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Screening of *Venturia inaequalis* virulence and resistance of *Malus* genotypes to apple scab using *in vitro* methodology

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Abstract. The apple scab induced by *Venturia inaequalis* is an economically significant disease of apples worldwide and is predominantly controlled by multiple fungicide applications. Therefore, resistant apple cultivation is important for long-standing disease control. The knowledge about cultivar resistance is mainly founded on their evaluation in orchard conditions and testing in a greenhouse that is laborious and requires a large space. This study evaluated apple cultivar resistance and the virulence of *V. inaequalis* strains by inoculating detached leaves and immature fruits. Nine *V. inaequalis* strains originating from different apple-growing regions and host genotypes were tested on eight apple genotypes. Microscopic and macroscopic symptom development and host tissue reactions were monitored during the experiments. The tested *V. inaequalis* strains and cultivars showed different levels of virulence and resistance, respectively. Cultivar 'Lobo' was scored as partially susceptible, with almost all strains tested. The incompatible interaction with or without host tissue reactions was observed only on the cultivar 'Priscilla' with all the strains and field populations tested. The results of this study using detached leaves and immature fruits were concordant with the data obtained in the cultivar evaluation trials in the orchards.
Key words: detached leaves, immature fruits, pathogenic fungi, plant disease

Introduction

The apple (*Malus domestica* (Suckow) Borkh., family *Rosaceae*) is an economically important and widely grown pome fruit species in temperate regions worldwide (O'Rourke, 2003). *Venturia inaequalis* (Cooke) G. Winter induces the apple scab, which is one of the most important apple diseases that may lead to substantial economic losses worldwide due to a decrease in apple yields and fruit quality up to 70% (Sivanesan, 1977; MacHardy, 1996; Schubert, Ritschel, & Braun, 2003; Biggs & Stensvand, 2014). Successful apple scab control relies on several fungicide sprays per season. Therefore, regular fungicide applications during the growing season are needed in almost all cultivation areas, both with traditional and integrated growing systems (Beresford & Manktelow, 1994; Holb, 2006). Even in integrated apple cultivation systems, fungicides are used more than ten times a season to control the spread of scabs; otherwise, without disease control measures, crop losses can reach up to 100% (Demeyere & De Turck, 2002; Soriano *et al*., 2009). In Latvia, 4–6 sprays are usually applied for apple scab control in orchards during the growing season (Rancane, Eihe, & Jankovska, 2008; Rancane, Vilka, & Bankina, 2013).

Successful apple scab control relies on fungicide sprays and growing scab-resistant cultivars essential for long-lasting disease control (MacHardy, 1996; Rossi, Giogue, & Bugiani, 2007; Holb, 2009; Höfer *et al*., 2021). An evaluation of apple genotype resistance to scab in orchard conditions is the primary method used. A favourable environment is needed for the plant pathogen to infect plants successfully (Francl, 2001). During vegetation, humid weather (air humidity around 99 %) is mainly needed for *V. inaequalis* spore germination and host infection (Gottleb, 1950; MacHardy & Gadoury, 1989; MacHardy, 1996). However, climatic conditions are unstable and uncontrollable, making it challenging to interpret cultivar resistance in the field. In addition, the infection background of the pathogen is difficult to quantify, and the pathogen's virulence can differ among the different

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regions (Sandskär & Liljeroth, 2005; Patocchi *et al*., 2020; Sokolova & Moročko-Bičevska, 2022). Tests in the greenhouse using an artificial inoculation of plants with the selected *V. inaequalis* strains are the standard technique for investigating the pathogen's virulence and host resistance (Chevalier *et al*., 2004; Chevalier *et al*., 2008; Lê Van *et al.,* 2011; Caffier *et al*., 2015). Although the establishment of inoculation experiments for the evaluation of cultivar resistance and pathogen virulence in a greenhouse is laborious and requires a large space and a quantity of inoculum, it provides the necessary favourable conditions for the disease development and allows the quantification of the pathogen's inoculum (Yepes & Aldwinckle, 1993a, b; Chevalier *et al*., 2004; Chevalier *et al*., 2008). Along with greenhouse experiments, *in vitro* experiments have been carried out using detached leaves, leaf discs and detached fruits to study the interaction of the *Venturia* species with their hosts (Yepes & Aldwinckle, 1993b; Bénaouf & Parisi, 1998; Stehmann, Pennycook, & Plummer, 2001; Caffier *et al*., 2012; Sokolova & Moročko-Bičevska, 2022). In this study, apple cultivar resistance and the virulence of *V. inaequalis* strains were evaluated by inoculating detached leaves and immature fruits with *V. inaequalis* strains originating from different geographic regions and host genotypes.

