

SHORT NOTES

Phlorizin released by apple root debris is related to apple replant disease

LIDIA NICOLA^{1,*}, URSKA VRHOVSEK¹, EVELYN SOINI¹, HERIBERT INSAM² and ILARIA PERTOT¹

¹ Research and Innovation Center, Fondazione Edmund Mach, 38010 San Michele all'Adige (TN), Italy

² Department of Microbiology, University of Innsbruck, 6020 Innsbruck, Austria

Summary. Autotoxic compounds are likely to be among the causes of apple replant disease, but their secretion is low during plant life. Using targeted metabolomics, the changes in soil phenolic profile were analyzed after the addition of apple roots, and their potential autotoxicity was assessed on apple seedlings. The addition of apple roots severely damaged the plants, attributed to autotoxic action of the phenolic compound phlorizin. Prolonged residence time of the roots in the soil before planting reduced their negative action, probably due to the degradation of phlorizin.

Key words: allelochemicals, phenolic compounds, soil, autotoxicity, continuous cropping obstacle.

Introduction

Apple replant disease (ARD) is a complex syndrome arising from the repeated replanting of apple trees in the same soil; the main symptom is reduced plant growth, particularly root biomass. This syndrome is related to biotic factors (i.e. increased concentrations of pathogenic fungi, decrease in plant growth promoting bacteria) and, possibly, abiotic factors in soil, although the precise etiology is still unclear (Mazzola and Manici, 2012). One of the possible biotic causes of ARD is autotoxicity, in which the phenolic compounds released by roots may play an important role (Huang *et al.*, 2013). The roots of apple trees can release several different phenolic compounds and some of them (phlorizin, *p*-hydroxybenzoic acid, *p*-hydroxy hydrocinnamic acid, phloroglucinol) were found in liquid cultures (Börner, 1959). However, root exudation of these substances is quite low during the lifespan of apple plants (Hofmann *et al.*, 2009). On the other hand, phenolic compounds

released from decomposing apple leaves and roots (1% in soil) may reach high concentrations, as demonstrated by Politycka and Adamska (2003). In the present study, we increased the quantity of root material added to soil by up to 20% of its volume.

In-field studies investigating the causes of ARD are of extremely difficult interpretation, because of the high number of factors that could be involved. We therefore studied the phenomenon with an artificial setup under controlled conditions, where only the factor 'effect of roots on new plants' varied. Sampling was performed at 0, 3 and 7 months at the most active temperature (20°C), to specifically identify and quantify the phenolic compounds released during the decay of apple roots, using Ultra High Performance Liquid Chromatography (UHPLC) coupled to a mass spectrometer. Furthermore, we tested root autotoxic potential on apple seedlings in soil.

Materials and methods

Experimental design and plant growth measures

Healthy roots (<3 mm diam) were collected from explanted apple trees (rootstock M26) in the Tren-

Corresponding author: L. Nicola
E-mail: lidia.nicola@fmach.it

tino-South Tyrol region (Italy) on 26 January 2015. They were ground and mixed (1:5, v:v) with sieved soil (loam; pH 7.7; 52 g kg⁻¹ of organic matter) taken from an uncultivated area (treatment R3). The soil was divided into two portions that were used to repeat the experiment twice in the same conditions. Sieved soil without any addition of ground roots served as an untreated control (treatment C3). After gentle watering (20 mL kg⁻¹ of soil), both soils (R3 and C3) were kept under controlled conditions (20°C) in the greenhouse for 90 d. The same protocol was repeated three months later (4 May 2015) using the soil collected in January, which was kept in natural conditions in the meantime, and a soil mixed with root debris (treatment R0) and an untreated control soil (C0) were obtained. Apple seedlings, grown in peat from seeds of the cv. Fuji in peat, were transplanted at the age of 90 d into the four treated soils (R0, C0, R3, C3), with three soil samples being collected from each soil treatment for analysis of phenolic compounds before transplanting (time T1). The soil samples were also checked for absence of the three main apple tree pathogens, *Armillaria* spp., *Phytophthora cactorum* and *Rosellinia necatrix*, using diagnostic PCRs, according, respectively, Lochman *et al.* (2004), Bhat and Browne (2010) and Pasini *et al.* (2016). Fifteen replicates (pots) per soil treatment, having one seedling each, were held at 20 ± 0.5°C in a greenhouse. After 120 d, the chlorophyll content of the apple seedling leaves was measured (SPAD502, Spectrum Technologies) and the fresh weights of whole plants and roots were assessed. At the same time, three soil samples per treatment were taken from the pots and subjected to phenolic compound analysis (time T2). During the experiment the plantlets did not show any symptoms ascribable to root infections of microbial pathogens.

