Field apple scab susceptibility of a diverse Malus germplasm collection identifies potential sources of resistance for apple breeding

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Abstract
Background: Breeding for resistance to apple scab (caused by Venturia inaequalis), the most devastating fungal disease of apples, relies on genetic resources maintained in germplasm collections.

Methods: To identify new sources of scab resistance, we evaluated 177 Malus accessions, including 27 primary and 13 hybrid Malus species from diverse geographical origins, in an orchard at Geneva, New York. We also screened a differential host set for 2 years to monitor for changes in the effectiveness of ten known scab resistance genes, which allowed us to confirm the presence of virulent pathogen races in the orchard.

Results: We found that ~37% of the wild Malus accessions and domesticated cultivars were resistant to apple scab in the field. Several of these accessions were unrelated to sources of previously known resistance genes and are promising for apple scab genetic research and resistance breeding. Cultivars carrying the Rvi6 (Vf) gene from Malus floribunda clone 821, e.g. 'Liberty' or 'Florina', remained resistant despite the breakdown of Rvi6. ‘Demir’, a Malus hybrid from Turkey, and ‘Chisel Jersey’, a traditional English hard cider cultivar, showed fewer symptoms than the Rvi6 resistant cultivar ‘Prima’. Races 1 to 7 and 9 of V. inaequalis were present in the orchard, but no scab was observed on the indicator host accessions for races 11 and 12.

Conclusions: Detailed and systematic screening of Malus germplasm identified resistant and moderately resistant donor accessions based on resistance reaction types. These accessions are promising for use in future genetic studies to identify novel sources of scab resistance alleles for apple breeding to develop cultivars with durable apple scab resistance.

Keywords: Venturia inaequalis, Disease resistance, Core collection, Differential hosts

Introduction
Disease susceptibility of commercial apple cultivars (Malus domestica Borkh.), and the continual emergence of new pathogenic races that overcome resistance genes, are major threats to the apple industry worldwide. Apple scab (causal agent: Venturia inaequalis Cke./Wint.) is the most devastating fungal disease of apples in humid areas throughout the world where apple is grown (González-Domínguez et al. 2017). The majority of apple cultivars grown commercially in the USA are susceptible to apple scab. Apple scab lesions on fruit mainly impact their cosmetic appearance, severely limiting their marketability. Growers must apply approximately 12–18 fungicide sprays per growing season to limit quality and yield loss due to apple scab (Peck et al. 2010; MacHardy et al. 2001). Frequent use of fungicides contributes significantly to production costs, and to negative human health and environmental impacts. Apple cultivars resistant...
to scab require fewer fungicide applications, saving on costs and reducing the environmental impact of disease control (Papp et al. 2019; Brown and Maloney 2008; MacHardy et al. 2001). Malus accessions and land races maintained in the US national germplasm repository are sources of diverse functional alleles that can be used to breed apple cultivars with enhanced and durable resistance (Byrne et al. 2018). In fact, large-scale screening of germplasm repositories is a common strategy to identify valuable traits for use in breeding for many major crop species (Girichev et al. 2018; Liang et al. 2015; Vasudevan et al. 2014). Conventional commercial apple production is driven mainly by desirable fruit quality traits including taste and shelf life, but development of new apple scab resistant, or tolerant, cultivars might allow reduction of disease management costs, fungicide resistance development, as well as reduce negative environmental and health impacts, and is especially critical for organic and low input production (Koutis et al. 2018; Kellerhals et al. 2004). Unfortunately, the introgression of disease resistance alleles from wild sources into apple cultivars with good fruit quality is a slow and challenging process and so the proportion of scab-resistant cultivars in commercial production remains low (Brown and Maloney 2013).

Genetic resistance to scab in apple is primarily guided by major resistance genes, in a classical gene-for-gene relationship with the Avr genes of the pathogen. To date, 20 resistance genes (Rvi genes) have been described in V. inaequalis, most of which were identified in wild Malus accessions and landraces (Bus et al. 2011; Khajuria et al. 2018). Only two of the Rvi genes, Rvi6 (receptor kinase gene) and Rvi5 (TIR-NBS-LRR gene) have been characterized and their functionally validated (Schouten et al. 2014; Jansch et al. 2014). Unfortunately, many of the resistance genes, including the well-characterized Rvi6 gene, have been overcome by novel virulent races of the scab pathogen (Papp et al. 2019; Parisi et al. 1993, 2004; Xu et al. 2008). A successful apple scab resistance breeding program in the USA between the Universities of Purdue, Rutgers and Illinois (the PRI initiative), used resistance genes from four sources, M. floribunda Sieb. ex Van Houtte clone 821 (Rvi6 and Rvi7) (Japanese crabapple), M. micromalus Makino (Rvi5) (Midget crabapple or Kaido crabapple), M. domestica sel. R12740-7A (Rvi2 and Rvi4), and the common apple ‘Antonovka’ (Rvi10, Rvi17, polygenic), to develop commercial scab-resistant cultivars and pre-breeding materials. These four genotypes became the foundation for later breeding work worldwide (Crosby et al. 1992). However, most modern scab resistant cultivars carry Rvi6 resistance from M. floribunda 821 (Brown and Maloney 2013).

Monitoring the virulence of pathogen races, as well as understanding the evolutionary and genetic mechanisms responsible for loss of host resistance, are essential both for managing disease resistance and developing durable resistance (Patocchi et al. 2020). In the USA, races 1 to 5 and 9 of V. inaequalis have been previously reported to overcome Rvi1, Rvi5 and Rvi9, respectively (Beckerman et al. 2009; Durham et al. 1999; Hagan et al. 2000; Shay et al. 1956; Williams and Kuc 1969), but there is no information regarding races of V. inaequalis with an ability to cause disease on apple genotypes with Rvi11 and Rvi12 resistance genes (derived from M. baccata (L.) Borkh. ‘jackii’ and ‘Hansens baccata #2’). The presence of races 6 and 7, which can infect M. floribunda 821, was suggested by Beckerman et al. (2009), but was only recently confirmed by characterization of monosporic isolates of V. inaequalis collected from M. floribunda 821 (Papp et al. 2019). According to the most recent update from monitoring scab resistance of differential indicator cultivars and accessions in 14 countries, the most promising R genes, exhibiting consistent resistance across locations to date, are Rvi5, Rvi11, Rvi12, Rvi14 and Rvi15 (Patocchi et al. 2020).