Materials and Methods

Plant material

In total, five individual tests (further noted with identification numers I, II, III, IV and V) were performed by inoculating young detached apple leaves and immature fruits from 2013 to 2016. Eight *Malus* genotypes with different origins and scab-resistance levels were selected for the study (Table 1). They included common commercial apple cultivars grown in Latvia ('Auksis', 'Beloruskoye Malinovoje', 'Lobo'), locally bred, scab-resistant cultivar 'Edite', common apple cultivars grown in other regions ('Golden Delicious', 'Priscilla', 'Gala') and ornamental apple *M. toringo*.

Young leaves and immature fruits were collected in the second half of June (Experiments IV, V) and the first half of July (Experiments I, II, III). The phenology of plants corresponded to development stages 33 (leaves) and 74-74 (fruits), according to the BBCH scale (Meier *et al*., 2001). Plant material was collected from trees grown in the germplasm collection at the Institute of Horticulture (Ceriņi, Doble, Latvia) without fungicide sprays. Apple leaves were tested in two (Experiments I, III) and fruits in three (Experiments II, IV, V) inoculation experiments. In each experiment, depending on availability in each year, two to five fruits and leaves

Table 1

***Malus* genotypes tested in this study, their origin and tolerance to *Venturia inaequalis* in field conditions in Latvia**

Genotype	Origin	Apple scab tolerance in the orchards in Latvia/ known resistance gene	Tested in experiments	
			fruits (I, II, V)	leaves (I, III)
Auksis	Lithuania	Partially resistant[d, e] / nd	I, V	I
Beloruskoye Malinovoye	Belarus	Partially resistant[d, e] / *Rvi13*[f]	II, V	I
Edite	Latvia	Resistant[g] / *Rvi6*[h]	II, IV, V	I, III
Gala[a]	New Zealand	Susceptible[e] / none[a]	II, IV	III
Golden Delicious[b]	United States	Partially susceptible[e] / *Rvi1*[b]	II, IV, V	III
Lobo	Canada	Susceptible / none[f]	II, IV, V	I, III
Malus toringo	Japan	Partially susceptible / nd	II, IV, V	I, III
Priscilla[c]	United States	Resistant[e] / *Rvi6*[c]	II, IV, V	III

[a] Differential host h(0) (Bus *et al*., 20011; Caffier *et al*., 2015).
[b] Differential host h(1) (Bus *et al*., 2011; Caffier *et al*., 2015).
[c] Differential host h(6) (Bus *et al*., 2011; Caffier *et al*., 2015).
[d] Grantiņa-Ieviņa *et al.*, 2015.
[e] According to observations in the orchards in Latvia, L. Ikase, personal communication.
[f] According to screening with molecular markers, G. Lācis, personal communication.
[g] Ikase *et al.*, 2022.
[h] Zelmene *et al.*, 2022.
[i] O. Sokolova, unpublished.
nd - no data.

Table 2

***Venturia inaequalis* strains and field populations tested in this study**

Strain identification number (race)	Host of origin	Organ	Habitat, location, country	Tested in experiments	
				on fruits (II, IV, V)	on leaves (I, III)
1634 (1, 4)[a, b]	TSR33T239[d]	nd	nd, Beaucouze, France	IV, V	III
2408 (7, 8)[a, b]	W188B	nd	nd, Havelock North, New Zealand	IV, V	III
147 (5, 7)[a, b]	9-AR2T196[e]	nd	nd, Beaucouze, France	IV, V	III
333[a, c]	TSR34T15[f]	nd	nd, nd, Germany	II, IV, V	I, III
M1.1.1	Gala[g]	fruit	Commercial orchard, Warsaw vicinity, Poland	II, V	I
M137.1.1	Auksis	fruit	Commercial orchard, Tērvete reg., Latvia	II, V	I
M139.1.1	Lobo	fruit	Commercial orchard, Talsi reg., Latvia	II, V	I, III
M142.1.1	Lobo	fruit	Commercial orchard, Kocēni reg., Latvia	II, V	III
M146.2.21	*M. toringo*	leaf	Experimental orchard: Greenery, Dobele reg., Latvia	II, V	I, III
Positive control	Field population, various genotypes	leaves, fruits	Germplasm collection, Dobele reg., Latvia	II, IV, V	I, III

[a] Reference strains obtained from Dr. Valérie Caffier, INRA, France, within Vinquest network (http://www.vinquest.ch/index.html).
[b] According to Bus *et al*., 2011; Caffier *et al*., 2015.
[c] Isolated from host h(2), V. Caffier, personal communication.
[d] Differential host h(4) (Bus *et al*., 2011; Caffier *et al*., 2015).
[e] Differential host h(5) (Bus *et al*., 2011; Caffier *et al*., 2015).
[f] Differential host h(2) (Bus *et al*., 2011; Caffier *et al*., 2015).
[g] Differential host h(0) (Bus *et al*., 2011; Caffier *et al*., 2015).
nd – no data.

were infected for each combination of *V. inaequalis* strain and genotype.

