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Evaluation of the susceptibility of *Prunus* rootstock genotypes to *Armillaria* and *Desarmillaria* species

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Abstract Variation in susceptibility of 28 *Prunus* rootstock genotypes to the causal agents of Armillaria root rot, *Armillaria mellea*, *A. solidipes, and Desarmillaria tabescens*, were studied by conducting in vitro root screening assays. Root segments with wounded and intact periderm were placed next to and on the top of the fungal cultures. At day 21, the percent success of fungal penetration and the circumferential and longitudinal lengths of fungal growth were measured. A parallel investigation using the inoculated root segments was carried out to characterize the active host defense mechanisms involved, including anatomical responses in bark and wood. Overall, the success of penetration and the longitudinal and circumferential spread of *Armillaria* spp. and *D. tabescens* were significantly different among various *Prunus* rootstock genotypes. None of the tested rootstock genotypes were completely resistant to infection. However, plum and plum derived rootstocks, and some of the cherry genotypes were less susceptible to infection compared to the peach genotypes. The host's ability to limit infection by *Armillaria* spp. and *D. tabescens* was not limited to a single mechanism but appeared to be regulated by several collective nonspecific host responses acting together. Differential levels of a series of non-specific coordinated events were triggered in *Prunus* genotypes such as the formation of new callus tissue on the root surface, a colored reaction zone, necrophylactic periderm, new cells, and new vascular cambium to compartmentalize the pathogen. The host responses were elevated in the genotypes with low level of infection as compared to highly infected genotypes.

Keywords Host response · Disease susceptibility · In vitro assay · Necrophylactic periderm

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Introduction

Stone fruits (*Prunus* spp.) are important specialty crops in the United States (U.S.) and include almond, apricot, sweet and sour cherry, peach, nectarine, and plum. According to the United States Department of Agriculture National Agricultural Statistics Service 2016, stone fruits are planted on approximately 7×10^5 ha of land. Peaches and cherries are widely planted stone fruits, planted to approximately 1×10^5 ha and valued at approximately $4.4 billion annually (USDA NASS 2016). California is the leading producer of peaches followed by South Carolina and Georgia (Zhao et al. 2017). Similarly, Michigan grows 70% of the tart cherries in the U.S. In 2018, this state alone produced sour cherries

with a value of $280.1 million (Michigan Agriculture Facts and Figures 2018). In these critical stone fruit-producing regions, damage to fruit trees and their fruits by pests and diseases, including Armillaria root rot (ARR), significantly reduces grower's profitability. Armillaria root rot, caused by *Armillaria solidipes*, *A. mellea*, (hereafter referred to as *Armillaria* spp.) and *Desarmillaria tabescens,* is regarded as one of the principal causes of premature mortality of stone fruit crops (Rhoads 1954; Proffer et al. 1988; Schnabel and Bryson 2005).

Armillaria solidipes is the principal causative agent of ARR in the Great Lakes region and has resulted in many cherry orchards going out of production (Michigan Tree Fruit Commission, personal communication). In the major peach growing areas in the southeastern U.S. (Georgia and South Carolina), ARR caused by *D. tabescens* is estimated to be responsible for 3–4% of the annual peach tree losses, which amounts to an $8 million loss annually (Georgia and South Carolina Peach Councils, personal communication). Similarly, *A. mellea* is the principal agent of ARR in the major stone fruit growing regions of California. This fungus is also present outside the U.S. and is known to severely affect peach, almond, apricot, and cherry in the major fruit-producing countries of Europe (Noviello and Zoina 1984).

Armillaria spp. and *D. tabescens* persist as mycelium in its vegetative stage within infected tree stumps and sub-surface root and woody tissues (Cleary et al. 2013). Infection of healthy tree roots occurs either by fungal mycelium through root-to-root contact and/or by means of thread-like fungal hyphae, a rhizomorph (rare in *D. tabescens*) (Cleary et al. 2012). *Armillaria* spp. and *D. tabescens* colonize healthy sapwood, either by infection through wounds, which provide a suitable infection court or direct penetration through root periderm (Thomas 1934). The fungus kills the cambium, degrades woody tissues, and can become established in the roots and lower trunks of trees subsequently leading to tree decline and death. After the tree dies, the fungus causes a white rot of wood, degrading major wood cell wall components and can survive in the dead roots/wood in the soil for decades.

Over the last five decades, multiple disease management approaches have been evaluated against ARR with limited success, including mechanical, chemical, biological, and genetic methods. Mechanical control includes extraction of the infected stump and roots from the soil and root collar excavation (Morrison et al. 1988; Schnabel et al. 2012). Though these methods are beneficial in reducing the infection, they are very costly and labor-intensive (Self and MacKenzie 1995). Also, when removing infected stumps, it is impossible to uproot every piece of the infected root from the soil, and even a 0.5 cm thick root is capable of reinitiating infection (Roth et al. 1980). Chemical control of *Armillaria* spp. and *D. tabescens* is not feasible as the fungus remains protracted in the infected wood and roots in the soil (Gubler 1992). Although injection of a systemic fungicide such as propiconazole to living trees may reduce infection (Amiri and Schnabel 2012), this method is uneconomical for large orchards. Specific strains of biological agents such as *Trichoderma* spp., *Bacillus* spp., and *Pseudomonas* spp. have been evaluated against ARR as they had been found to exhibit in vitro antagonism; however, they have not shown disease prevention and control in the field (Baumgartner and Warnock 2006; Downer and Faber 2019). Planting of less susceptible rootstocks (genetic control) is suggested as the most cost-effective, sustainable strategy for long-term mitigation of tree decline and death from ARR (Beckman and Pusey 2001).

The species diversity within the genus *Prunus* offers the opportunity to search for rootstocks that are less susceptible to ARR. In addition, various rootstock breeding programs have released new rootstocks that are either interspecific hybrids or complex species hybrids. The fact that peach and almond scions have good cross and graft compatibility with a wide variety of *Prunus* species gives breeders more options to develop disease-tolerant rootstocks. For example, plums are generally known to be less susceptible to *A. mellea* (Guillaumin et al. 1991), and plums and peach × plum hybrids are graft compatible with peach. In contrast, cherries are not considered graft compatible with plums, which supports the need to search for less susceptible species within cherry. By studying the response of many *Prunus* rootstocks to *Armillaria* spp. and *D. tabescens*, it might be possible to find individuals that are less susceptible to ARR.

Current methods of screening susceptibility of *Prunus* species to ARR involve an artificial inoculation technique to transfer the fungus directly into the root tissues of seedlings grown in the field (Proffer et al. 1988), glasshouse (Singh 1980), and in growth chambers in vitro (Baumgartner et al. 2018). Greenhouse and field inoculation techniques are labor and time intensive.

Growth chamber assays utilize inoculation of the plants developed from explants in vitro, which may not correctly represent the type of root tissue in the field. Furthermore, these screening techniques only focused on the tree/seedling mortality associated with the infection. However, work done in woody trees species other than *Prunus* suggests that study of nonspecific responses of host roots following *Armillaria* infection such as the formation of periderm (Mullick 1977), barrier zone (Cleary et al. 2012), callus tissue (Shigo and Tippett 1981), reaction zone, and new vascular cambium (Oven and Torelli 1999) that aid to compartmentalize the pathogen in the infected tissue or impede further pathogen growth are crucial in disease resistance screening. More recently, an in vitro assay utilizing freshly excavated root segments from the field was developed by Devkota and Hammerschmidt (2019). This method, which is rapid and allows the evaluation of root infection and assessment of associated host defense responses, was utilized for resistance screening in the current study.

By mimicking two modes of natural infection (i.e., through a wound and direct penetration through periderm) to inoculate *A. solidipes* as described by Devkota and Hammerschmidt (2019), we examined the relative susceptibility of roots from 28 *Prunus* rootstock genotypes including, plum, peach, cherry, and their interspecific hybrids to *A. mellea, A. solidipes,* and *D. tabescens*. These *Prunus* genotypes represent several cultivars that are available to plant in the Great Lakes region and the southeastern U.S. as well as many untested rootstock genotypes and potentially some new rootstock selections. In this study, 28 *Prunus* rootstock genotypes were screened with two *Armillaria* spp. and a *Desarmillaria* sp. that cause ARR in stone fruit growing regions of the U.S. The relative disease susceptibility was evaluated by comparing: i) the percent success of fungal penetration and the subsequent longitudinal and circumferential spread of the fungus in the root tissue, and ii) the associated anatomical changes in the root bark, cambium, and xylem.