Screening the existing apple germplasm collections for scab resistance can contribute to the identification of additional scab resistance gene resources, and if utilized, eventually to the development of new resistant cultivars with good fruit quality (Papp et al. 2019). The national Malus collection at the USDA (United States Department of Agriculture) Plant Genetic Resources Unit (PGRU) is the world’s largest apple germplasm repository, with 5004 unique Malus accessions growing in the field and 1603 seedlots representing M. domestica, 33 Malus species, and 15 hybrid species from around the world (Volk et al. 2015a). Approximately 2500 accessions in the collection have been evaluated for a 28-trait descriptor set (Volk et al. 2015a). The collection exhibits broad diversity for a large range of morphological descriptors (e.g., leaf, shoot, flower and bark characteristics), economically important horticultural traits (e.g., tree vigor, shoot traits, ploidy, flowering, fruiting characteristics), disease and pest resistance, and fruit quality traits (Forline and Aldwinckle 2001, 2003; Harshman et al. 2017; Hokanson et al. 2001; Jurick et al. 2011; Khan and Chao 2017; Luby et al. 1996, 2002; Momol et al. 1999; Myers et al. 2008; Norelli et al. 2013; https://www.ars-grin.gov).

Comprehensive genetic characterization of the entire USDA-PGRU apple collection for disease resistance is laborious and logistically challenging, especially for disease evaluation in the field. Some wild Malus accessions of the USDA-PGRU collection, particularly M. sieversii (Ledeb.) M.Roem. (Aldwinckle et al. 1997; Hokanson et al. 1997; Volk et al. 2005) and M. orientalis Uglitzk. ex Juz. (Volk et al. 2008), have been screened for apple scab resistance, and a considerable number of the accessions
showed resistance to *V. inaequalis* in those studies. In general, and with the exception of a few specifically bred cultivars, *M. domestica* cultivars show a low level of scab resistance (Aldwinckle et al. 1997; Brown and Maloney 2008, 2013). Development of core collections, representing maximum genetic and trait diversity in the gene pool of various crop species, has been widely adopted to lower the maintenance costs and evaluation of crop germplasm (Escribano et al. 2008; Liang et al. 2015; Schoen and Brown 1995). To this end, a core collection of 258 individual *Malus* accessions, representing genetic diversity of the whole collection, was established at five field locations in the U.S.A. to assess disease resistance, fruit quality, and horticultural traits (Luby et al. 1996; Potts et al. 2012). With regard to disease resistance, the *Malus* core collection has been evaluated for fire blight resistance in the greenhouse (Khan et al. 2013), but no extensive evaluation has yet been initiated to screen the 258 *Malus* accessions for resistance to apple scab.

Screening accessions in the field is the most direct method to assess scab resistance relevant to production systems. However, for successful infection with the pathogen, not only are a virulent race of *V. inaequalis* and a susceptible host required, but a favorable environment is also needed, so as to satisfy the host/pathogen/environment interactions (Francl 2001). Particularly humid years are conducive to spore germination (Machardy and Gadoury 1989) and offer an excellent opportunity to identify scab resistance in the field; indeed, the identification of *Vf* resistance occurred in such a season (Crosby et al. 1992). The growing season was wet in 2019, and the resulting favorable weather conditions for development of epidemics of scab may have contributed to the development and occurrence of new races of *V. inaequalis* with the ability to infect the previously resistant *M. floribunda* 821 (Papp et al. 2019).

In this study, firstly, we evaluated the scab resistance of 177 diverse *Malus* accessions in the field, including wild species, cultivars, and hybrid selections to identify new sources of scab resistance. Secondly, we assessed scab on a differential host set of ten apple genotypes to monitor the breakdown of resistance for each of the ten known scab resistance genes, and also to monitor the presence of virulent pathogen races in the orchard.

**Materials and methods**

**Plant material**
The apple scab resistance reaction types of 177 accessions of the *Malus* core collection were evaluated in the research orchard at Cornell AgriTech, Geneva, New York (42° 52′ 38″ N, 77° 03′ 08″ W). The orchard comprises four replicated blocks of the core collection. The core collection is derived from the USA national *Malus* germplasm repository and includes 27 primary wild *Malus* species and 13 interspecific hybrid species, 61 domestic apple cultivars/landraces and 36 unspecified hybrid selections (Table 1). Most of the hybrid selections were developed by the PRI Initiative and other breeding programs, or are crabapples of unknown parentage. The 177 accessions represent the major part of a core collection of 258 individual *Malus* accessions that was established at five field locations in the U.S.A. to evaluate disease resistance, fruit quality, and horticultural traits (Khan et al. 2013; Luby et al. 1996; Potts et al. 2012). Some trees were lost over time at some orchard locations and were not replaced. The unique Plant Introduction (PI) number of each accession used in this study (Table 1) was obtained from the USDA Germplasm Resources Information Network (GRIN) database (https://www.ars-grin.gov), and can be used to compare accessions evaluated in different studies. In addition, the research orchard includes ten differential host accessions with known apple scab resistance genes to identify previously characterized races of *V. inaequalis*. If a differential host was not available as proposed by Bus et al. (2011), alternative hosts (some with more than one R gene) present in the core collection were evaluated as surrogates for that resistance reaction type (Table 2). Information on *Malus* taxonomy for each species was obtained from the GRIN database, which adheres to the *Malus* systematics of Rehder (1915) and Langenfeld (1970).

**Orchard maintenance and weather data**

*Malus* accessions in the core collection are grafted on ‘M9’ rootstocks and are planted in 12 consecutive rows. The 12 rows are divided into 4 blocks (replications) with each replication arranged in 3 rows of trees. Accessions within each replicated block are planted using a randomized block design; in each block there is only one replicate of any accession (i.e., a single tree of each accession per block). Trees have 1.8 m in-row spacing and 3.9 m between-row spacing with a four-wire training system. Trees are approximately 15–20 years old. No pesticides (i.e., insecticides, fungicides, and antibiotics) have been applied in the orchard since 2017 to avoid any possible pathogen, host and pesticide interactions and to observe pathogen isolates and host resistance responses under natural epidemic and selection environments. Trees were occasionally pruned, and a regular mowing schedule was maintained for laneways. The orchard was not fertilized or irrigated during the study period.