Strains and inoculum preparation

In total, nine *V. inaequalis* strains were tested. Four single-conidial strains originating from different apple genotypes and regions in Latvia and one strain from Poland were tested (Table 2). In addition, four reference strains for races (2) (147), (4) (333), (5) (1634), and (8) (2408), which have been isolated from apple genotypes having resistance genes *Rvi2*, *Rvi4*, *Rvi5*, and *Rvi8* (Table 2; Bus *et al*., 2011; Caffier *et al*., 2015; V. Caffier, personal communication for strain 333), were also included. The strain maintenance and inoculum preparation was performed as described previously (Sokolova & Moročko-Bičevska, 2022).

The conidial suspension of the *V. inaequalis* field population was used as a positive control, made from scabbed fruits and leaves randomly collected from different genotypes each year in the experimental orchard (Table 2) without fungicide treatment. Sterile distilled water was used in each experiment as the control treatment without the pathogen.

Inoculation, incubation, and observations

In general, inoculation and incubation conditions followed the procedure described previously (Sokolova & Moročko-Bičevska, 2022). Shortly, detached leaves (Experiments I, III) and immature fruits (Experiments II, IV, V) were inoculated by applying droplets of inoculum with a pipette (Experiments I, II) or a paintbrush (Experiments III, IV, V). In experiments I and II, fruits and leaves were rinsed in distilled water three times without sterilisation to maintain their surface unchanged, whereas, in experiments III, IV, and V, they were surface sterilised for 1 min in 70% ethanol, rinsed three times in sterile distilled water before inoculation.

In experiments, I and II, apple leaves and fruits were kept on moistened filter paper in Petri plates (leaves) or transparent plastic boxes with caps (fruits). In experiments III - V, the incubation was on florist

foam blocks (Oasis® Floral Products, Sunderland, UK) moistened with sterile distilled water in aluminium foil trays, which were separately wrapped in polypropylene bags (Type P2G35, Propafilm). Incubation took place in a climate chamber (Binder KBW 720) at 20-22 °C in the day-light cycle and 18 °C during the dark cycle, with air relative humidity of 80%, 16 hours in light and 8 hours in dark mode.

Macroscopic and microscopic observations and the scoring of the interactions were done as described by Sokolova & Moročko-Bičevska (2022). In summary, the observations were done once a week, starting one week after inoculation and continuing until week six, when the final scoring was made following the score 0-4 developed by Chevalier, Lespinase, & Renaudin (1991). Interaction phenotypes were further categorised following Caffier *et al.* (2015) as incompatible if scores were 0, 1, 2 and 3a and reflected as "-" (resistant), "(-)" (partially resistant). If scores were 3b and 4, the interaction phenotype

Table 3

Interaction phenotypes between *Malus* genotypes and strains of *Venturia inaequalis* on detached apple fruits and leaves

