'	0.0 ± 0.0	4.3 ± 0.7	4.6 ± 3.5	16.0 ± 7.8	3.0 ± 0.4	17.8 ± 5.3	27.8 ± 7.5	43.0 ± 37.5	0.0 ± 0.0	8.0 ± 7.5	9.1 ± 2.5	9.1 ± 2.5

* Weeks followed by the same letters within each year are not significantly different ($P < 0.05$) according to the post hoc Tukey's test

From week 4 onward, *Ea* was spread from the site of infection through the plants (Fig. 3 a-d).

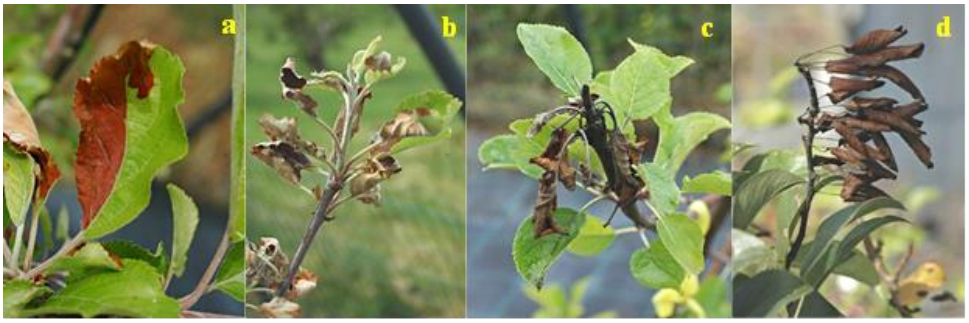

Fig. 3 Symptoms of fire blight on apple cultivars after artificial inoculation of randomly selected terminal shoots with a mixture of *Erwinia amylovora* bacterial strains

(**a**) Symptoms on the apple cultivar 'Telse' 4 weeks after artificial inoculation. (**b**) Wilting terminal shoot on the apple cultivar 'Telse' 8 weeks after inoculation. (**c**, **d**) Necrotic terminal shoots at week 12 on the apple cultivars 'Lyra' and 'Bonita,' respectively

The progression is expressed through slowly increasing, stagnating, or even decreasing *TS* values, depending on the cultivar (Table 3). The *TS* values ranged from 2 to 50 at week 12. For the positive control cultivar 'Gala', the highest *TS* value at week 12 was 43. 'Orange Crisp®', 'Ghiva', 'Lucy' were classified as very low susceptible (class 1) to terminal shoot infection throughout the trial (Fig. 4).

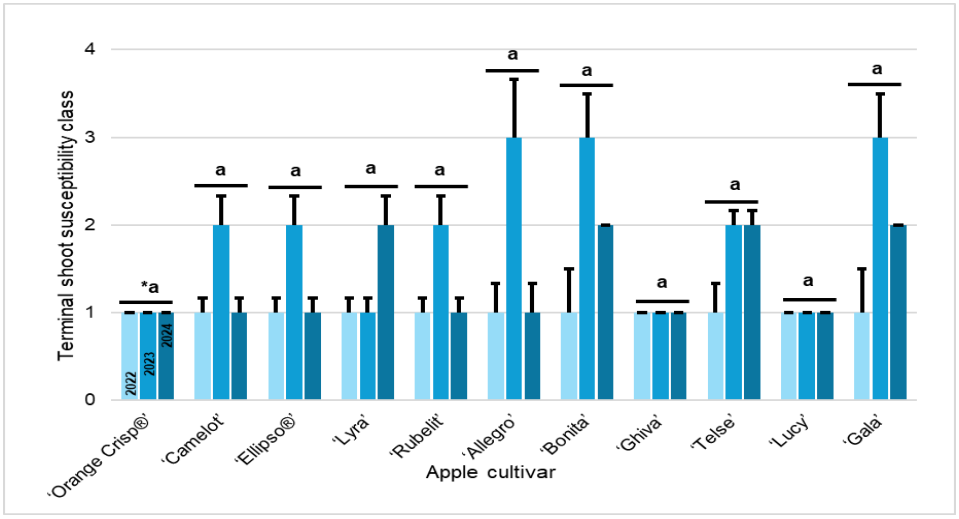

Fig. 4 Terminal shoot susceptibility (*TS*) classes in 10 apple cultivars to the causal agent of fire blight, *Erwinia amylovora*, evaluated on potted trees in a net house from 2022 to 2024

Each bar represents annual *TS* class ± SD, calculated from the mean of three replicates determined within the 2022-2024 study; * groups of bars labelled with the same letters are not significantly different at the 0.05 level according to Fisher's LSD test