A Hobo RX3000 weather station equipped with temperature (Temp) and relative humidity (RH) sensors (Onset Computer Corporation, Bourne, MA) was located approximately 800 m east of the core collection research orchard and was used to collect weather
Table 1  Apple scab resistance of accessions from a *Malus* core collection in a research orchard at Geneva, New York

| PI number  | Name                        | *Malus* species | pi number  | Name                        | *Malus* species |
|------------|-----------------------------|-----------------|------------|-----------------------------|-----------------|
| PI589763   |                             |                 | PI589820   | Prairie Fire                | hybrid          |
| PI589838   | Hansen’s #2                 | baccata         | PI58819    | PR1 2050-2                  | hybrid          |
| PI594110   | Jackii                      | baccata         | PI588866   | Kerr                        | hybrid          |
| PI286599   |                             | baccata         | PI589181   | Prima                       | hybrid          |
| PI589956   | Antonovka 172670-B          | domestica       | PI588991   | Bechel Crab                 | iomensis        |
| PI590183   | Dayton                      | domestica       | PI590015   |                             | iomensis        |
| PI588747   | Florina                     | domestica       | PI589999   |                             | iomensis        |
| PI589962   | Jonafree                    | domestica       | PI58932    | M-84                        | prunifolia      |
| PI594111   | Redfree                     | domestica       | PI589390   |                             | sikkimensis     |
| PI589726   | Britegold                   | domestica       | PI589420   | M. hartwigii                | sp.             |
| PI588868   | florentina                  |                 | PI589958   | MA # 4                      | toringo         |
| PI589933   |                             |                 | PI483254   |                             | x dawsoniana    |
| PI594105   |                             |                 |           |                             |                 |
| PI589972   |                             | halliana        | PI594099   |                             | asiatica        |
| PI589246   | Parkman                     | halliana        | PI594107   |                             | asiatica        |
| PI589408   |                             | hupehensis      | PI437055   | Flexilis                    | baccata         |
| PI589522   |                             | hupehensis      | PI588930   | Macorcarpa                  | bhutanica       |
| PI588870   | Dolgo                       | hybrid          | PI589170   |                             | brevipes        |
| PI589572   | E14-32                      | hybrid          | PI589976   |                             | coronaria       |
| PI590072   | E31-10                      | hybrid          | PI280400   | Anna                        | domestica       |
| PI590070   | E7-54                       | hybrid          | PI589596   | Calville Blanc              | domestica       |
| PI589794   | PRI 1754-2                  | hybrid          | PI588848   | Cortland                    | domestica       |
| PI589807   | PRI 1773-6                  | hybrid          | PI588853   | Cox's Orange                | domestica       |
| PI589807   |                             |                 |           |                             | Pippin          |
| PI589792   | PRI 1850-4                  | hybrid          | PI589024   | Crimson Beauty              | domestica       |
| PI589777   | PRI 1918-1                  | hybrid          | PI589841   | Delicious                   | domestica       |
| PI588992   | White Angel                 | hybrid          | PI590179   | E.8                         | domestica       |
| PI589570   | E36-7                       | hybrid          | PI280401   | Ein Shemer                  | domestica       |
| PI590008   |                             | iomensis        | PI588842   | Empire                      | domestica       |
| PI594097   |                             | kansiensis      | PI588785   | Esopus                      | domestica       |
| PI589955   |                             | micromalus      | PI392303   | Gala                        | domestica       |
| PI594096   |                             | micromalus      | PI590184   | Golden Delicious            | domestica       |
| PI588933   |                             | prattii         | PI588880   | Granny Smith                | domestica       |
| PI588832   | Xanthocarpa                 | prunifolia      | PI589469   | Haralson                    | domestica       |
| PI589402   |                             | prunifolia      | PI588841   | Idare                       | domestica       |
| PI588761   |                             | sargentii       | PI589441   | Ingol                       | domestica       |
| PI594094   |                             | toringo         | PI589122   | Kimball McIntosh            | domestica       |
| PI589395   |                             | tschonoskii     | PI589053   | Lady                        | domestica       |
| PI588757   |                             | x hartwigii     | PI588998   | Marshall McIntosh           | domestica       |
| PI588959   |                             | x magdeburgensis| PI589486   | Murray                      | domestica       |
| PI589415   | Hoopesii                    | x platycarpa    | PI588872   | Northern Spy                | domestica       |
| PI588825   | Robusta 5                   | x robusta       | PI589478   | Novosibirski                | domestica       |
|             |                             |                 |           |                             | Sweet           |
|             |                             |                 |           |                             |                 |
| PI588960   | Rockii                      | baccata         | PI588255   | Redspur Delicious           | domestica       |
| PI322713   | Mandshurica 2330            | baccata         | PI483257   | Reimette                    | domestica       |
| PI588943   | Liberty                     | domestica       | PI589520   | Rhode Island                | domestica       |
| PI589490   | Trent                       | domestica       | PI588850   | Rome Beauty Law             | domestica       |
data in 2018 and 2019. Temp and RH readings were collected at 5-min intervals and transmitted in real-time to the HOBO RX3000 base station, which uploaded data at 15-min intervals to HOBOlink. Datasets were generated in HOBOlink for download, with hourly summarizations and subsequent analysis. Wet periods (RH > 90%) represented infection risk and were used to calculate Mills periods, based on the Temp and RH readings.

Grouping of accessions into different classes is based on a visual assessment of the scab response type on visible leaves throughout the tree canopy on each tree in four replicated blocks and three sampling dates in 2018 and 2019. Assessments were based on an ordinal scale developed by Chevalier et al. (1991). Plant Introduction (PI) number of each accession is provided according to the USDA Germplasm Resources Information Network (GRIN).
period duration according to MacHardy and Gadoury (1989).