Cultivar (resistance gene)/ Organ (experiment), strain (race)	Lobo (none)[c]		Auksis (nd)		Golden Delicious (*Rvi1*)[a]		Belorusskoye Malinovoye (*Rvi13*)[c]	
	fruits (II, IV, V)	leaves (I, III)	fruits (II, V)	leaves (I)	fruits (II, IV, V)	leaves (I, III)	fruits (II, V)	leaves (I)
1634 (1, 4)[a]	na/**(+)/(+)**	na/-	na/(-)	na	na/-/-	**(+)**	na/(-)	na
2408 (7, 8)[a]	na/**(+)/(+)**	na/+	na/(-)	na	na/(-)/-	**(+)**	na/(-)	na
147 (5, 7)[a]	na/(-)/(-)	na/-	na/-	na	na/(-)/-	-	na/-	na
333[b]	**(+)/+/(+)**	(-)/(-)	-/-	+	(-)/(-)/-	**(+)**	-/-	(-)
M1.1.1 (nd)	-/na/-	-/na	-/-	-	+/na/-	na	**(+)**/-	-
M137.1.1 (nd)	(-)/na/(-)	-/na	-/(-)	-	**(+)**/na/**(+)**	na	-/-	(-)
M139.1.1 (nd)	-/(+)/na	-/-	-/na	-	-/**(+)**/na	(-)	-/na	-
M142.1.1 (nd)	na/+/**(+)**	na/**(+)**	na/(-)	na	na/-/-	(-)	na/-	na
M146.2.21 (nd)	na/+/**(+)**	na/(-)	na/-	na	na/(-)/-	(-)	na/-	na
Field population	(-)/**(+)**/+	**(+)**/**(+)**	-/(-)	(-)	(-)/(-)/-	na	-/-	-
Water control	-/-/-	-/-	-/-	-	-/-/-	-	-/-	-
Cultivar/Strain, organ (experiment)	Priscilla (*Rvi6*)[a]		*M. toringo* (nd)		Edite (*Rvi6*)[d]		Gala (none)[a]	
	fruits (II, IV, V)	leaves (III)	fruits (II, IV, V)	leaves (I, III)	fruits (II, IV, V)	leaves (I, III)	fruits (II, IV)	leaves (III)
1634 (1, 4)[a]	na/-/-	-	na/(-)/(-)	na/(-)	na/-/-	na/-	na/-	**(+)**
2408 (7, 8)[a]	na/-/-	na	na/-/(-)	na/(-)	na/-/-	na/-	na/**(+)**	**(+)**
147 (5, 7)[a]	na/-/-	-	na/-/-	na/(-)	na/-/-	na/(-)	na/-	**(+)**
333[b]	-/-/-	-	-/+/-	-/(-)	-/-/-	-/-	-/-	**(+)**
M1.1.1 (nd)	-/na/-	na	-/na/-	-/na	-/na/-	-/na	+/na	na
M137.1.1 (nd)	-/na/-	na	(-)/na/(-)	-/na	**(+)**/na/-	-/na	-/na	na
M139.1.1 (nd)	-/-/na	na	+/+/na	+/(+)	**(+)**/-/na	-/(-)	-/**(+)**	-
M142.1.1 (nd)	na/-/-	na	na/na/-	na/(-)	na/-/-	na/-	na/-	-
M146.2.21 (nd)	na/-/-	na	na/(-)-	na/(+)	na/-/-	na/-	na/**(+)**	(-)
Field population	-/-/-	-	-/-/-	(-)/(-)	**(+)**/-/(-)	-/-	-/-	-
Water control	-/-/-	-	-/-/-	-/-	-/-/-	-/-	-/-	-

[a] Bus *et al*., 2011; Caffier *et al*., 2015.
[b] Isolated from host h(2), V. Caffier, personal communication.
[c] According to screening with molecular markers, G. Lācis, personal communication.
[d] Ikase *et al*., 2002; Zelmene *et al*., 2022.
Scores: - nd - no data.

was categorised as compatible and reflected as "(+)" (partially susceptible) and "+" (susceptible). Host tissue responses and intensity of *V. inaequalis* sporulation were confirmed by microscopic examination with binocular and stereomicroscope (Leica DMLS).

Results

The first signs of the *V. inaequalis* and *Malus* interaction were observed one to two weeks after inoculation in all experiments. In general, the obtained results coincided between the tests when the same plant organ was inoculated, although few combinations of strains and host genotypes expressed a variation between inoculation tests on the same host organ and between fruits and leaves (Table 3).

About 80 % of the combinations tested were incompatible and expressed necrosis or/and chlorosis without pathogen sporulation or with limited sporulation (Table 3). All strains and field populations were incompatible with the cultivar 'Priscilla'. Cultivars 'Priscilla' and 'Edite' in combinations with field population (experiment 4) and strain M146.2.21 (experiment 4), respectively, showed also resistance reactions resembled as pin-points on fruits (Figure1). Among interactions considered incompatible, 27 % developed scab lesions with limited pathogen sporulation (Table 3).

Most genotypes were scored as partially susceptible in the host genotype and strain combinations showing compatible phenotypes, expressing necrosis or chlorosis with abundant pathogen sporulation (Table 3, Figure 2). Fruits of cultivar 'Lobo' were compatible (partially susceptible) with most of the tested strains. For the rest of the tested cultivars, compatible interactions developed only in combination with one or a few strains. The phenotype considered a fully compatible interaction, expressed as lesions with abundant conidial sporulation and no reaction of host tissue, was visible only on some genotypes inoculated with certain strains and some experiments (Table 3; Figure 2D-F).

A considerable difference in virulence among the strains was detected. Each tested strain showed different virulence on a combination of apple genotypes ranging from one to five (Table 4).