Materials and methods

Roots from the 28 *Prunus* rootstock genotypes (Table 1) were included in screening utilizing in vitro wounded and intact root bark inoculation assays developed by Devkota and Hammerschmidt (2019). Uniform sized roots (i.e. 16 ± 4 mm-diameter) that had undergone secondary growth and had fully developed periderm were collected. Root samples were collected from healthy 6 year (except *P. mahaleb* i.e., 12 year) old trees with no visual signs and symptoms of disease planted at research farms in both Michigan and South Carolina in summer 2018 when approximately 500 growing degree days (base temperature: 40 °C) had been reached. At the Clarksville Horticultural Research Center, Clarksville, MI, U.S., root samples for cherry were collected from three individual trees (genotypes) belonging to each of three cherries and one plum tree species. Root samples for the other 16 *Prunus* genotypes were collected from the Musser Fruit Research Center, Seneca, SC, U.S. In vitro root inoculation assays were performed using single isolates of *A. mellea*, *D. tabescens*, and *A. solidipes* that originated in either South Carolina or Michigan (Table 2).

To prevent the loss of moisture and maintain viability, root samples were transported to the lab in an ice-filled box and stored at 4 °C until the start of the experiment. Inoculation assays were initiated within a few days after root collection. Root samples were gently washed with running water to remove external soil, washed with sterile distilled water, immersed in 70% ethanol for 1 min, washed with running sterile distilled water, and blot dried. Surface sterilized root segments were utilized for the wounded root inoculation assay and the intact root periderm inoculation assay.

Wounded root inoculation

Fungal cultures were prepared in approximately 200 Magenta™ GA-7 Plant Culture Boxes (7.62 cm × 7.62 cm × 10.16 cm) as described by Devkota and Hammerschmidt (2019). Four (5 mm-diameter) mycelial agar plugs each of *A. mellea*, *D. tabescens*, and *A. solidipes* were taken from the leading edge of 14-day old Malt Extract Agar (MEA) culture plates and placed at the thicker end of Dichloran-Benomyl-Streptomycin Malt Extract Agar (Worrall 1991) slant in Magenta boxes (Fig. 1a and b). Healthy roots with no visual signs and symptoms of disease were cut into 6 cm long segments and used for inoculation. Diameters of individual segments were measured and used as a covariate in the data analysis. To allow uniform unidirectional growth of test fungus in the root, a free end of the root segment was sealed with paraffin wax, and the exposed end was placed next to the 14-day-old culture

Table 1 *Prunus* rootstock genotypes used in in vitro *Armillaria* resistance screening assays. All rootstocks are seed propagated unless otherwise noted to be vegetatively propagated. *Prunus* species with labels 1–4 represent unique accessions

Species origin	Genotype[a]	Common name
Peach (*P. persica*)	Guardian®	Peach
	Lovell	Peach
	Bailey	Peach
	Pisa P.S.B2	Peach
	Pisa P.S.A5	Peach
	Pisa P.S.A6	Peach
	Tennessee Natural	Peach
	S-37 Stribling	Peach
	BY520–8	Peach
	Kakamas	Peach
Peach × Peach (*P. persica* × *P. davidiana*)	Nemaguard	Peach
Plum (*P. munsoniana*)	*P. munsoniana* #1	Wild goose plum
	P. munsoniana #2	Wild goose plum
	P. munsoniana #3	Wild goose plum
	P. munsoniana #4	Wild goose plum
Plum (*P. cerasifera*)	*P. cerasifera* #1	Myrobalan plum
	P. cerasifera #2	Myrobalan plum
	P. cerasifera #3	Myrobalan plum
Plum × Peach (*P. umbellata* × *P. persica*)	MP-29[b]	Plum x peach
Cherry (*P. avium* - mazzard)	*P. avium* #1	Sweet cherry
	P. avium #2	Sweet cherry
	P. avium #3	Sweet cherry
Cherry (*P. maackii*)	*P. maackii* #1	Amur cherry
	P. maackii #2	Amur cherry
	P. maackii #3	Amur cherry
Cherry (*P. mahaleb*)	*P. mahaleb* #1	Mahaleb cherry
	P. mahaleb #2	Mahaleb cherry
	P. mahaleb #3	Mahaleb cherry

[a] *Prunus avium*, *P. maackii*, *P. cerasifera*, were collected from Michigan. All other *Prunus* genotypes were collected from South Carolina. *Prunus* species with labels 1–4 represent roots collected from individual unique accessions (genotype) of that species

[b] Vegetatively propagated rootstock

Table 2 Fungal isolates used in the Armillaria root rot resistance screening assays

Fungi	Isolate name	Isolation source	Isolation location	Isolation year
A. mellea	SC.00i49	Peach tree	Spartanburg County, SC	2000
D. tabescens	SC.GJ2.02	Peach tree	Cherokee County, SC	2002
A. solidipes	Warren	Tart cherry tree	Grand Traverse County, MI	2006

SC, South Carolina; *MI* Michigan

of three fungi in Magenta boxes (Fig. 1b). Two to four root segments were inoculated in each box and nine root segments from each *Prunus* genotype were inoculated next to each fungus. The culture boxes were incubated at 23 °C in the dark.

At 21 days, the periderm of the root samples was peeled back and the presence of visible fungal mycelia and infection under the bark was recorded as successful penetration. The longitudinal and circumferential lengths of the fungal growth and infection were measured. Then the percentage of the root circumference infected by the fungus (percentage of circumferential

infection) in each segment was calculated using the formula:

$$\textit{Percentage of circumferential infection} = \frac{\textit{Circumference of the root infected by the fungus}}{\pi \textit{ x Diameter of the root}} x100\%$$

All roots were morphologically examined for the formation of callus on the surface (external callus). Percentage of the sample that formed callus in each genotype was calculated. For external callus formation, genotypes were assessed according to a 0–3 scale: 0 = no external callus formation, 1 = callus initials were starting to form, 2 = callus initials were fully formed, and 3 = callus was formed and starting to produce root initials. Roots were examined for formation of any colored zones. Formation of a colored reaction zone around the infection point was evaluated according to a 0–3 scale: 0 = no presence of colored reaction zone, 1 = presence of an unclear reaction zone but further fungal infection beyond that zone, 2 = presence of a distinct reaction zone but further fungal infection beyond that zone, and 3 = presence of a distinct zone and no further fungal infection beyond that zone. In addition, a fraction of each segment was sectioned using a sledge microtome (80–100 μm) where the infection front was centered on a radial plane. An aqueous solution of phloroglucinol-hydrochloric acid (PG-HCl: 1 volume of concentrated HCl (37 N) to two volumes of 3% phloroglucinol in ethanol) was used to detect lignin in the cell walls. Suberin was detected using a saturated solution of Sudan Black B in 70% ethanol. The sections were visualized using a microscope (DM2500, Leica) with the bright field (BF) and ultraviolet fluorescence.

To study the detailed view of the anatomical changes in bark, cambium, and xylem, a set of samples were transversely trimmed (10 mm) to have the infection front centered on the radial plane. Immediately after trimming, samples were fixed in FAA (formaldehyde: acetic acid: 70% ethanol, 5:5:9 *v*/v/v) for histological examination. Following the fixing for at least 2 days, the samples were kept in 50% ethanol for 3 h and dehydrated through ethanol: xylene series on a vacuum infiltrating tissue processor (Sakura VIP, 200) (Jensen 1962). The samples were then embedded in paraffin wax with a melting point of 55–60 °C. Processed samples were sectioned (section thickness: 5–6 μm) using a rotary microtome (model RM2245, Leica Biosystems) and placed on a positively charged glass slide and stained with 0.1% Toluidine blue to visualize the fungal hyphae and the host defense responses. The anatomical changes in the periderm, cambium, and xylem tissue in response to infection by *Armillaria* spp. and *D. tabescens* were described. Host defenses such as formation of any barrier zones, necrophylactic periderm (new periderm formed after wounding or infection), new cells, and callus were visualized in the sections using a light microscope (DM2500, Leica).