Assessment of apple scab symptoms

Symptoms of apple scab on the 177 *Malus* accessions and the 10 differential apple host genotypes were evaluated three times a year in June, July, and August in 2018 and 2019. Evaluations consisted of a careful examination of the visible leaves in the tree canopy of each of the 177 trees and 10 differentials, in each of the 4 replicated blocks. The scab evaluations were used to ascertain the resistance response types of each of the accessions in the orchard, using a previously developed rank ordering of scab symptoms (Chevalier et al. 1991). The classes of the ordinal scale are as follows:

0—no symptoms; 1—pin point pits; 2—chlorotic lesions; 3a—necrotic and some chlorotic lesions, very weak sporulation; 3b—clearly sporulating chlorotic and necrotic lesions; and 4—abundantly sporulating lesions covering most of the leaf area. Based on the symptom classes we distinguished four response categories: 1—Resistant (symptom class: 0, 1, 2), 2—moderately resistant (3a), 3—Moderately susceptible (3b), 4—susceptible (4).

In addition, the 10 differential host accessions in the four replicated orchard blocks were evaluated for scab severity in early July and August of 2019. The most severely infected tree of each host accession in the four replicated blocks was used to represent the accession. The scab severity within the tree canopy was visually evaluated using a 9 point ordinal scale described by Lateur and Populer (1994) and adapted by Patocchi et al. (2009), taking the most severely infected tree of each accession as the representative sample. These differential host accessions have known apple scab resistance genes (Bus et al. 2011) corresponding to specific races of *V. inaequalis*. Plant Introduction (PI) number of each accession is provided according to the USDA Germplasm Resources Information Network (GRIN) and adapted by Patocchi et al. (2020).

### Table 2 Severity of apple scab at two timepoints in 2019 on a scab differential host set in the research orchard at Geneva, New York

| Differential host | Host name                     | PI number | R gene       | Severity (1–9, July) | Severity (1–9, August) |
|-------------------|-------------------------------|-----------|--------------|----------------------|------------------------|
| h0                | Gala                          | 392303    | –            | 5                    | 6                      |
| h1                | Golden Delicious              | 590184    | Rvi1         | 7                    | 7                      |
| h2                | TSR34t15 (syn. PRI 384–1)     | 589780    | Rvi2         | 4                    | 4                      |
| h3                | *Malus* × ‘Geneva’            | 589079    | Rvi3.1, Rvi3.2, Rvi3.3 | 3          | 4                      |
| h4                | TSR33t239                     | N/A²      | Rvi4         | 5                    | 5                      |
| h5                | OR4ST132 (syn. PRI 333–9)     | 589829    | Rvi5         | 4                    | 4                      |
| h6                | Priscilla                     | 589965    | Rvi6         | 1                    | 1                      |
| h7                | *Malus floribunda* sel. 821   | 589827    | Rvi6, Rvi7   | 4¹                  | 4¹                    |
| h9                | *Malus* × ‘Dolgo’             | 588870    | Rvi9, unknown | 4                    | 4                      |
| h11               | *Malus baccata* ‘jackii’      | 594110    | Rvi11, unknown | 1          | 1                      |
| h12               | *Malus baccata* ‘Hansens baccata’ #2| 589838 | Rvi12   | 1                    | 1                      |

The scab severity within the tree canopy was visually assessed in four replicate trees of each accession using a 9 point scale described by Lateur and Populer (1994) and adapted by Patocchi et al. (2009), taking the most severely infected tree of each accession as the representative sample. These differential host accessions have known apple scab resistance genes (Bus et al. 2011) corresponding to specific races of *V. inaequalis*. Plant Introduction (PI) number of each accession is provided according to the USDA Germplasm Resources Information Network (GRIN).

| a Differential apple hosts carrying specific Rvi scab resistance genes as described in Patocchi et al. (2020) |
| b Not available in the USDA-PGRU GRIN database |
| c Scab severity reported by Papp et al. (2019) |

Data analyses and visualization

Statistical analyses were performed to evaluate the difference between the weather conditions of the 2 years, and to test differences in scab resistance of the accessions. The scab resistance reaction type data collected at three time points in 2018 and 2019 were used to assess scab susceptibility of each of the accessions. Scab resistance reaction type and severity data collected over 2 years for the differential hosts with ten known scab resistance genes were used to assess whether any local races of *V. inaequalis* were able to overcome the genetic resistance in hitherto resistant differentials and to identify novel sources of apple scab resistance. Results of daily RH and Temp data from April to October and scab
resistance responses of accessions/Malus taxonomy groups for each species were visualized in R version 3.6.2 (R Core Team 2020) using the inbuilt functions and the ggplot2 package (Wickham 2016). The effect of different genotypes and different time points within and across years on the disease severity inferred from the scab resistance reactions was explored using an ordinal logistic regression model in IBM SPSS Statistics v.25.0 (Arkmonk, NY). Furthermore, the total number of plants in each of the four categories of resistance response symptom classes for each month (June, July and August) in 2018 and 2019 and the corresponding weather variables were used to perform principal component analysis (PCA) analysis to study the effect of weather variables on disease susceptibility. The PCA results were explored graphically using biplots with the packages FactoMineR (Husson et al. 2020) and factoextra (Kassambara and Mundt 2017) in R version 3.6.2. Weather variables used included: average RH (RHave), minimum RH (RHmin), maximum RH (RHmax), average temperature (Tave), minimum temperature (Tmin), and maximum temperature (Tmax) for June, July and August 2018 and 2019. Pearson’s correlation analysis among these variables was performed in R version 3.6.2. A chi-square test was performed to evaluate relationship among Malus taxonomic groups and scab severity.