Two strains, M139.1.1 and M142.1.1, isolated from 'Lobo', were virulent on 'Lobo', but only M139.1.1 was virulent on *M. toringo*, 'Edite' and 'Gala'. Strain

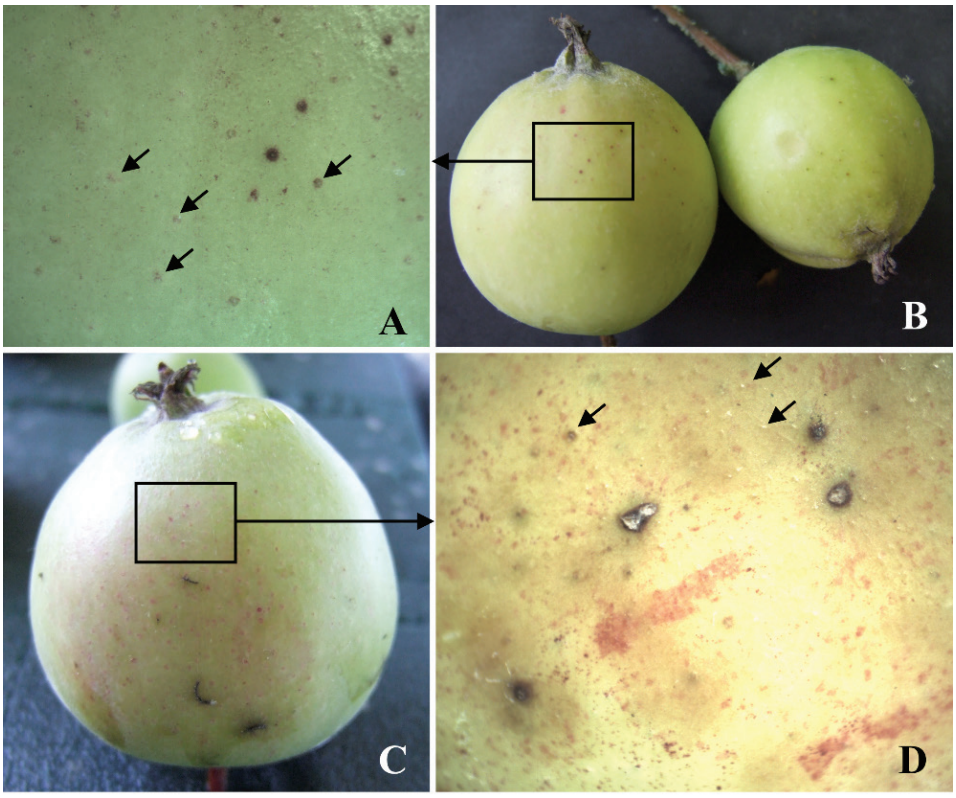


Figure 1. The incompatible phenotype of apple genotypes inoculated with *Venturia inaequalis*: (A, B) Necrosis and spots resembling pin-points (some indicated by arrows) on 'Priscilla' fruits in experiment IV two weeks after inoculation with the field population; (C, D) Spots resembling pin-points (some indicated by arrows) and necrosis on 'Edite' fruits in experiment IV one week after inoculation with M146.2.21.