Analysis of phenolic compounds

Samples were extracted as described in Vrhovsek *et al.* (2012). After evaporation of methanolic fractions, samples were applied to a preconditioned ENV+ Isolute C18 SPE column. Preconditioning was performed by purging the column with 10 mL of methanol and 20 mL of water. After loading a sample onto the column, it was washed with 10 mL of water. Polyphenols, retained in the column, were eluted with 20 mL of methanol. Solvent was evaporated using a rotavapor and the residues were dissolved in

500 µL of a methanol/water mixture (2:1). Samples were injected before and after concentration using SPE. Phenolic compounds were analyzed according to Vrhovsek *et al.* (2012), with a method that allows the detection of a total of 135 different phenolic compounds. Briefly, UHPLC (Waters Acquity UPLC - Milford) coupled to a mass spectrometer (Waters Xevo TQMS - Milford) was used. Separation of the compounds was achieved on a Waters Acquity HSS T3 column 1.8 µm, 100 mm × 2.1 mm (Milford), kept at 40°C. Mobile phase A was water containing 0.1% formic acid; mobile phase B was acetonitrile containing 0.1% formic acid. The chemicals used for the analysis were purchased from Sigma Aldrich.

Statistical analyses

Statistical analyses was performed with PAST, version 2.17 (Hammer *et al.*, 2001) and Statistica 9 software (StatSoft). An F-test was used to demonstrate non-significant differences between the two repetitions of the experiment ($P > 0.05$) and data on plant growth were pooled. Since the distribution of data was not normal, statistically significant differences between treatments ($P < 0.05$) were assessed with the Kruskal-Wallis test with Mann Whitney pairwise comparisons (Bonferroni corrected). During analysis of the phenolic compounds, values below the Limit Of Detection (LOD) were substituted with LOD/√2 (Verbovšek, 2011). Once homogeneity of variance assessed with Levene's test ($P > 0.05$) was satisfied, non-metric multidimensional scaling (NMDS), one-way analysis of similarities (ANOSIM), similarity percentage analysis (SIMPER) and the Wilcoxon test were employed to assess the difference in composition in the phenolic profile of soils. Pearson's correlation was calculated to determine the relationship between the concentrations of phenolic compounds and plant weights.

Results and discussion

Diagnostic PCRs (*Armillaria* spp., *Phytophthora cactorum*, *Rosellinia necatrix*) did not amplified any products, therefore we excluded the presence of apple root pathogens in the soil treated with roots. The soil treatments affected seedling growth. In particular, seedlings planted in soil immediately after mixing with root debris (treatment R0) showed lower chlorophyll content and total seedling weight com-

pared with all other treatments (Table 1, Kruskal-Wallis and Mann Whitney pairwise test, $P < 0.05$). The mean root weight in the R0 treatment was only significantly less than R3 and C3 treatments. The addition of apple roots to soil just before planting therefore significantly impaired the health of the seedlings, showing marked autotoxic effects on the plants and not just on their root systems.

Our results indicate that this autotoxic effect of roots on new plants was visible in the soil, and not only in water cultures (Börner, 1959). In contrast, Politycka and Adamska (2003) found a stimulating or slightly inhibiting effect of apple roots on radical growth of cucumber, results that could be due to the use of a different plant species and/or lower concentrations of apple roots in the soil. The artificial experimental set up allowed us to separate the effect of roots on new plants, without confounding effects from other factors.