Necrophylactic periderm (np, i.e. wound periderm) formation was assessed according to a 0–2 scale: 0 = no np formation, 1 = thin layer of np initials forming, and 2 = thick layer of np being formed. Cambial activity was recorded as 0 and 1 if cambium was in an inactive state (dead and non-functional) or an active state (functional), respectively. Root sections were also inspected for the presence or absence of newly formed callus in the cambial region (i.e. internal callus). Internal callus formation was assessed according to a 0–3 scale: 0 = no internal callus formation, 1 = initiation of internal callus initials formation, 2 = single layer of callus formed, and 3 = callus fully formed and inhibiting the radial spread of the pathogen. The collective strength of the measured active host responses in the root bark (Strength of HR) of each genotype was calculated using the following formula:

$$\textit{Percentage strength of active host responses in the bark} = \frac{\textit{observed scores of } (ec + crz + np) \textit{of each germplasm}}{\textit{sum of maximum score of each response}} x100\%$$

The strength of the measured active host responses in the cambial region in the bark of each genotype was calculated using the following formula:

$$\textit{Percentage strength of active host responses in the cambium} = \frac{\textit{observed scores of } (ac + ic) \textit{ of each germplasm}}{\textit{sum of maximum score of each response}} x100\%$$

Where ec = external callus, crz = colored reaction zone, np = necrophylactic periderm, ac = activity of cambium, and ic = internal callus.

Intact root periderm inoculation

Healthy roots from each genotype were cut into 15 three-cm segments and securely sealed at both ends with paraffin wax (melting temperature 50–55 °C) to understand the relative ability of intact periderm of various genotypes to block the fungal invasion. Twenty-five ml

Fig. 1 In vitro wounded and intact root inoculation assays. **a** - Actively growing culture of *A. mellea* for wounded root inoculation assay; **b -** Wounded roots of S-37 Stribling placed next to the edge of actively growing *A. mellea* in fungal culture box; **c** - *A. mellea* culture with fungal rhizomorph formation in oak twig amended media for intact root inoculation assay; and **d** - Intact roots segments of Pisa P.S.B2 on the top of *D. tabescens* culture

of the MEA was poured into each of 100 mm × 15 mm plastic Petri dishes. Two sterile twigs (~3 mm-long) of oak (*Quercus alba*) were immediately placed at the two edges of the plate to allow rapid mycelial growth and rhizomorph formation (Fig. 1c). A mycelial plug (diameter ~ 5 mm) of test fungus was placed at the center of the plate. After incubation in the dark at 23 °C for 14 days, oak twig segments were replaced with five root segments from each genotype by securely placing them on the top of the fungal hyphae or rhizomorph culture plates of each of the three fungi (Fig. 1d). At 21 days after incubation at 23 °C in the dark, the root samples were evaluated for the success of the fungal infection. In addition, each segment was sectioned using a sledge microtome with the infection front centered on a radial plane and visualized using a light microscope. To observe the active host responses, some segments were sectioned using the rotary microtome as described above. The visible fungal infection below the bark was recorded as successful penetration. Percentage success of fungal infection for each genotype was calculated using the following formula:

Percentage success of fungal infection

$$= \frac{\textit{Number of samples with successful fungal infection}}{\textit{Total number of samples}} \; x\; 100\%$$

Data analysis

The data were analyzed using a general linear model and the Proc GLM procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Initially, the data were checked for normality and homogeneity of variance and log transformations were performed for the longitudinal fungal growth in the wounded root segments. Tukey's Honest Significant differences were calculated to determine the statistical differences among the *Prunus* genotypes at $\alpha = 0.05$. The following statistical model was used:

$$Y_{ij} = \mu + Cov + A_i + P_j + AP + E_{ij} \quad (1)$$

Where, Y_{ij}=response variable (for example : success of fungal penetration, the longitudinal and circumferential percentage of fungal growth, percentage of sample with external callus etc.), μ = mean of parameters, Cov = root/shoot diameter as a covariate, A_i=effect of three fungal treatments, P_j = effect of *Prunus* genotype, AP = interaction effect of *Prunus* genotype and fungal treatments, and E_{ij} = residual with mean zero and constant variance (random error).

Results

Fungal infection

The wounded and intact root periderm inoculation assays resulted in successful infection of the roots. In root samples with successful fungal infection, a white mycelial mat was present underneath the root bark both longitudinally and circumferentially (Figs. 2a-d). The mycelial mat was accompanied by dark necrotic zones (Fig. 2c and d). In the wounded root inoculation assay, the overall success of the fungal infection underneath the bark was significantly different among the roots of the different *Prunus* genotypes [$F_{(27,\ 641)} = 17.26$, $P < 0.0001$)]. The data from three fungi were pooled together to determine the overall success of infection in each *Prunus* genotype. *Armillaria mellea* and *D. tabescens* had higher ability to successfully penetrate through the wounded surface as compared to *A. solidipes* [$F_{(2,\ 641)} = 8.01$, $P = 0.0004$]. However, within the roots of each genotype, the three fungi had differential success of infection [$F_{(54,\ 641)} = 2.52$, $P < 0.0001$]. Root segments of S-37 Stribling, Pisa P.S.A5, Nemaguard, Kakamas, and BY520–8, exhibited high susceptibility to infection, whereas fungi had lower success invading wounded root segments of *P. avium*, *P. cerasifera*, *P. maackii*, *P. munsoniana* #1, and *P. munsoniana* #4 (Table 3).

The overall percent of the circumference that showed fungal infection (Fig. 2a) was significantly different among the wounded roots of the different *Prunus* genotypes [$F_{(27,\ 641)} = 26.54$, $P < 0.0001$; Table 4]. The circumferential growth of *A. mellea* and *D. tabescens* was higher than that of *A. solidipes* [$F_{(2,\ 641)} = 6.68$, $P = 0.0014$]. Moreover, the genotype × fungal interaction was also significant [$F_{(54,\ 641)} = 2.96$, $P < 0.0001$]. Genotypes such as *P. munsoniana* #1–2, Bailey, and Lovell had lower percentage of circumferential infection with *A. solidipes* as compared to *A. mellea* and *D. tabescens*. Similarly, Kakamas had a high level of infection due to *D. tabescens* and compared to *A. mellea* and *A solidipes*. Overall, *P. maackii*, *P. avium*, *P. cerasifera*, and *P. munsoniana* #1 and #4, had a lower percentage of circumferential fungal infection and were therefore considered less susceptible genotypes (Table 3). Bailey, Pisa P.S.A6, MP-29, *P. mahaleb* #1–3, Pisa P.S.A5, BY520–8, and Guardian® were moderately tolerant to circumferential infection; whereas, S-37 Stribling and Nemaguard had more than 50% of their circumference infected with the fungus. The overall longitudinal length of the fungal growth underneath the bark was significantly different among the roots of different *Prunus* genotypes [$F_{(27,\ 641)} = 20.82$, $P < 0.0001$; Table 3]. In the wounded roots, the longitudinal growth rate of the three fungi was significantly different. *Armillaria mellea* was able to grow significantly faster as compared to *A. solidipes* and *D. tabescens* [$F_{(2,\ 641)} = 4.51$, $P < 0.01138$]. *Prunus* genotype and fungal interaction was also significant [$F_{(54,\ 641)} = 2.60$, $P < 0.0001$]. The overall length of fungal growth was significantly shorter in roots of *P. cerasifera*, *P. munsoniana* #3, and *P. munsoniana* #4, *P. avium*, and *P. maackii* as compared to other *Prunus* genotypes (Table 3). The overall fungal growth was longest in roots of S-37 Stribling and Nemaguard.