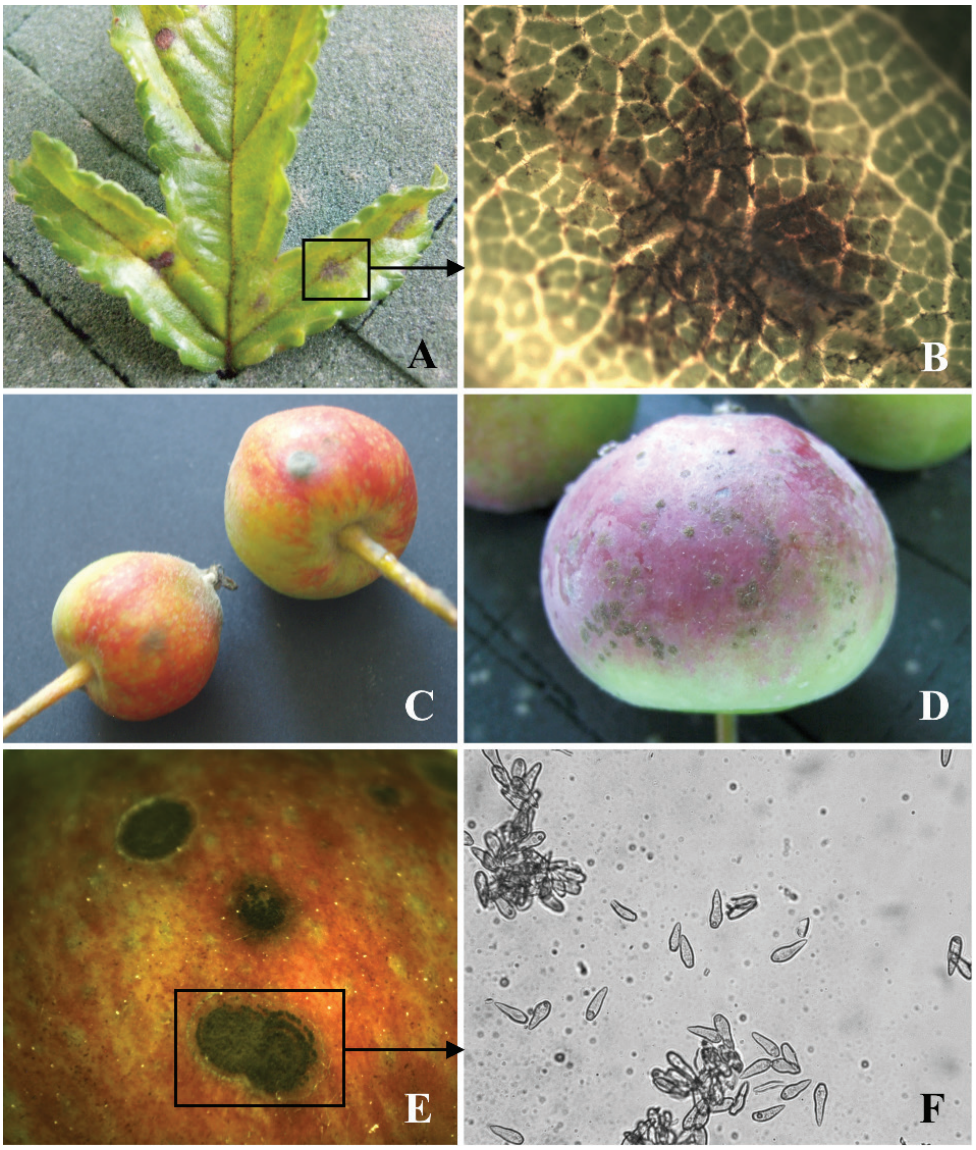


Figure 2. The compatible and incompatible phenotype of apple genotypes inoculated with *Venturia inaequalis*: (A, B) Chlorosis and necrosis with abundant conidial sporulation on *Malus toringo* leaf in experiment III four weeks after inoculation with M146.2.21; (C) Scab lesions with limited conidial sporulation on 'Lobo' fruits in experiment V two weeks after inoculation with M137.1.1; (D) Sab lesions with conidial sporulation on 'Lobo' fruit in experiment IV one week after inoculation with M146.2.21; (E, F) Scab lesions with abundant conidial sporulation on 'Lobo' fruits in experiment V two weeks after inoculation with field population. Magnifications: (B, E) 35x; (F) 400x.

M146.2.21 isolated from *M. toringo* was virulent on 'Lobo', *M. toringo* and 'Gala' (Table 4). Isolate M1.1.1 isolated from the cultivar 'Gala' in Poland was virulent on 'Gala' and 'Golden Delicious' and 'Belorusskoye Malinovoye' but not on other cultivars widely grown in Latvia or on cultivars with *Rvi6*. None of the tested strains or field populations were compatible with 'Priscilla' (*Rvi6*) in any of the experiments (Tables 3, 4). However, the locally bred cultivar 'Edite' (*Rvi6*) expressed a compatible interaction when inoculated with Latvian origin strains, M137.1.1 and M139.1.1, and the field population.

Discussion

The mixed reproduction system of *V. inaequalis* leads to significant variation in the pathogen populations, adaption and breakdown of cultivar resistance (McDonald & Linde, 2002; Guérin *et al*., 2004). This phenomenon has resulted in the formation of highly diverse *V. inaequalis* populations that may

Table 4

Summary of compatible interactions of apple genotypes and *Venturia inaequalis* evaluated on inoculated leaves and fruits *in vitro*[a]

Strain (race)/ Genotype (resistance gene)	Lobo (none)[d]	Auksis (nd)	Golden Delicious (*Rvi1*)[b]	Belorusskoye Malinovoye (*Rvi13*)[d]	Priscilla (*Rvi6*)[b]	*M. toringo* (nd)	Edite (*Rvi6*)[e]	Gala (none) [b]
1634 (1, 4)[b]	x		x					x
2408 (7, 8)[b]	x		x					x
147 (5, 7)[b]								x
333[c]	x	x	x			x		x
M1.1.1 (nd)			x	x				x
M137.1.1 (nd)			x				x	
M139.1.1 (nd)	x		x			x	x	x
M142.1.1 (nd)	x							
M146.2.21 (nd)	x					x		x
Field population	x						x	

[a] Considered compatible and marked with "x", if interaction + or (+) was noted at least in one of the experiments.
[b] Bus *et al*., 2011; Caffier *et al*., 2015.
[c] Isolated from host h(2), V. Caffier, personal communication.
[d] According to screening with molecular markers, G. Lācis, personal communication.
[e] Ikase *et al*., 2002; Zelmene *et al*., 2022.
nd - no data.