Other tested cultivars and the positive control varied among class 1, class 2 and class 3 throughout the trial. The highest *TS* classes were determined in year 2023 (Fig. 4). The effect of the vegetation season was statistically significant (F = 0.59, n = 3, P < 0.05). 'Orange Crisp®' was classified as having very low susceptibility, while 'Lyra' and 'Telse' were determined to have low susceptibility to *Ea* infection in both blossoms and terminal shoots (Fig. 5). Most of the new apple cultivars (60 %) were low susceptible to blossom infection and very low susceptible to shoot infection, like the positive control. Only 'Allegro' was low susceptible to blossom infection and moderately susceptible to terminal shoot infection.

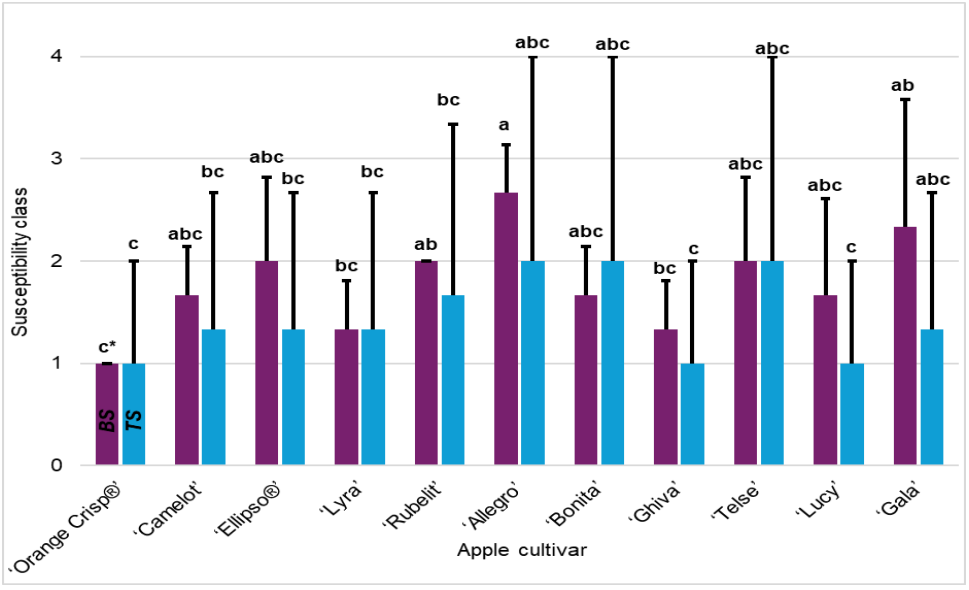

Fig. 5 Comparison of blossom (*BS*) and terminal shoot (*TS*) susceptibility classes of apple cultivars to causal agent of fire blight, *Erwinia amylovora*

Each bar represents the mean ± SD of three replicates of susceptibility class determined in the 2022–2024 study; * bars assigned the same letters are not significantly different at the 0.05 level according to Fisher's LSD test

DISCUSSION

This study contributes to ongoing breeding efforts by identifying new apple cultivars with improved resistance to the major pathogens and with high potential for application in agricultural practice globally. The three-year study evaluating *Ea* susceptibility in apple cultivars revealed critical insights into infection dynamics and resistance mechanisms. Artificial inoculation of blossoms and terminal shoots under net house conditions demonstrated genotype-specific variation in disease progression, consistent with *Ea*'s known pathogenesis strategies such as the production of phytotoxins and the utilization of the type III secretion system (T3SS) to inject effector proteins into host cells, which are vital for the suppression of plant immune responses (Hulin *et al.* 2018). This mechanism was particularly following the inoculation of blossom clusters by an

almost identical hypersensitive reaction. Initial symptoms in blossoms (5–7 days post-inoculation) and shoots (2–8 weeks) aligned with *Ea*'s rapid colonization via nectaries or wounds. The observed "symptom-free period" after the hypersensitive reactions to artificial inoculation has disappeared, correlates with necrotrophic survival of *Ea* in dead tissue. *Ea* also maintains viability through biofilm formation and oxidative stress resistance mechanisms (Santander and Biosca 2017, Sobiczewski *et al*. 2017). Such adaptations allow the formation of reservoirs for subsequent seasonal infections and overwintering in necrotic tissues, (Santander and Biosca 2017). This "symptom-free period" varies in length depending on the susceptibility of the cultivars and weather conditions in the given year. The physiological response to shoot infection was observed to be slower than that of blossoms, which are more succulent and possess a nutrient-rich environment conducive to rapid bacterial multiplication due to higher moisture content and metabolic activity (O'Malley and Anderson 2021).