The percent success of fungal penetration through intact root periderm was significantly different among screened *Prunus* genotypes [$F_{(27,\ 316)} = 7.43$, $P < 0.0001$); Table 4]. The ability of *A. solidipes* to penetrate through intact root periderm was significantly lower when compared to *A. mellea* and *D. tabescens* [$F_{(2,\ 316)} = 4.61$, $P = 0.0106$]. The genotype × fungal

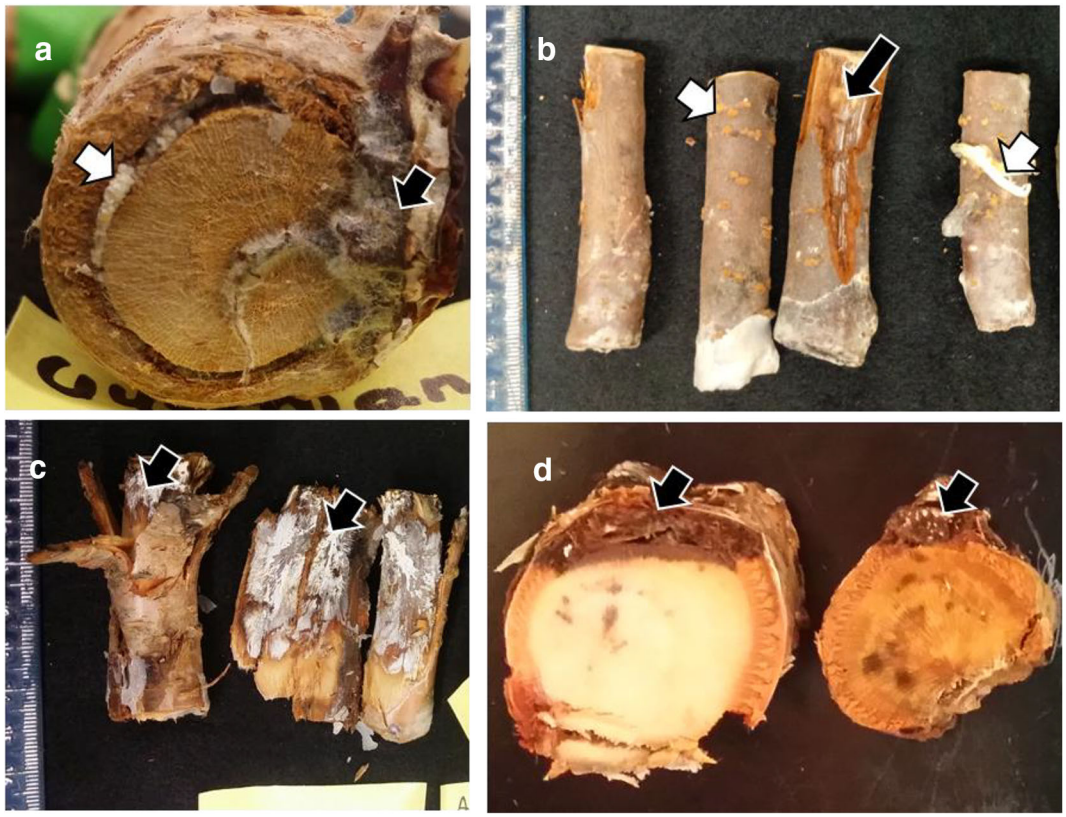

Fig. 2 *Armillaria* spp. infection of root tissues using the in vitro wounded and intact root assay. Black arrows identify the presence of fungal infection whereas white arrows identify the internal (**a**) and external callus formation (**b**), respectively. **a** - Wounded root segment of Guardian® infected with *D. tabescens* at the radial plane; **b** and **c** - Wounded root segments of *P. cerasifera* and S-37 Stribling, respectively, infected with *D. tabescens* 21 days following inoculation; and **d** - Cross-sections of intact root segments of S-37 Stribling roots showing *D. tabescens* infection

interactions were also significant [$F_{(54, 316)} = 1.72$, $P = 0.0023$] as the success of *A. mellea* and *D. tabescens* penetration was higher compared to *A. solidipes* in many of the genotypes. Overall, the three fungi were unable to penetrate through the intact root periderm of MP-29, *P. avium*, Pisa P.S.B2, Guardian®, *P. munsoniana* #1, *P. munsoniana* #2, and *P. munsoniana* #3 in the study period. The success of fungal invasion through the intact root of S-37 Stribling was 100%, therefore S-37 Stribling was regarded as highly susceptible genotype.

Histological and anatomical observations

After 21 days of incubation, host responses were evident on the surface, within, and below the root bark. The host responses were elevated in the roots from the wounded root inoculation assay as compared to the intact root inoculation assay. On the root surface, growing masses of unorganized plant cells (i.e., external callus) were evident (Fig. 2b). The frequency of root samples that formed external callus differed among genotypes. More than 90% of the root samples from *P. munsoniana* #3, *P. cerasifera*, MP-29, Tennessee Natural, and Bailey formed external callus (Table 5). The score of the callus formation, however, varied among the genotypes. *Prunus cerasifera* and MP-29 formed masses of callus. Irrespective of the fungal infection underneath the bark, roots from these genotypes formed callus on the surface and some of the calli were already organizing into growing points to give rise to new roots (Fig. 2b).

In response to fungal infection, the bark and cambium of the root compartmentalized the pathogen through a two-part process. Part 1 was the formation of a reaction zone, (i.e. colored reaction zone) which served to limit the fungal infection (Fig. 3a). Part 2 was the formation of barrier zones (i.e. a protective tissue) in the following forms: i) cellular hypotrophy at bark

Table 3 Least square means of the success of infection, longitudinal length and circumferential percent infection of the wounded root segments by all three overall fungi, *Armillaria mellea*, *Desarmillaria tabescens*, and *A. solidipes* within each *Prunus* rootstock genotype

		Overall fungi			*A. mellea*			*D. tabescens*			*A. solidipes*		
Genotype	Common name	SI%	L(mm)	CP%	SI%	L(mm)	CP%	SI%	L(mm)	CP%	SI%	L(mm)	CP%
P. maackii #1	Amur cherry	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
P. avium #3	Sweet cherry	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a	0a
P. maackii #2	Amur cherry	7a	1a	2a	0a	0a	0a	22ab	2a	5a	0a	0a	0a
P. avium #2	Sweet cherry	7a	1a	2a	0a	0a	0a	22ab	4a	5a	0a	0a	0a
P. avium #3	Sweet cherry	15ab	0a	0a	11ab	0a	0a	33bc	0a	0a	0a	0a	0a
P. maackii #3	Amur cherry	18abc	5a	11ab	11ab	1a	2a	11ab	2a	3a	33bcd	13bc	28de
P. cerasifera #3	Myrobalan plum	30bcd	5a	10a	33cd	7ab	9a	33bc	5a	12ab	22bc	0a	8a
P. cerasifera #2	Myrobalan plum	30bcd	6a	10a	22bc	6a	6a	56cd	11ab	23bc	11ab	2a	2a
P. munsoniana #1	Wild goose plum	41cde	6a	12ab	78def	14bc	30de	44bc	3a	7a	0a	0a	0a
P. munsoniana #4	wild goose plum	44cde	5a	10a	67de	8ab	19bc	44bc	4a	8ab	22bc	1a	2a
P. cerasifera #1	Myrobalan plum	52def	8ab	16bc	22bc	5a	6a	56cd	12ab	22bc	11ab	8ab	21bc
P. mahaleb #3	Mahaleb cherry	56def	8ab	20bc	78def	14bc	24cd	44bc	5a	16bc	44cde	6a	18bc
Pisa P.S.A6	Peach	63ef	12bc	22cd	60de	12bc	22cd	40bc	12ab	26bcd	83fgh	11b	18bc
P. munsoniana #2	Wild goose plum	63ef	7a	16bc	100fg	12bc	29cde	89ef	9ab	19b	0a	0a	0a
P. mahaleb #1	Mahaleb cherry	63ef	13bc	31de	78def	14bc	32de	56cd	10ab	26bcd	56def	11b	35ef
P. mahaleb #2	Mahaleb cherry	63ef	12bc	25cde	78def	21de	33de	22ab	2a	14ab	89fgh	12b	28de
MP-29	Plum x Peach	67fg	19cd	24cde	67de	21de	28cde	67cde	23bcd	26bcd	67ef	12b	18bc
Bailey	Peach	67fg	7a	20bc	100fg	13bc	40ef	67cde	7ab	17bc	33bcd	2a	4a
P. munsoniana #3	Wild goose plum	67fg	7a	16bc	67de	14bc	15bc	89ef	11ab	23bc	44cde	5a	11ab
Pisa P.S.B2	Peach	74fgh	16bcd	32de	67de	10ab	27cde	75de	25bcd	41cde	80fgh	13bc	28de
Tennessee Natural	Peach	78 fgh	12bc	38ef	89ef	11abc	42efg	67cde	14abc	45efg	78 fg	14bc	28de
Guardian®	Peach	78 fgh	13bc	36efg	78def	10ab	41ef	89ef	14abc	40def	67ef	14bc	28de
Lovell	Peach	81fgh	12bc	42 fg	100fg	16cd	55gh	89ef	11ab	48efg	56def	9ab	24cde
BY520–8	Peach	81fgh	12bc	34ef	89ef	17cd	39def	89ef	8ab	38def	67ef	13bc	26cde
Kakamas	Peach	94gh	19cd	47fgh	100fg	14bc	38def	100f	26cd	79i	83fgh	17c	29de
Nemaguard	Peach	96h	31f	60hi	100fg	24def	52fgh	100f	38e	66hi	89gh	50d	62h
Pisa P.S.A5	Peach	100h	28ef	36efg	100fg	17cd	29cde	100f	14abc	29cde	100 h	52d	50g
S-37 Stribling	Peach	100h	36f	64i	100fg	24def	46fgh	100f	38e	69hi	100 h	50d	81i

SI %: Percentage success of infection; L: Length of infection, CP%: Circumferential percentage of infection. Different letters indicate Tukey's Honest Significant differences among different genotypes within each column at $\alpha = 0.05$

margin (Fig. 3a), ii) necrophylactic periderm in the bark (Fig. 3b), iii) new cells in the bark (Fig. 3c), and iv) internal callus around the cambial region (Fig. 3d). The formation of a barrier zone was more evident in wound inoculated roots as compared to the roots from the intact bark inoculation assay.