differ in their virulence depending on the region and cultivars grown (Melounová *et al*., 2004; Didelot, Brun, & Parisi, 2007). Cultivar disease resistance is one of the major factors for long-term sustainable scab control in the apple industry, and it is the focus of apple breeding programs (Bénaouf & Parisi, 2000; Bowen *et al*., 2011; Kaufmane *et al*., 2013; Höfer *et al*., 2021). So far, seventeen *Malus* resistance genes (*Rvi*) have been identified and 14 *V. inaequalis* races overcoming resistance genes reported in different regions (Bus *et al*., 2011; Caffier *et al*., 2015; Patocchi *et al*., 2020). In Latvia, various apple cultivars are grown, including local and introduced cultivars (mainly from Russia, Belarus, Lithuania, and Estonia). Besides, cultivars from other countries, e.g. Canada, Czech Republic, France, Germany, New Zealand, Scandinavia, the United States and Ukraine, have been introduced for breeding purposes (Ikase *et al*., 2015; Ikase, Drudze, & Lācis, 2022). In different regions, including Latvia, most of the data about *V. inaequalis* virulence and host resistance are based on the observations in the field trials. According to the observation in the orchards, commercially grown apple cultivars in Latvia have different degrees of susceptibility to apple scab (Grantiņa-Ieviņa *et al*., 2015). This study evaluated the apple cultivar responses and virulence of *V. inaequalis* strains by inoculating detached leaves and immature fruits. Four strains of *V. inaequalis* from different regions and host genotypes in Latvia, including widely grown commercial cultivars and ornamental apples, were tested on eight apple genotypes. Four reference strains with known virulence and one from Poland isolated from 'Gala' were also included for comparison. The tested apple genotypes included commercial cultivars grown in Latvia and popular cultivars from other regions with known responses to different *V. inaequalis* races.

In our experiments, inoculating immature fruits and detached leaves, we noted similar interaction phenotypes as reported from plant inoculation experiments, such as host resistance reactions, showing tissue chlorosis and/or necrosis without or limited conidial sporulation of the pathogen, and susceptibility without or with host tissue reaction with abundant pathogen sporulation (Chevalier, Lespinasse, & Renaudin, 1991; Martinez-Bilbao *et al*., 2012; Bus *et al*., 2011; Caffier *et al*., 2015). In our experiments, we noted all five previously described interaction phenotypes (Chevalier, Lespinasse, & Renaudin, 1991; Caffier *et al*., 2015), indicating that *V. inaequalis* strains have different virulence and resistance or susceptibility of the tested *Malus* genotypes. Most combinations of *V. inaequalis* strains and *Malus* genotypes were incompatible, showing necrosis and/or chlorosis with limited or no conidial sporulation. Notably, quite a large proportion of interactions considered incompatible were scored as 3a (moderately resistant), whereas most interactions considered compatible were

noted as partially susceptible (score 3b). The two types of interactions with scores 3b (partially susceptible, considered as compatible) and 3a (partially resistant) were possible to differentiate with confidence only when the evaluation was supplemented with microscopic examination, as noted before in similar studies (Pierantoni *et al*., 2007; Sokolova & Moročko-Bičevska, 2021). Similarly, as in other studies (Caffier *et al*., 2015), susceptible phenotype (score 4 reflected as "+") was rarely observed and often was not consistent among the tests. These findings that most apple genotypes were evaluated as partially resistant or partially susceptible correspond to the field observations (Table 1; Univer, 1999; Urbanovich & Kazlovskaya, 2008; Gelvonauskienė *et al*., 2005; Grantiņa-Ieviņa *et al*., 2015).

Among the tested cultivars, only 'Priscilla' (differential host (h6), carrying *Rvi6*) was incompatible with all strains included in the experiments and field populations of each year, indicating that the local *V. inaequalis* population does not contain virulence to *Rvi6* hosts. This finding is supported by five-year observations in the orchard with *V. inaequalis* races differential *Malus* genotypes (Sokolova & Moročko-Bičevska, 2022) at the same location where field populations were sampled in this study. In contrast, the local cultivar 'Edite', reported as resistant and carrying *Rvi6* (Ikase, Drudze, & Lācis, 2022), showed a compatible interaction with two Latvian-origin strains and field populations on fruits, indicating a possible resistance breakdown. However, this phenotype was not consistent among the experiments and was not observed in leaf tests; therefore, more studies are needed, including plant inoculations with more strains from different regions. Apple cultivar 'Edite' is one of five cultivars created in Latvia's scab resistance breeding program (Ikase, Drudze, & Lācis, 2022). It has been evaluated as resistant to apple scab in field conditions in Latvia; it has been registered for commercial growing since 2009 and has been grown in organic orchards and home gardens since 2011 (Ikase *et al*., 2015). Cultivars 'Auksis' and 'Belorusskoye Malinovoye' are recommended for commercial growing in Latvia and have been reported as partially resistant to scab in field conditions in Latvia (Grantiņa-Ieviņa *et al*., 2015), Lithuania (Gelvonauskienė *et al*., 2005), and Belorussia (Urbanovich & Kazlovskaya, 2008). In our study, all Latvian-origin strains and field populations tested were incompatible with these cultivars, confirming field observations. Despite a susceptibility to apple scab, the cultivar 'Lobo' is still widely cultivated in Latvia and neighbouring countries (Univer, 1999; Urbanovich & Kazlovskaya, 2008; Grantiņa-Ieviņa *et al*., 2015). Similarly, in our study, this cultivar showed compatible interactions with most of the strains and field population tested, by developing scab lesions with abundant pathogen sporulation without or with tissue reaction, except with the local strain M137.1.1 from 'Auksis', M1.1.1 from 'Gala' in Poland and reference strain 147 virulent on h(5), h(7).