Fourteen phenolic compounds were detected in soil samples at time T1 (preplanting). The concentrations of these compounds were generally low, with the exception of phlorizin, phloretin and narigenin (Table 2). An NMDS (stress = 0.078, R^2 axis 1 = 0.992, axis 2 = 0.085) on Euclidean distances of the dataset indicated that data points representing the samples from R0 soil clustered together, separated from the other cluster, which comprised samples from the R3, C0 and C3 treatments (Figure 1A). A one-way ANOSIM with Bonferroni-corrected pairwise comparisons, confirmed the difference between the phenolic profile of R0 samples and all the other samples ($P < 0.05$).

The concentration of four phenolic compounds, *p*-coumaric acid, quercetin-3-rhamnoside, phloretin and phlorizin, significantly increased in R0 treatment

soils, compared to C0 (Wilcoxon test, $P < 0.05$). These compounds are all considered to be allelochemicals in apple and in other plants (Huang *et al.* 2013; Inderjit and Dakshini, 1995). In the R0 treatment, the concentrations of all these compounds, but not *p*-coumaric acid, were also significantly greater than those in R3, meaning that after 3 months of roots in the soil, these substances had degraded. A significant negative correlation was found between the sum of the concentrations of the single phenolic compounds measured at T1 and total plant weight (Pearson correlation $r = -0.89$, $P < 0.05$), so a high concentration of polyphenols at planting corresponded to diminished plant growth. In order to detect which phenolic compounds were most responsible for the difference in R0 soils, SIMPER was used. This indicated phlorizin as the phenolic compound contributing to more than 90% of inter-group dissimilarity between R0 and the other treatments, and phloretin as the second most important compound (approximately 5%). In the R0 samples, phlorizin and phloretin reached average concentrations, respectively, of $77.4 (\pm 8.0)$ and $3.7 (\pm 0.9) \mu\text{g g}^{-1}$, while in the other samples phlorizin concentrations were $< 0.1 \mu\text{g g}^{-1}$ and phloretin $< 0.06 \mu\text{g g}^{-1}$.

We therefore confirm the trend for polyphenol concentrations observed by Politycka and Adamska (2003), although they measured total phenolic content, which also comprises other high molecular weight polyphenols, such as proanthocyanidins. Phlorizin and phloretin are the main flavonoids produced by apple plants and are usually stored in bark and roots (Gosch *et al.*, 2010). These polyphenols inhibit root and shoot growth in water culture (Börner, 1959), and phlorizin can specifically inhibit the respiratory rate and enzyme activities of the tricarboxylic acid cycle in apple roots (Wang *et al.*, 2012; Yin *et*

Table 1. Means (\pm standard errors) of measurements for apple seedlings after 4 months growth in soils amended with old apple roots at different times and in control soils. Letters in each column indicate statistically significant differences ($P < 0.05$). R3 = soil with roots amended 3 months before planting; C3 = control soil of the R3 treatment; R0 = soil with roots amended just before planting; C0 = control soil of the R0 treatment.

| Treatment | Whole plant fresh weight (g) | Root fresh weight (g) | Chlorophyll content (SPAD) |
|-----------|------------------------------|-----------------------|----------------------------|
| R3 | 5.53 ± 0.34 a | 3.29 ± 0.26 a | 33.9 ± 0.8 a |
| C3 | 6.70 ± 0.52 a | 3.69 ± 0.31 a | 38.0 ± 0.9 b |
| R0 | 3.19 ± 0.16 b | 2.21 ± 0.14 b | 24.5 ± 1.2 c |
| C0 | 5.90 ± 0.54 a | 2.93 ± 0.24 ab | 38.6 ± 1.1 b |

Table 2. Mean concentrations ($\mu\text{g g}^{-1}$, \pm standard errors) of the phenolic compounds in soil at planting time (T1), measured with UHPLC and mass spectrometry. R3 = soil with roots amended 3 months before planting; C3 = control soil of the R3 treatment; R0 = soil with roots amended just before planting; C0 = control soil of the R0 treatment.