When the wound inoculated root bark was peeled back, a colored reaction zone was distinctly visible around the fungal infection in root barks of *P. avium*, *P. cerasifera*, *P. maackii*, Kakamas, Guardian®, Pisa P.S.A5, and Pisa P.S.A6 (Table 5). No colored reaction zone was observed in genotypes S-37 Stribling, *P. mahaleb*, Nemaguard, Lovell, *P. munsoniana* #1, BY520–8, and *P. munsoniana* #2 (Table 5). Of the genotypes that formed a colored reaction zone, *P. avium*, *P. maackii*, and Pisa P.S.A5 formed the most distinct zones. There was no evidence of fungal infection beyond these zones in these genotypes. These colored reaction zones were also visible in the root cross-sections (Fig. 3a-b).

Table 4 Least square means of the percentage of fungal success in penetrating the intact root periderm of various *Prunus* rootstock genotypes

Genotype	Common name	Success of fungal penetration (%)			
		Overall fungi	*A. mellea*	*D. tabescens*	*A. solidipes*
Guardian®	Peach	0a	0a	0a	0a
MP-29	Plum x peach	0a	0a	0a	0a
P. avium #1	Sweet cherry	0a	0a	0a	0a
P. avium #2	Sweet cherry	0a	0a	0a	0a
P. maackii #3	Amur cherry	0a	0a	0a	0a
P. munsoniana #1	Wild goose plum	0a	0a	0a	0a
P. munsoniana #2	Wild goose plum	0a	0a	0a	0a
P. munsoniana #4	Wild goose plum	0a	0a	0a	0a
Pisa P.S.B2	Peach	0a	0a	0a	0a
P. maackii #2	Amur cherry	7a	0a	0a	20bc
P. maackii #1	Amur cherry	17ab	0a	25bc	25bc
P. munsoniana #3	Wild goose plum	17ab	50cd	0a	0a
Bailey	Peach	20bc	0a	60de	0a
P. avium #3	Sweet cherry	20bc	40cd	20bc	0a
P. cerasifera #2	Myrobalan plum	20bc	50de	60de	40cd
Pisa P.S.A5	Peach	25bc	0a	25bc	50d
P. cerasifera #1	Myrobalan plum	27bc	40cd	40cd	0a
Nemaguard	Peach	30bcd	0a	20bc	0a
P. mahaleb #3	Mahaleb cherry	33bcd	25bc	33cd	40cd
Lovell	Peach	37cd	20bc	40cd	50d
BY 520–8	Peach	47cde	40cd	100f	0a
P. mahaleb #1	Mahaleb cherry	50de	100f	25bc	25bc
P. mahaleb #2	Mahaleb cherry	50de	60de	50de	40cd
Pisa P.S.A6	Peach	50de	80ef	20bc	50d
Tennessee Natural	Peach	50de	50de	100f	0a
Kakamas	Peach	67e	75e	100f	25bc
S-37 Stribling	Peach	100f	100f	100f	100e

Different letters indicate Tukey's Honest Significant differences among different genotypes within each column at $\alpha = 0.05$

As a first indication of barrier zone formation process, cellular changes indicated by tissue hypertrophy were seen internally adjoining the primary infection point in roots with both wounded and intact bark in all the evaluated materials (Fig. 3a). Internal to the hypertrophied cells, a new necrophylactic periderm had formed or was initiating to form in the bark of most of the genotypes except *P. avium* #2, Tennessee Natural, Nemaguard, and S-37 Stribling (Table 5; Fig. 3a). Necrophylactic periderms were forming around phellem (cork cambium) cells (Fig. 3b), but the development was not always continuous along the periphery of the infection zone. In roots that formed necrophylactic periderm, newly restored phellogen had produced thin layers (few cell layers) of phellem cells (Fig. 4a-c). Although most of the genotypes were forming necrophylactic periderm, its formation was not always successful to halt the advancement of the fungal infection to the cambium (Fig. 4f). Necrophylactic periderm formation was more frequently successful to halt the fungal infection in *P. cerasifera* #1–3, *P. munsoniana* #1–4, MP-29, *P. avium* #1 and #3. Additionally, below the infection point in the bark new cells were being formed in the spatial positions (Figs. 3c and 5a) in all the genotypes. These new cells forming at the spatial locations were able to successfully capsulate around the fungal hyphae

Table 5 Description of host responses following three fungal inoculation for the wounded bark assay. ec - percentage of sample with external callus; s-ec - scores of ec; s-crz - colored reaction zone; s-np - necrophylactic periderm; s-irb - strength of induced response in bark; s-ic - level of internal callus; ac - activity of cambium; s-irc - strength of induced response in cambium

Prunus genotype	Common name	ec (%)	s-ec	c-crz	s-np	s-irb (%)	s-ic	ac	s-irc (%)
P. munsoniana #3	Wild goose plum	100 a	2	0	2	50	1	1	50
P. avium #1	Sweet cherry	100 a	1	3	1	63	1	1	50
P. avium #2	Sweet cherry	100 a	1	2	1	50	0	1	25
P. cerasifera #1	Myrobalan plum	100 a	3	1	1	63	3	1	100
P. cerasifera #2	Myrobalan plum	96.3 a	3	1	1	63	3	1	100
P. cerasifera #3	Myrobalan plum	96.3 a	3	1	1	63	2	1	75
P. munsoniana #1	Wild goose plum	92.59 a	1	0	2	38	1	1	50
MP-29	Plum x peach	92.59 a	3	0	1	50	2	1	75
Tennessee Natural	Peach	88.89 ab	2	1	1	50	0	0	0
Bailey	Peach	83.33 b	1	2	0	38	1	1	50
Pisa P.S.B2	Peach	48.14 c	2	1	1	50	0	1	25
BY520–8	Peach	47.62 c	2	0	1	38	2	1	75
P. avium 3	Sweet cherry	44.83 c	1	2	1	50	1	1	50
P. munsoniana #2	Wild goose plum	44.45 c	1	0	2	38	1	1	50
P. munsoniana #4	Wild goose plum	44.44 c	1	1	2	50	0	1	25
Kakamas	Peach	22.22 d	1	1	1	38	0	1	25
Guardian®	Peach	14.81 e	0	0	1	13	1	1	50
Pisa P.S.A6	Peach	12.5 e	2	1	1	50	1	1	50
Lovell	Peach	11.11 e	2	0	1	38	1	1	50
Nemaguard	Peach	4.34 f	0	0	0	0	1	0	25
Pisa P.S.A5	Peach	0 f	0	2	1	38	1	1	50
S-37 Stribling	Peach	0 f	0	0	0	0	0	0	0
P. maackii #1	Amur cherry	0 f	0	3	1	50	1	1	50
P. maackii #2	Amur cherry	0 f	0	3	1	50	1	1	50
P. maackii #3	Amur cherry	0 f	0	3	1	50	1	1	50
P. mahaleb #1	Mahaleb cherry	0 f	1	0	1	25	1	1	50
P. mahaleb #2	Mahaleb cherry	0 f	1	0	1	25	1	1	50
P. mahaleb #3	Mahaleb cherry	0 f	1	0	1	25	1	1	50

in genotypes such as *P. cerasifera* (Fig. 3c) and MP-29 (Fig. 5a) but not in Guardian (Fig. 5b), Lovell (Fig. 5c), S-37 (Fig. 4d), and others. The roots of the genotype *P. cerasifera* showed reactions that were consistent with attempts to close the wound and limit the infection. The host roots that were able to form a thick layer of new cells around the margin of the wound had no necrosis extending down to the vascular cambium (Fig. 5a).