Latvian-origin *V. inaequalis* strains showed some preferences and were more compatible with cultivars commonly grown in Latvia, indicating an adaptation of local populations. This finding was evident by the fact that only two of the Latvian origin strains were compatible with 'Gala', whereas all other strains caused lesions and were able to produce abundant sporulation. Similarly, only two strains from Latvia were virulent on 'Golden Delicious'. In Latvia, cultivars 'Gala' and 'Golden Delicious' are rarely grown, only in germplasm collections, whereas in other European countries, they are widely grown (O'Rourke, 2003; Hancock *et al*., 2008). The observed host tissue reactions of 'Gala' scored as 3a and 3b coincided with the observations in the field in races differential *Malus* orchard where scab lesions with pathogen's sporulation and chlorosis or/and necrosis of tissues were also noted (Sokolova & Moročko-Bičevska, 2022). Perhaps it could be related to ontogenic resistance observed previously, where the formation of sporulation or tissue susceptibility was reduced when the age of host tissues increased (Gessler & Stumm, 1984; Xu & Robinson, 2005). Although no resistance genes are known in 'Gala', it has been highlighted that there could be putative resistance genes in this host and a truly resistant apple cultivar is not expected to exist (Caffier *et al*., 2015).

Most results among the experiments were concordant with some variations, especially between fruits and leaves, which could be the effect of plant phenology in each sampling year. The effect of leaf and fruit ontogenic resistance to *V. inaequalis* infection is well-known and has been reported in several scientific reports (Keitt & Jones, 1926; Schwabe, 1980; MacHardy & Jeger, 1983; MacHardy, 1996; Beloshapkina, Kumakhova, & Wakhsheh, 2014). The outcome of the experiments in some cases could also be affected by inoculation, e.g. increasing or decreasing the concentration of spores in some sites and causing changes in tissue reaction (Caffier *et al*., 2015; Pétriacq, Stassen, & Ton, 2016). Similarly, as in experiments with pear detached fruits and leaves (Sokolova & Moročko-Bičevska, 2021), maintenance of leaves for more than two to three weeks was not possible that was needed to develop typical scab lesions with abundant pathogen sporulation. Such problems were not observed in tests with fruits. Other studies report that resistance symptoms appeared two and three weeks after inoculation (Yepes &

Aldwinckle, 1993b; Caffier *et al*., 2015). Our studies noted the resistance reactions on fruits and leaves as chlorosis and necrosis during the first two weeks. Although a limited number of strains have been used in this research, the strains with diverse origins were included, and it provides the first insight into the virulence of Latvian strains on locally widely grown cultivars and less known cultivars, which are common in other apple cultivation regions. More studies are needed, including more strains and inoculation of plants, to characterise *V. inaequalis* virulences in Latvia, especially to verify the possible breakdown of resistance of the cultivar 'Edite'.

Conclusions

The noted variable phenotypes indicate that the tested *V. inaequalis* strains differ in their virulence on the tested apple genotypes. The cultivar 'Priscilla' (differential host (h6), carrying *Rvi6*) was incompatible with all the strains included in the experiments and field populations of each year, confirming that the local *V. inaequalis* population does not contain virulence to *Rvi6* hosts. Commercial cultivars, 'Auksis' and 'Belorusskoye Malinovoye', were incompatible with all tested Latvian-origin strains and field populations, confirming field observations that these cultivars are partially resistant. The observed host tissue resistance reactions of 'Gala' (differential host (0), no resistance genes known) only to Latvian-origin strains confirmed the observations in the field in the races differential *Malus* orchard located in Dobele, and it demonstrates that local *V. inaequalis* population is not adapted to this host. The results showed that detached fruits and leaves could be used for the fast screening of host genotype and *V. inaequalis* strain interaction.

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