| Phenolic compound | R3 | C3 | R0 | C0 |
|------------------------|---------------------|---------------------|----------------------|---------------------|
| Anthranilic acid | 0.0015 \pm 0.010 | 0.0009 \pm 0.0004 | 0.0040 \pm 0.0024 | 0.0023 \pm 0.0014 |
| 4-Aminobenzoic acid | 0.0004 \pm 0.002 | 0.0002 \pm 0.0000 | 0.0003 \pm 0.0001 | 0.0002 \pm 0.0000 |
| P-hydroxybenzoic acid | 0.0086 \pm 0.0037 | 0.0160 \pm 0.0074 | 0.0235 \pm 0.0096 | 0.0053 \pm 0.0027 |
| Cinnamic acid | 0.0736 \pm 0.0616 | 0.0734 \pm 0.0726 | 0.0957 \pm 0.0796 | 0.0173 \pm 0.0170 |
| Vanillin | 0.0048 \pm 0.0005 | 0.0040 \pm 0.0004 | 0.0056 \pm 0.0005 | 0.0050 \pm 0.0002 |
| Vanillic acid | 0.0008 \pm 0.0002 | 0.0009 \pm 0.0001 | 0.0010 \pm 0.0002 | 0.0009 \pm 0.0002 |
| 2,6-Dioh-benzoic acid | 0.0217 \pm 0.0114 | 0.0109 \pm 0.0021 | 0.0551 \pm 0.0416 | 0.0436 \pm 0.0348 |
| P-coumaric acid | 0.0479 \pm 0.0334 | 0.0496 \pm 0.0465 | 0.0916 \pm 0.0553 | 0.0211 \pm 0.0185 |
| Caffeic acid | 0.0010 \pm 0.001 | 0.0058 \pm 0.0023 | 0.0036 \pm 0.0011 | 0.0034 \pm 0.0016 |
| Ferulic acid | 0.0707 \pm 0.0433 | 0.0392 \pm 0.0381 | 0.1339 \pm 0.0852 | 0.1092 \pm 0.1081 |
| Phloretin | 0.0107 \pm 0.080 | 0.0024 \pm 0.0016 | 3.6734 \pm 8.8509 | 0.0104 \pm 0.0091 |
| Phlorizin | 0.0707 \pm 0.0000 | 0.0707 \pm 0.0000 | 77.4076 \pm 8.0480 | 0.0707 \pm 0.0000 |
| Naringenin | 0.1536 \pm 0.1275 | 0.0230 \pm 0.0195 | 0.1752 \pm 0.1153 | 0.1683 \pm 0.1648 |
| Quercetin-3-rhamnoside | 0.0124 \pm 0.0059 | 0.0243 \pm 0.0097 | 0.1562 \pm 0.0786 | 0.0119 \pm 0.0084 |