In roots exhibiting expansion of the infection up to the cambium, a thin to a thick layer of the new cells or internal callus was evident (Fig. 3d). Bark and cambial cell death associated with the *Armillaria* spp. and *D. tabescens* infection stopped before it circled the circumference of the root (Fig. 2a) in most of the genotypes. The cambium beyond the point of infection was still active in all genotypes except S-37 Stribling, Nemaguard, and Tennessee Natural (Table 5). The living cambium beyond the lateral and vertical limits of the infected area showed evidence of new internal callus formation that would result in continued secondary growth of the root, and/or a limit to the radial and circumferential spread of the pathogen into the healthy tissues (Fig. 3e). *Prunus cerasifera* formed high levels of internal callus followed by MP-29 and BY520–8

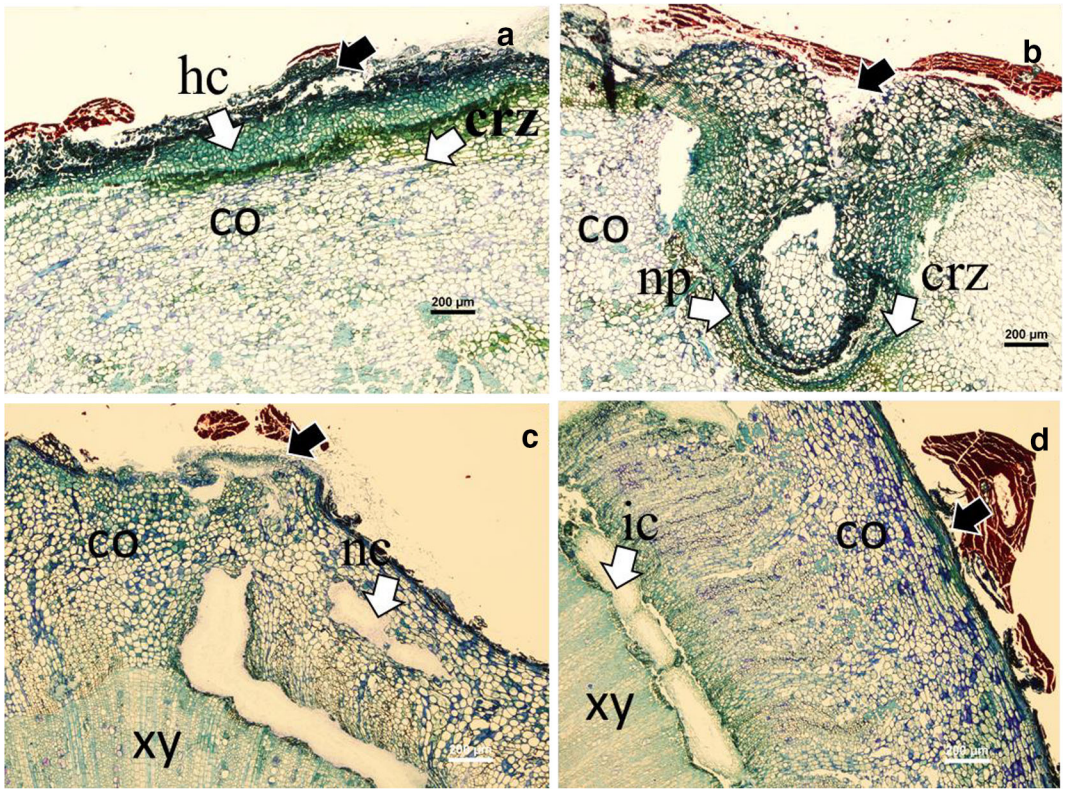

Fig. 3 Transverse cross-sections of *P. avium* and *P. cerasifera* root segments with wounded root inoculation assay. Black arrows identify the fungal infection/hyphae and white arrows identify active host responses. **a** - Formation of hypertrophied cells (hc) and colored reaction zone (crz) in the cortex (co) of *P. avium* inoculated with *A. mellea*; **b** - Formation of necrophylactic periderm (np) in *P. avium* root inoculated with *A. mellea*; **c** - Formation of new cells (nc) in the bark of *P. cerasifera* root enclosing *D. tabescens* infection; and **d** - Formation of internal callus (ic) in the cambial region of *P. cerasifera* root inoculated with *A. mellea.* xy: Xylem, Bars = 200 µm

(Table 5). The overall strength of the measured induced response in cambium (S-IRC) was highest in *P. cerasifera* #2–3, followed by *P. cerasifera* #1, MP-29, and BY520–8 (Table 5).

All three inoculated fungi were being compartmentalized either in the root bark or cambial region of some of the genotypes (Figs. 3, 4, and 5) with the frequency of the success of compartmentalization lower in the highly susceptible genotypes as compared to the less susceptible genotypes. Compartmentalization was more evident in wound inoculated roots as compared to the roots from the intact bark inoculation assay. The percentage of the collective active responses (external callus, colored reaction zone, and np) in the bark was highest in *P. avium* #1 and *P. cerasifera* #2–3, and was intermediate in *P. munsoniana* #1–4, MP-29, *P. avium* #2–3, *P. maackii* #1–3, Pisa P.S.B2, and Pisa P.S.A6 (Table 5). In the roots from the intact bark inoculation assay, these host responses were not well developed as the epidermis of the roots were not completely breached to provoke induced host responses compared to wounded roots and are therefore not described exclusively.

Lignification and suberization of the tissues were frequently detected around the infection site. Although such cellular changes did not appear to fully limit the extending fungal infection in most of the genotypes the frequency of infection beyond the lignified cells was very low in *P. munsoniana* #1, 2, 3, and 4 (Fig. 5f-g). Genotype *P. munsoniana* had low level of infection even though other host defenses were low. In the periderm of MP-29, there was an accumulation of granular starch-like particles around the inoculation site (Fig. 5d). Furthermore, a thin reaction zone visualized as a distinct darker band referred to as zone line was observed in the xylem of some of the genotypes. Zone line formation is regarded as a fungal strategy to capture host resources rather than a host defense strategy.

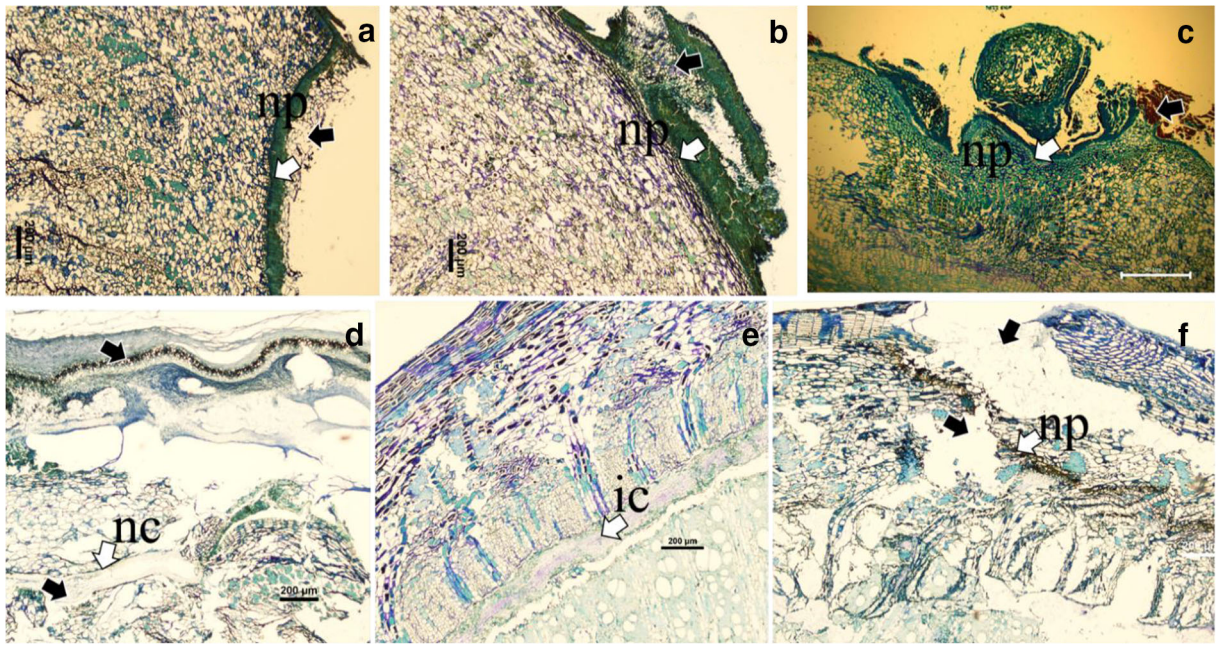

Fig. 4 Transverse cross-sections of various *Prunus* genotypes for un-inoculated control and *Armillaria/Desarmillaria* inoculated wounded and intact periderm root assays. Black arrows identify the presence of fungal infection/hyphae and white arrows identify active host responses. **a** - Necrophylactic periderm (np) formed by root of *P. munsoniana* #1 under *A. solidipes* wound inoculation; **b** - *A. solidipes* being walled off by intact bark of *P. munsoniana* #1; **c** - *D. tabescens* infected tissue being compartmentalized by np formation in wound inoculated root of *P. munsoniana* #4; **d** - *A. mellea* infection spreading around the bark of S-37 Stribling and thin layer of newly formed cells (nc) unable to compartmentalize the fungal spread and infection; **e** - Healthy wounded root of *P. mahaleb* #1 with internal callus (ic) formation around the cambial region; and **f** - Spread of *D. tabescens* infection in *P. mahaleb* #1 root following inoculation with wounded root inoculation assay. Bars = 200 µm (a, b, d, e, and f) and 50 µm (c)