Results
Impact of weather conditions on apple scab severity
In 2019, a higher proportion of the Malus core collection trees were infected with scab, which reached a plateau of approximately 50% of all the trees in the orchard, in contrast to 25% in 2018 (Figs. 1 and 2). The ordinal logistic model showed significant \( P < 0.0001 \) difference among the genotypes and the six timepoints in apple scab susceptibility. According to the dispersion of Mills periods across the two study years, 2019 experienced conditions favorable to infection by apple scab 2 weeks earlier (data not shown). During the early vegetative phase of growth, both average Temp and RH (%) were higher in 2019 compared to in 2018 (Fig. 3). Later in the season, the relative difference in temperature between the 2 years shifted, but was close to the 16 to 23.9 °C optimum for scab development. The high Temp peaks in June and July of 2018 were > 32 °C, higher than the more consistent daily temperatures experienced in 2019. The average monthly RH was higher in April, May, and July in 2019, and RH was > 90% more often throughout the whole season in 2019, providing more suitable conditions for spore germination of V. inaequalis.

The PCA biplot showed a strong positive association among RHave, RHmin and Tmin, and negative association between RHmax and Tmax (Fig. 4). The variables RHave and RHmin, RHave and Tmin, and RHmin and
Tmin showed positive correlations ($r$) of 0.96, 0.86 and 0.93, respectively, whereas RHmax and Tmax showed a negative correlation ($r$) of $-0.61$. The first two PCs (principal components) explained 86.6% of the variation; PC1 and PC2 explained 53.6% and 33% of the variation, respectively. Apple scab susceptibility was found to be
influenced by RHmax, RHave, RHmin and Tmin, whereas apple scab resistance was found to be influenced by Tmax as depicted by the location of RHmax in relation to the moderately susceptible accessions, RHave, RHmin, Tmin, and the susceptible accessions, and Tmax and the resistant accessions in the same quadrant of the PCA biplot i.e. quadrant II, I and IV of the PCA biplot, respectively. The presence of RHmax and susceptible accessions, and Tmax and resistant accessions in close proximity to each other in the PCA biplot shows strong association of RHmax and scab symptom development, and Tmax and disease resistance respectively (Fig. 4).

Scab severity on the differential set

The indicator accessions with ten known scab resistance genes were used to monitor the severity of scab infection in relation to races of *V. inaequalis* present in the orchard (Table 2). ‘Gala’ showed heavy scab infection in both years. Scab severity on ‘Golden Delicious’, carrying *Rvi1*, was even higher (class 7) compared to severity on the susceptible control. Differential hosts 2 (TSR34t15), 3 (M. × ‘Geneva’), 4 (TSR33t239), 5 (OR45T132) and 9 (M. × ‘Dolgo’) were all severely diseased with scab, indicating the presence of races 1 to 5 and race 9. Scab severity was as high as class 4, except for host differential indicator for race 4, which was rated in scab severity class 5 by the end of the season. *M. baccata* ‘Jackii’ (indicator for race 11) and ‘Hansens baccata #2’ (indicator for race 12) were free of scab. Severe scab was observed on *M. floribunda* 821, the source of *Rvi6* and *Rvi7* resistance, as reported earlier by Papp et al. (2019), but ‘Priscilla’ (*Rvi6*) was free of scab symptoms. Overall, resistance response types were consistent over the period of assessment, with only slight differences in severity.

Sources of novel scab genetic resistance in the core collection

Of the 177 *Malus* accessions evaluated for apple scab symptom, a total of 49, 17, 32, and 79 accessions were free of scab or resistant (ordinal scale classes 0, 1, 2), had weak sporulation (3a), had well-developed sporulating lesions (3b), or were completely susceptible (4), respectively (Table 1; Additional file 1: Table S1). The 49 resistant accessions are mostly primary *Malus* species (46.9%), hybrids from breeding programs (22.4%), secondary *Malus* sp. (14.3%), and *Rvi6* resistant cultivars (12.2%); there were two apple ‘Antonovka’ landraces (4%). In contrast to the large number of resistant accessions among the primary apple species, no modern domestic cultivar lacking a known major resistance gene was found to be completely free of scab. In the most susceptible category (n=79), 51.8% were domestic cultivars. Out of 61 *M. domestica* accessions included in the study, 8 were resistant to apple scab (‘Antonovka 172670-B’, ‘Antonovka 43470 lb’, ‘Britegold’, ‘Dayton’, ‘Florina’, ‘Jonafree’, ‘Liberty’, and ‘Redfree’). Most of these accessions with scab
resistant phenotypes can be traced back to PRI breeding materials and are considered to have Rvi6 resistance from *M. floribunda* 821. Sporulation of scab lesions was noticed on Rvi6 cultivars ‘Prima’ and ‘Nova Easygro’ and has been reported previously (Papp et al. 2019). In addition, PRI cultivars ‘Murray’ (Rvi5), ‘Trent’ (Rvi6) and ‘Viking’ were found to show scab symptoms. Only 5 of the 19 hybrid selections listed by their PRI codes did not show symptoms during the 2 years. The selections PRI 333-9 (syn. OR45T132, Rvi5) and PRI 384-1 (syn. TSR34T15, Rvi2) were both severely scabbed. Besides the PRI derivates, two cultivars: ‘Demir’ and ‘Chisel Jersey’, were evaluated as moderately resistant to scab. Traditional heritage cultivars including ‘Gravenstein Washington Red’ and ‘Irish Peach’ or ‘Burgundy’ had less severe symptoms compared to ‘McIntosh’, ‘Granny Smith’, ‘Gala’, or ‘Golden Delicious’.

The highest number of scab infected accessions are in the *Malus* section. In August 2019, scab severity was the highest in the *Malus* section at all time points; approximately 81% of the genotypes in the *Malus* section showed scab susceptibility (Additional file 1: Table S1). The percentage of scabbed genotypes for the interspecific hybrids was approximately 41%, and for the sections Chloromeles, Gymnomeles, and Sorbomalus was 35%, 28%, and 18% of trees, respectively (Fig. 5). No completely susceptible accession was identified in the sections Sorbomalus and Dyconiopsis. However, the section Dyconiopsis, comprised only one accession with 3 samples, which showed moderate susceptibility in 2018, but not in 2019. Scab resistance differed significantly among six taxonomic groups ($\chi^2$ (d.f. 4) = 40.365, $p < 0.001$). Although the proportion of trees with apple scab increased from 2018 to 2019, this did not affect the relative differences between the scab susceptibilities of the taxonomic groups.