Genotypic differences in susceptibility were evident when comparing 'Orange Crisp®', which showed consistent resistance (*BS*/*TS* class 1) throughout the trial, while others varied. The anticipated outcome was supported by field trials in areas with the natural occurrence of *Ea* in orchards (unpublished data from trials in Switzerland and Germany) and the genetic origin of resistance loci donors such as *Malus floribunda* 821 (Durel *et al*. 2009) and 'Red Delicious' (Le Roux *et al*. 2010). Furthermore, the assumption was also confirmed in the sibling cultivar 'Ghiva', which similarly exhibited increased resistance (predominantly *BS*/*TS* class 1). Interestingly, for both most resistant cultivars, the maternal variety was 'Fuji', generally considered to be moderately resistant (Khan et al. 2007, Kostick *et al*. 2019), whereas for the moderately resistant 'Lucy', it was at the paternal level. The second parent was always the moderately susceptible 'Topaz' (Forejtová *et al.* 2024, Khan *et al*. 2007) or 'Heliodor', a descendant of the moderately susceptible 'Golden Delicious' and 'Topaz' (Khan *et al.* 2007, Kostick *et al*. 2019). On the other hand, both blossom and shoot *Ea* susceptibility of cultivars like 'Rubelit', 'Allegro' do not suggest the expected quantitative trait locus (QTL) genetic predisposition to fire blight resistance from 'Cox's Orange Pippin' (Khan *et al.* 2007). Shoot infections showed annual variability, with *TS* values stagnating or declining after week 8 in some cultivars, suggesting partial resistance mechanisms. This variance aligns with observations that *Ea* employs a type III secretion system (T3SS) to deliver effector proteins into host cells, modulating plant immunity and virulence (Hulin *et al.* 2018, Zhao *et al*. 2022). Notably, 'Allegro's divergent susceptibility (low in blossoms, moderate in shoots) underscores tissue-specific defence responses, potentially linked to differential expression of host-regulated *Ea* genes involved in effector-triggered immunity (ETI) or other defence pathways across different tissues during infection (Zhao *et al.* 2022). The control cultivar 'Gala' confirmed the expected susceptibility (*BS* up to 65.0, *TS* up to 43.0), indicating the use of a virulent and viable *Ea* inoculum capable of epiphytic growth on stems and systemic spread via

xylem, as well as the suitability of the chosen method for evaluating the susceptibility of new apple cultivars to blossom and shoot blight.

The symptom progression observed from localized necrosis to systemic spread parallels *Ea*'s staged infection strategy (blossom → shoot → tree). However, even though, controlled net house conditions may underestimate field complexities, such as pollinator-mediated stigma colonization or overwintering in holdover cankers, the low QTL-dependent phenotypic variation of *Ea* resistant response is also problematic in breeding for fire blight resistance (Durel *et al.* 2009). Integrating multi-year phenotypic data with molecular analyses (e.g., high depth sequencing, diagnostic marker development) could refine resistance breeding programs, particularly for the offspring of cultivars like 'Orange Crisp™' and 'Lucy', which exhibit durable low susceptibility. This underscores the need to pair artificial inoculation with field trials capturing *Ea*'s ecological adaptability. The evaluation of new apple cultivars under net house conditions reveals promising resistance profiles against *Ea*, with all tested cultivars demonstrating lower susceptibility compared to the positive control ('Gala'). Notably, cultivars classified as very low included in class 1, including 'Orange Crisp®', 'Ghiva', 'Lucy' and those with low susceptibility in class 2, such as 'Telse', exhibit resistance levels comparable to or exceeding those reported in recent laboratory and field trials conducted by collaborating institutions and commercial partners, particularly in Switzerland and Germany.

CONCLUSIONS

Altogether, 60% of the tested cultivars were classified as having low susceptibility to blossom infection and very low susceptibility to shoot infection by *Erwinia amylovora*, indicating strong potential for disease-resilient apple production. The apple cultivars 'Orange Crisp®', 'Ghiva', and 'Lucy' consistently exhibited very low susceptibility (class 1) to both blossom and terminal shoot infections, making them top candidates for cultivation in fire blight-prone regions.Resistance traits observed align with known genetic backgrounds, particularly in cultivars derived from resistant ancestors like *Malus floribunda* 821 and 'Red Delicious'. These apple cultivars offer strong commercial potential in global markets due to their reduced need for chemical treatments and their ability to promote sustainable, eco-friendly apple production.

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