Discussion

This study identified variation in susceptibility of *Prunus* rootstock genotypes to three causal species of ARR: *A. mellea*, *A. solidipes*, and *D. tabescens*, based on two previously developed assays using field-grown roots (Devkota and Hammerschmidt 2019). The wounded root inoculation assay permitted an assessment of the relative levels of fungal infection and the early evaluation of varying degrees of associated active host root responses among the genotypes, whereas the intact root periderm inoculation assay permitted an assessment of the relative ability of secondary plant tissues (rhytidome and periderm) of each genotype to resist the fungal infection. Considering both assays, none of the tested genotypes were completely resistant to *Armillaria* and *Desarmillaria* spp. Although the wounded roots of *P. maackii* #1 and *P. avium* #3 showed no successful infection, they were successfully infected through the intact bark, implying that those genotypes were also not completely resistant. One possible explanation for this result is that in wounded roots, layers of periderm are exposed, which may lead to a rapid accumulation of antifungal metabolites and other enhanced host defense responses around the inoculation point. Although MP-29, Guardian®, *P. avium* #1 & #2, *P. maackii* #3, *P. munsoniana* #1, #2, and #4 did not show any fungal penetration through the intact root periderm, the fungus was able to grow both longitudinally and circumferentially when the bark was wounded. The susceptibility of the tested *Prunus* rootstock genotypes to *Armillaria* spp. and *D. tabescens* infection was manifested in the root's living bark and cambium.

As a group, the peach rootstocks were most susceptible to *A. mellea, A. solidipes,* and *D. tabescens* infection for both of the assays, with S-37 Stribling being the most susceptible. Not only was the fungus able to breach the root periderm of this genotype, but it was able to spread substantially around the root circumference. This may be because the strength of induced responses in the bark and cambium following fungal inoculation was weakest in this genotype. Genotypes Pisa P.S.A5, Pisa P.S.A6, and Bailey were relatively less susceptible to *D. tabescens* as the fungus was able to spread to a

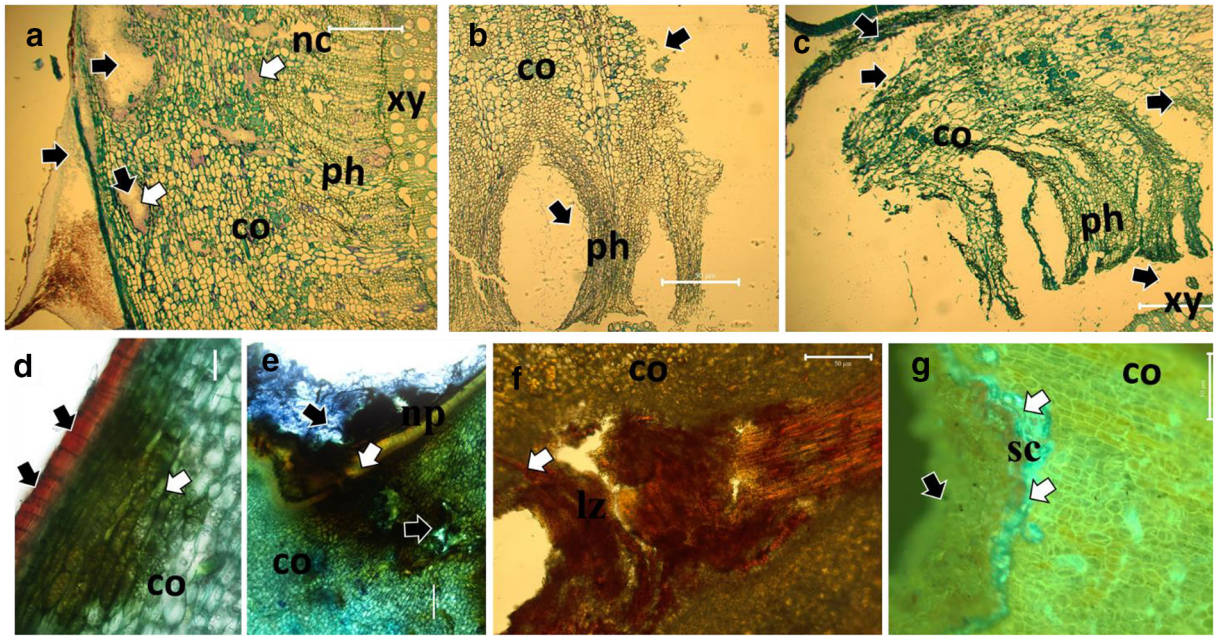

Fig. 5 Transverse cross-sections of MP-29, Guardian®, and *P. munsoniana* #1 under *Armillaria* spp. inoculated wounded and intact periderm root inoculation assay. Black arrows identify the fungal infection/hyphae and white arrows identify the active host responses. **a** - Newly formed cells in the cortex of MP-29 enclosing spread of *A. mellea* under wound inoculation assay; **b** - Spread of *A. mellea* up to xylem after wound inoculation of Guardian®; **c** - Spread of *A. mellea* infection in Lovell following *A. mellea* inoculation in wounded root assay; **d** - Accumulation of starch-like particles in the intact periderm of *D. tabescens* inoculated MP-29; **e** - Fungal penetration breaching the necrophylactic periderm and lignified cells in *D. tabescens* inoculated Lovell root under intact periderm inoculation assay; **f** and **g** - *P. munsonina*#1 root with lignified zone (lz) and suberized cells (sc) around the infection point in *A. mellea* inoculated wounded root. Bars = 50 μm (a, b, c, f, and g) and 100 μm (d-e)

limited length in the root circumference of these genotypes. In these genotypes, internal callus, a visible colored reaction, and activity of cambium may have contributed to limiting *D. tabescens* spread. In contrast, the highly susceptible S-37 Stribling had inactive cambium, breached internal callus, and no visible presence of a colored reaction zone. The colored zone may be indicative of the accumulation of constitutive secondary metabolites that may impede the fungal growth. Bailey, which appeared to be less susceptible than the other peach rootstocks in the inoculation assays, has not been reported susceptible or tolerant to *Armillaria/Desarmillaria* where it is planted in the northern states. It is likely that the reaction zone that was observed in the bark of this genotype may consist of secondary metabolites that may or may not have antifungal properties against *Armillaria*/*Desarmillaria*. Antifungal properties of such secondary metabolites must be studied before making any conclusion. In other peach rootstocks, Nemaguard, Lovell, and Guardian®, which are also known to be susceptible to *D. tabescens* in the southeastern U.S. (G. Reighard, personal communication), the strength of the induced response observed in the bark and cambium was so low that it provided little protection against infection.

In general, the roots of two of the three cherry species, *P. avium*, and *P. maackii* showed relatively low susceptibility to *Armillaria* spp. compared to peach and the cherry species, *P. mahaleb*. All three *P. maackii* genotypes and *P. avium* #1 exhibited a high level of colored reaction zone, suggesting the possibility of antifungal activity of these genotypes. Proffer et al. (1988) conducted a 27-month trial to compare the susceptibility of *P. avium*, *P. mahaleb*, and 17 *Prunus* hybrid rootstocks with *A. mellea, A. solidipes, and A. gallica*. They found that *P. avium* was relatively less susceptible to these *Armillaria* spp. compared to *P. mahaleb*. Field observations also indicated that ARR is more common on cherry trees grafted to *P. mahaleb* than *P. avium* (Proffer et al. 1988). Similarly, Warnstrom et al. (2011) reported a low susceptibility of *P. maackii* to *A. solidipes* in vitro, which was further corroborated by our findings. However, preliminary field trials suggest that *P. maackii* does not show evidence of reduced

susceptibility to *A. solidipes* compared to *P. mahaleb* (A. Iezzoni, personal communication).