**Discussion**

Detailed and systematic screening of diverse *Malus* germplasm can identify donor accessions for sources of novel and durable apple scab resistance. We have identified potential new sources of scab resistance among *M.*
inaequalis races 1, 7 and 9 have all been reported previously, genes have been derived from small-fruited crabapples: Rvi2 and Rvi4 from M. pumila R12740-7A, Rvi5 from M. × atrosanguinea Schneid. sel. 804 and M. micromalus 245–38, Rvi6 and Rvi7 from M. floribunda 821, Rvi8 from M. sieversii GMAL4302-X8, Rvi11 from M. baccata ‘jackii’, Rvi12 from M. baccata ‘Hansen’s 2#’ and Rvi9 from M. × ‘Dolgo’, possibly a clone of M. baccata or M. prunifolia (Wild.) Borkh. (Bus et al. 2011). We lack molecular and genomic data to characterize the genetic basis of the resistance of many of these promising accessions, although it is possible that these resistances were due to already-described major genes or by polygenic quantitative resistance. Novel single gene-based resistance can be easier to characterize and provide new opportunities for apple breeding. At the same time new technologies including biotechnology, genomics, marker assisted selection and novel breeding methods might make polygenic resistance sources more accessible and reliable for apple breeding in the future.

We observed the breakdown of major resistance genes Rvi1 to Rvi7 and Rvi9 in the research orchard. The V. inaequalis races 1, 7 and 9 have all been reported previously from the U.S.A. (Beckerman et al. 2009; Durham et al. 1999; Papp et al. 2019; Shay and Williams 1956; Williams and Kuc 1969). No scab was observed on the differentials of race 11 and 12, although of these, only host 11 (alongside the host for race 15, which was not included in the study) were assigned as ‘not overcome’ by a recent update on worldwide race distributions (Patočchi et al. 2020). In the case of hosts for races 2 (PRI 384-1) and 5 (PRI 333-9, ‘Murray’, Malus sp. ‘Prairie Fire’), all other genotypes screened that possessed known R genes have confirmed breakdown; in the case of Rvi6, many cultivars remained resistant (e.g. ‘Liberty’, ‘Florina’). It should be noted that the only apple cultivars with good fruit quality, and that have scab resistance are those with the Rvi6 gene. We do not yet know why Rvi6 cultivars retain their resistance when the original host source of the Rvi6 resistance can be infected by V. inaequalis. Investigating the reason why infection of Malus floribunda 821, the source of Rvi6 resistance, is possible, but infection of descendant cultivars is not will be of value for informed development of scab resistant cultivars in the future.

In our study, we have identified a clear relationship between the taxonomic affiliation and the scab susceptibility of Malus germplasm. The relationship might reflect host frequency-based selection on the pathogen, hypothesizing that scab susceptibility of wild species is directly related to their genetic proximity to the domesticated apple. The domestication of apples started in Central Asia, where the primary progenitor, M. sieversii exists in large natural populations. Congruent with this host distribution, V. inaequalis populations infecting domesticated apples originated from Central Asia and coevolved with M. sieversii during the domestication process (Gladiieux et al. 2008, 2010). Populations of the pathogen tend to show distinct genetic structure related to their original Malus host species, and the breakdown of resistance genes derived from wild Malus species might be caused by divergent pathogen populations emerging from wild apple reservoirs, as has been demonstrated in the case of Rvi6 resistance (Gladiieux et al. 2010; Lemaire et al. 2016; Leroy et al. 2016; Michalecka et al. 2018). M. sieversii accessions (including the accession of M. kirghisorum syn. M. sieversii var. kirghisorum) were susceptible to apple scab in our study. There is substantial genetic evidence that the European wild apple M. sylvestris, M. orientalis with gene centers to the west of Central Asia, as well as M. baccata with gene centers in East Asia, have hybridized with domestic apple during the domestication process (Cornille et al. 2012, 2014; Duan et al. 2017; Volk et al. 2015b). Overall, accessions of M. domestica and M. sieversii, two closely related species, had considerably more severe scab compared to the accessions of the other wild Malus species. Despite this finding, the inoculum of V. inaequalis from the Malus core collection research orchard might simply not be diverse enough to reflect larger scale trends and might represent races specialized to domestic cultivars. Larger-scale genetic studies and genome-based analysis of local isolates will contribute to understanding the pathogen in relation to host specificity patterns in the Malus-Venturia pathosystem.

A consequence of modern production systems with large acreage of monocultures could facilitate specialization of pathogen strains on particular cultivars, the build-up of large populations on uniform host populations with rapid dissemination of the new strain (McDonald and Stuktenbrock 2016). The sexual and asexual phases of V. inaequalis can allow it to evolve rapidly and in the context of modern high-density
apple orchards, it has the ability to quickly overcome genetic resistance in apples. Therefore, it is critical to continue to identify and characterize new sources of both qualitative and quantitative scab resistance and pyramid multiple resistance sources in order to develop apple cultivars with durable scab resistance. The scab resistance screening data from this study can be combined with previous assessments for fire blight resistance, fruit quality, and horticultural traits (Khan et al. 2013; Luby et al. 1996; Potts et al. 2012) available for the majority of the accessions in the Malus core collection to identify potential sources for introgression of multiple traits for cultivar development. At the same time, it is also important to understand the relationship between the evolutionary potential of V. inaequalis and current disease management practices for enhancing the durability of the resistance genes. Disease management practices to decrease population sizes of the pathogen, limiting production of sexual inoculum, may also contribute to reducing the pathogen’s evolutionary potential (McDonald 2015).

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s43170-020-00017-4.

Additional file 1: Table S1. Apple scab resistance response type of 177 Malus accessions in four blocks (replications) across six sampling dates in 2018 and 2019. Resistance response type was evaluated in a research orchard at Geneva, New York by visually assessing scab severity on single leaves throughout the visible tree canopy of single trees/accessions in each replicated block. Plant Introduction (PI) number of each accession is provided according to the USDA Germplasm Resources Information Network (GRIN).

Acknowledgments
Not applicable.