The plum and plum hybrids exhibited low susceptibility to *Armillaria* spp. and *D. tabescens* compared to the peach genotypes. All plum genotypes were less susceptible to *A. solidipes* compared to *A. mellea* and *D. tabescens*. Genotypes, *P. munsoniana* #2, *P. munsoniana* #3, *P. cerasifera* #1–3 were more susceptible to infection by *D. tabescens* compared to *A. mellea*. *P. munsoniana* #4 was less susceptible to *A. mellea* infection compared to *D. tabescens* suggesting that the effect of fungi may be *Prunus* genotype specific. The variation in relative susceptibility between peach and plum genotypes is consistent with previously reported field trials (Beckman and Pusey 2001; Beckman et al. 1998; Guillaumin et al. 1991; Proffer et al. 1988). Guillaumin et al. (1991) conducted a 10-year field trial in France to determine the relative susceptibility of peach and plum derived ungrafted rootstocks to *A. mellea* and found peach derived rootstocks to be relatively susceptible compared to plum. In our in vitro assay, we found similar results for the plum rootstocks, *P. cerasifera* and *P. munsoniana*. Low susceptibility to *Armillaria spp.* may be a dominant characteristic of plum. Guillaumin et al. (1991) suggested that the levels of ARR susceptibility of the hybrids *P. cerasifera* × peach and *P. cerasifera* × (*P. cerasifera* × peach) is about the same as that found with *P. cerasifera*.

Low susceptibility of plum rootstocks to ARR may be due to their ability to generate a high level of external callus which can organize into new root growing points (i.e., *P. cerasifera*, MP-29), to impede the fungal growth by forming thick barrier zones such as necrophylactic periderm (i.e., *P. munsoniana*) and to generate new cells or internal callus that can compartmentalize the fungus and prevent the further growth of the pathogen (i.e. *P. cerasifera*) inside the root tissue.

Necrophylactic periderm formation was nearly always complete in *P. munsoniana* #1, 2, 3, and 4, and breaching was rarely observed. The ability of roots to compartmentalize the pathogen (i.e. *P. munsoniana*) and form new growing points from external callus (*P. cerasifera*) on the surface may be an important characteristic for root fitness and survival of the tree infected with *Armillaria*/*Desarmillaria* spp. Additionally, *A. mellea*-host interactions was shown to involve a compensatory mechanism, where pathogen attack was compensated for by induction of regrowth of new root tissues without a concomitant reduction in root fitness (Shigo and Tippett 1981). This may be the reason plums continued to survive in the field with low levels of fungal infection in field studies by Beckman and Pusey (2001) and Guillaumin et al. (1991). Our conclusions are in accordance with Oven and Torelli (1999), who reported that the ability of the trees to successfully contain *Armillaria* spp. may depend on the rate of development and efficacy of these barriers and callus production. The barrier zone formed by cambium after infection has been reported to serve to disconnect the infected wound from the healthy wood that continues to form after the zone is completed (Pouzoulet et al. 2013).

Peach tree short life (PTSL) is another complex disease problem for peach growers in the southeastern U.S. Guardian® is a commercially popular peach rootstock due to its improved resistance to PTSL. Guardian® and MP-29 (a clonal plum-peach interspecific hybrid released in 2011) are reported to have a similar level of field tolerance to PTSL. MP-29 is preferred over Guardian® due to its low field susceptibility to ARR (Beckman 2008; Beckman et al. 2012). A field trial in Georgia revealed that trees on Guardian® had mortality exceeding 50% due to ARR after only six growing seasons, whereas MP-29 did not reach this threshold until 12 growing seasons. In our wounded root inoculation assay, we observed that MP-29 had a relatively lower level of circumferential *Armillaria*/*Desarmillaria* infection as compared to Guardian®. The relatively low susceptibility of MP-29 compared to Guardian® in the field and our screening test might be because of the relatively high strength of induced responses present in the bark of MP-29. Also, some granulated starch-like particles accumulated in the cortex (possibly a key process in host resistance) of MP-29 but not in Guardian®. The role of accumulation of such particles around the infection site in ARR resistance of MP-29 merits further investigation. Also, the ability of MP-29 to form external callus was extraordinarily high compared to Guardian®. Although we did not look at the tree mortality due to ARR, our results suggest that the force of the host response is higher than the infection force of *Armillaria* spp. and *D. tabescens* in MP-29 compared to Guardian®. Shigo and Tippett (1981) suggested that when the *Armillaria* infection force is stronger than the compartmentalizing force of the tree, the tree declines and may die. This information also emphasizes the value of describing the host resistance responses along with observations of the fungal infection when screening *Prunus* genotypes for ARR susceptibility.

Evaluation of the circumferential spread of the infection was an important observation, as the genotypes varied in their ability to hinder the pathogen spread around the root circumference. Although the growth of *Armillaria* spp./*D. tabescens* is primarily spreading longitudinally, the circumferential growth was limited only to a certain portion of the root circumference in all the genotypes. In the less susceptible genotypes such as *P. cerasifera*, *P. munsoniana*, *P. avium*, and *P. maackii,* fungal spread and the infection stopped before it circled a larger percentage of the root. The activity of the living cambium beyond the point of infection played a partial role in limiting the circumferential spread of the pathogen in all genotypes. Shigo and Tippett (1981) conducted large-scale assessments of root systems of *A. mellea* infected trees within eight different genera. They reported that bark killing associated with the spread of *A. mellea* stopped before it circled the root or root collar area as living cambium beyond the limits of *Armillaria* infected bark area has the ability to produce xylem cells that develop into the barrier zones. They further suggested that as the healthy/uninfected circumference of the root or root collar area decreases, any additional *A. mellea* infection may lead to tree death. Thus, it appeared that evaluating the circumferential percentage of the fungal spread is important in screening susceptibility of *Prunus* rootstock genotypes to *Armillaria*/*Desarmillaria* because in the field tree death is more likely as the fungus-infected circumference in a root increases.

Lignification and suberization of the cells around the infection point were evident in most of the material in this study. In highly susceptible genotypes (e.g. S-37, Lovell), such cellular changes appeared to limit pathogen ingress at a lesser frequency as compared to less susceptible genotypes (e.g. *P. munsoniana*). The fungus was also able to grow through these barriers to spread into the surrounding tissues more frequently in susceptible genotypes. In the case of plum genotypes, there were cases where infection was absent even when host responses were not induced, and this could be because of the cell wall constituents of the epidermis that acted as the first line of barrier against infection. The composition of lignin and suberin and other cell-wall constituents was not determined in the genotypes studied, suggesting a need for future biochemical analysis of these components in the root bark.

Host resistance to *Armillaria/Desarmillaria* spp. was not limited to a single mechanism, but several collective host responses were identified. The strength of these collective responses influenced the process of compartmentalization of the pathogen or the infected tissue. Effective compartmentalization of the pathogen confers low pathogen susceptibility in any host. In general, ARR infected roots of MP-29 and *P. cerasifera* could rapidly form new cells to prevent the further growth of pathogen. They also form high level of external callus tissues and some of callus cells may organize into growing points to give rise to roots. Plums, *P. munsoniana* #1 and 4, had some properties in the root epidermis that blocked the fungal infection and could rapidly form new periderm, which also could block fungal infection. Cherry roots (*P. avium* and *P. maackii*) showed distinct colored reaction zones possibly with deposition of phenolic compounds. Comprehensive studies, including time-course, biochemical analysis of cell-wall constituents, and histological and subcellular transcriptome analysis to explore reaction-zone and barrier zone formation in less susceptible vs. highly susceptible genotypes, are needed to establish their roles in the defense mechanisms of *Prunus* genotypes to *Armillaria/Desarmillaria* spp. susceptibility.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical statement All authors have approved the manuscript and agreed with its submission to European Journal of Plant Pathology. The submitted work is original and have not been submitted or published elsewhere. The manuscript has been prepared following principles of ethical and professional conduct. The study does not involve human participants or animals.

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