Authors’ contributions
AK designed the experiment, supervised the research and revised the manuscript. DP and LG collected the apple scab data in 2018 and 2019 respectively. DP analyzed the data and wrote the manuscript, RT helped with manuscript. DP and LG designed the experiment, supervised the research and revised the manuscript. AK, Girichev V, von Reth M, Hanke MV, Höfer M, Schulte E, Flachowsky H. Evaluation of Rubus genetic resources on their resistance to cane disease. Genet Resour Crop Evol. 2018;65(1):179–93.

Khan SA, Zhang XG, Afoufa-Bastien D, Sanhueza RMV, Sbaghi M, Le Cam B. New insight into the history of domesticated apple: second-order contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet. 2012;8(6):e1002703.

Cornille A, Gladieux P, Smulders MJ, Roldán-Ruiz I, Laurens F, Le Cam B, Nersesyan A, Clavel J, Olofsson M, Feugey L, Gabrielyan I, Zhang XG, Tenallon MI, Giraud T. New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. PLoS Genet. 2012;8(6):e1002703.

Durham RE, McNeil RE, Hartman JR, Potter DA, Fountain WM. The flowering crabapple. Univ. of Kentucky, Coop. Ext. Svc. Pub. ID-68. 1999.

Escribano P, Viruel MA, Hormaza JI. Comparison of different methods for disease resistance and horticultural traits. In: XI eucarpia conference. N Y Fruit Q. 2013;21(1):3–10.

Forsline PL, Aldwinckle HS. Evaluation of apple scab resistance of Malus sieversii populations from Central Asia. HortScience. 1997;32:440.

Franchi LJ. The disease triangle: a plant pathological paradigm revisited. Plant Health Instr. 2001. https://doi.org/10.1094/PHI-T-2001-0517-01.

Gladieux P, Zhang XG, Roldán-Ruiz I, Caffier V, Leroy T, Devaux M, van Glabeke S, Coart E, Le Cam B. Evolution of the population structure of Venturia inaequalis, the apple scab fungus, associated with the domestication of its host. Mol Ecol. 2010;19(4):658–74.

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Received: 19 June 2020  Accepted: 9 October 2020
Published online: 28 October 2020

References
González-Domínguez E, Armengol J, Rossi V. Biology and epidemiology of *Venturia* species affecting fruit crops: a review. Front Plant Sci. 2017;8:1496. https://doi.org/10.3389/fpls.2017.01496.

Hagan AK, Tilt KM, Williams JD, Akridge JR. Susceptibility of crabapple cultivars to several diseases in the Gulf Coast region of Alabama. J Environ Hort. 2000;18(4):192–203.

Harshman JM, Evans KM, Allen H, Potts R, Flamenco J, Aldwineck HS, Wisniewski NE, Norelli JL. Fire blight resistance in wild accessions of *Malus sieversii*. Plant Dis. 2017;10(10):1738–45.

Hokanson SC, McFerson JR, Forsline PL, Lamboy WF, Luby JJ, Aldwineck HS, Djangaiev AD. Collecting and managing wild *Malus* germplasm in its center of diversity. HortScience. 1997;32:173–6.

Hokanson SC, Lamby WF, Szewcz-McFadden AK, McFerson JR. Microsatellite (SSR) variation in a collection of *Malus* (apple) species and hybrids. Euphytica. 2001;113(3):281–94.

Husson F, Josse J, Le S, Mazet J, Husson MF. Package “FactoMineR”. 2020. https://cran.r-project.org/web/packages/FactoMineR/index.html.

Jänsch M, Paris R, Amoako-Andoh F, Keulemans W, Davey MW, Pagliarani K, Khan MA, Chao T. Wild apple species as a source of fire blight resistance in *Venturia inaequalis*. J Appl Hortic. 2011;130(4):481–6.

Kassambara A, Mundt F. Factoextra: extract and visualize the results of multivariate data analyses. R Package Version. 2017;1(4):2017.

Khan MA, Zhao Y, Korban SS. Identification of genetic loci associated with fire blight resistance in *Venturia inaequalis*. J Hortic. 2000;663(1):107–13.

Kathera M, Bertschinger L, Gessler C. Use of genetic resources in apple breeding and for sustainable productivity of apple orchards. N York Fruit Q. 2015;33(3):458–73.

Kellerhals M, Bertschinger L, Gessler C. Parasitic and biological fitness of *Venturia inaequalis* (spp.) germplasm collection. J Am Soc Hortic Sci. 2005;130(2):203–10.

Khan MA, Papp D, Singh J, Gadoury DM, Khan MA. New North American isolates of *Venturia inaequalis* can overcome apple scab resistance of *Malus Runbundia* 821. Plant Dis. 2019;104(3):649–55.

Khajuria YP, Lisinovsky NE, Norelli JL. Fire blight resistance in wild accessions of *Malus sieversii* in *Malus* core germplasm collection. In: II international symposium on discovery and development of innovative strategies for postharvest disease management, vol. 1053. 2013. p. 199–203.

Liang W, Dondini L, De Franceschi P, Paris R, Sansavini S, Tartarini S. Genetic association mapping. Physiol Plant. 2013;148(3):344–53.

Llayan GT, Armengol J, Rossi V. Biology and epidemiology of *Venturia inaequalis* (spp.) accessions with resistance to the postharvest decay pathogens *Penicillium expansum* and *Colletotrichum acutatum*. Plant Breed. 2011;130(4):481–6.

Lubin E, Bower BH. Collecting and managing wild *Malus sieversii*. J Am Soc Hortic Sci. 1999;154:821. Plant Dis. 2019;104(3):649–55.

MacHardy WE, Gadoumy DM, Gessler C. Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. Plant Dis. 2001;85(10):1036–51.

McDonald BA. How can research on pathogen population biology suggest disease management strategies? The example of barley scald (*Rhyzochosium commune*). Plant Pathol. 2015;64(5):1005–13.

McDonald BA, Stukinbrock EH. Rapid emergence of pathogens in agro-ecosystems: global threats to agricultural sustainability and food security. Philos Trans R Soc B Biol Sci. 2016;371(1709):20160026.

MacHale M, Masny S, Leroy T, Pulavskova J. Population structure of *Venturia inaequalis*, a causal agent of apple scab, in response to heterogeneous apple tree cultivation. BMC Evol Biol. 2018;18(1):S1.

Momol MT, Forsline PL, Lamby WF, Aldwineck HS. Fire blight resistance and horticultural evaluation of wild *Malus* populations from Central Asia. Acta Hortic. 1999;489:229–34.

Myers CT, Harvey Reissig W, Forsline PL. Susceptibility of fruit from diverse apple and crabapple germplasm to attack from apple maggott (*Diptera: Tephritidae*). J Econ Entomol. 2008;101(1):206–15.

Norelli JL, Wisniewski M, Droby S. Identification of a QTL for postharvest disease resistance to *Penicillium expansum* in *Malus sieversii*. In: II international symposium on discovery and development of innovative strategies for postharvest disease management, vol. 1053. 2013. p. 199–203.

Papp D, Singh J, Gadoury DM, Khan MA. New North American isolates of *Venturia inaequalis* can overcome apple scab resistance of *Malus Runbundia* 821. Plant Dis. 2019;104(3):649–55.

Papp D, Singh J, Gadoury DM, Khan MA. New North American isolates of *Venturia inaequalis* can overcome apple scab resistance of *Malus Runbundia* 821. Plant Dis. 2019;104(3):649–55.

Parisi L, Lepisanne Y, Guillaumes J, Krüger J. A new race of *Venturia inaequalis* virulent to apples with resistance due to the W gene. Phytopathology. 1993;83(5):533–7.

Parisi L, Foullet V, Schouten HJ, Groenwold R, Lauwers F, Didelot F, Evans K, Fischer C, Gennart F, Kemp H, Lateur M, Patocchi A, Thissen J, Tisponoudis C. Variability of the pathogenicity of *Venturia inaequalis* in Europe. Acta Hortic. 2004;663(1):107–13.

Patocchi A, Frei A, Frey JE, Kellerhals M. Towards improvement of marker assisted selection of apple scab resistant cultivars: *Venturia inaequalis* virulence surveys and standardization of molecular marker alleles associated with resistance genes. Mol Breed. 2009;24(4):337.

Patocchi A, Wehrli A, Dubuis P-H, Auwerkerken A, Leida C, Cipriani G, Passey T, Staples M, Didelot F, Philion V, Peil A, Laszakovits H, Rüther T, Boeck K, Baniulis D, Strasser K, Vávra R, Guerra W, Masny S, Russel F, Le Berre F, Nybomb H, Tarrasini S, Spornberger A, Pikunova A, Bus V. Ten years of VINQUEST: first insight for breeding new apple cultivars with durable apple scab resistance. Plant Dis. 2020;104(8):2074–81.

Peck GM, Merwin IA, Brown MG, Agnello AM. Integrated and organic fruit production systems for Liberty apple in the Northeast United States: a systems-based evaluation. HortScience. 2010;45(7):1038–48.

Potts SM, Han Y, Khan MA, Kushad MM, Rayburn AL, Korban SS. Genetic diversity and characterization of a core collection of *Malus* germplasm using simple sequence repeats (SSRs). Plant Mol Biol Rep. 2012;30(4):827–37.

R Core Team. A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2020.

Rehder A. Rosaceae. Subfam. Pomoideae. In: Sargent CS, editor. Plantae Wilsonianae 2. Publications of the Arnold Arboretum, Cambridge (Mass), vol. 4. 1915. p. 263–345.

Schoen DJ, Brown AHD. Maximising genetic diversity in core collections of wild relatives of crop species. In: Hodgkin T, Brown AHD, van Hintum TJL, Morales EAV, editors. Core collections of plant genetic resources. Chichester: Wiley; 1995. p. 55–76.

Schouten HJ, Brinkhuis J, van der Burgh A, Schaart JG, Groenwold R, Broginni GA, Gessler C. Cloning and functional characterization of the *Vri15* (*V*) gene for apple scab resistance. Tree Genet Genomes. 2014;10(2):251–60.

Shay JR, Williams EB. Identification of three physiologic races of *Venturia inaequalis* affecting fruit crops: a review. Front Plant Sci. 2018;9:251–60.

Vachon D, Gessler C. Parasitic and biological fitness of *Venturia inaequalis*: relationship to disease management strategies. Plant Dis. 2001;85(10):1036–51.

Vr2 (*Vri15*) gene for apple scab resistance. Plant Dis. 2001;85(10):1036–51.

Walsh JC, Bruegger MA, Goodwin CA, Stiller HA. Corn Pol II (V) gene for apple scab resistance. Tree Genet Genomes. 2014;10(2):251–60.

Way RE, Williams EB. Identification of three physiologic races of *Venturia inaequalis* affecting fruit crops: a review. Front Plant Sci. 2018;9:251–60.

Wright IL, Polygenia P. Subfam. Pomoideae. In: Sargent CS, editor. Plantae Wilsonianae 2. Publications of the Arnold Arboretum, Cambridge (Mass), vol. 4. 1915. p. 263–345.
Volk GM, Richards CM, Reilley AA, Henk AD, Reeves PA, Forsline PL, Aldwinckle HS. Genetic diversity and disease resistance of wild Malus orientalis from Turkey and Southern Russia. J Am Soc Hortic Sci. 2008;133(3):383–9.
Volk GM, Chao CT, Norelli J, Brown SK, Fazio G, Peace C, McFerson J, Zhong GY, Bretting P. The vulnerability of US apple (Malus) genetic resources. Genet Resour Crop Evol. 2015a;62:765–94.
Volk GM, Henk AD, Baldo A, Fazio G, Chao CT, Richards CM. Chloroplast heterogeneity and historical admixture within the genus Malus. Am J Bot. 2015b;102(7):1198–208.
Wickham H. ggplot2: elegant graphics for data analysis. Berlin: Springer; 2016.

Williams EB, Kuc J. Resistance in Malus to Venturia inaequalis. Annu Rev Phytopathol. 1969;7(1):223–46.
Xu X, Yang J, Thakur V, Roberts A, Barbara DJ. Population variation of apple scab (Venturia inaequalis) isolates from Asia and Europe. Plant Dis. 2008;92(2):247–52.

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