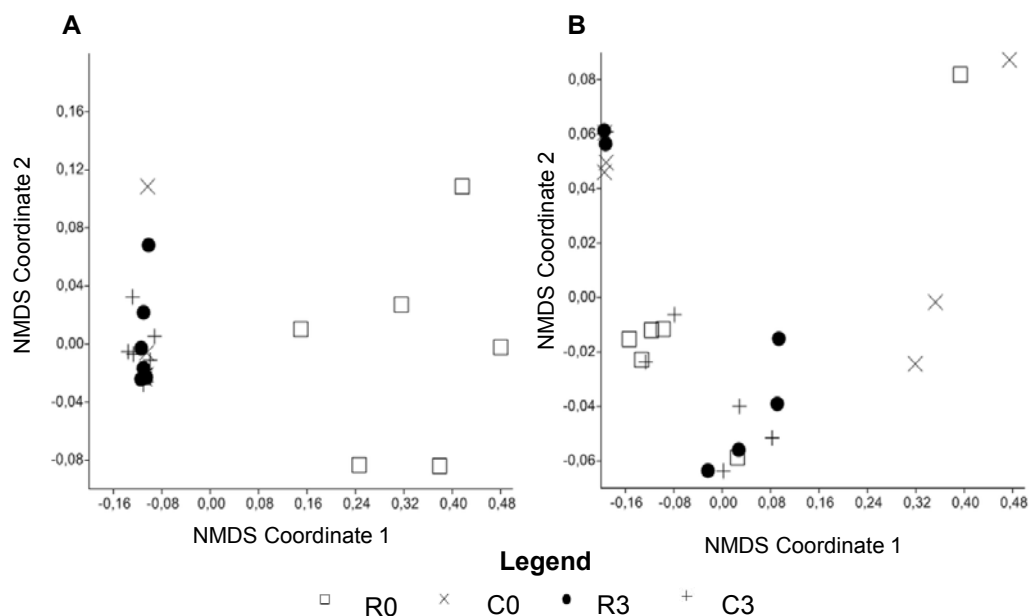


Figure 1. Non Metric Multidimensional Scaling (NMDS) based on Euclidean distances of soil samples amended with old apple roots at different times and control soils. R3 = soil with roots amended 3 months before planting; C3 = control soil of the R3 treatment; R0 = soil with roots amended just before planting; C0 = control soil of the R0 treatment. Each point represents the phenolic profile of one sample. a) at planting time (T1); b) after 4 months of seedlings growth (T2).

al., 2016). The concentration of phlorizin and phloretin in R3 treatment soils was comparable with that in control soils, indicating that the 3 months when the ground roots remained in the soil were sufficient to allow degradation of these compounds.

These results suggest that in orchards the concentration of phlorizin in soil should be measured before replanting to assess the level of autotoxicity, using this compound as an indicator of soil health. We ascertained that concentrations of $77 \mu\text{g g}^{-1}$ in soil were detrimental for apple seedlings. Leaving several months between explanting and replanting is also recommended, especially because the degradation of phenolic compounds is much slower in winter, when the soil temperatures are low (Politycka and Adam-ska, 2003), and the release of phenolic compounds from intact roots could be gradual.

Eleven phenolic compounds were detected in soils from sampling at time T2. Again in this case, the concentrations were low (Table 3). As compared to T1, a lower number of benzoic acid derivatives was found. At this time, the NMDS on Euclidean distances (stress = 0.01, R^2 axis 1 = 0.99, axis 2 = 0.1) did not show any clustering of the samples (Figure 1B), a fact that was confirmed by one-way ANOSIM, which found no significant differences in the phenolic profile in the different treatments ($P > 0.05$). The

only phenolic compound that significantly increased in all soil treatments at T2 as compared to T1 was vanillic acid (Wilcoxon test, $P < 0.05$), suggesting possible exudation from seedling roots, as happens in other plant species (Kong *et al.*, 2006). Four months after planting the seedlings, the concentrations of phlorizin and phloretin in R0 soils, which were very high in T1, dropped significantly (Wilcoxon test, $P < 0.05$), although weights of seedlings planted in this soil were reduced. This suggests that the initial stress caused by high concentration of phlorizin can impair plant health for long periods, as the plants remained stunted even when the concentration of the compound decreased significantly.

In conclusion, this study confirmed that the presence of apple root debris in soil can significantly impair the growth of apple seedlings, and that this negative effect disappears when phenolic compounds (mainly phlorizin and phloretin) have degraded. If the seedlings are planted just after the addition of roots, the initial negative impact on subsequent growth persists over time, despite the reduction in concentrations of phenolic compounds. Assessment of phlorizin could therefore be the basis for developing an indicator of ARD risk in orchard soils, or to determine the appropriate time for replanting to avoid ARD.

Table 3. Mean concentrations ($\mu\text{g g}^{-1}$; \pm standard errors) of phenolic compounds in soil after 4 months of seedlings growth (T2), measured with UHPLC and mass spectrometry. R3 = soil with roots amended three months before planting; C3 = control soil of the R3 treatment; R0 = soil with roots amended just before planting; C0 = control soil of the R0 treatment.

| Phenolic compound | R3 | C3 | R0 | C0 |
|-----------------------|---------------------|---------------------|---------------------|---------------------|
| P-hydroxybenzoic acid | 0.0206 \pm 0.0018 | 0.0265 \pm 0.0063 | 0.0271 \pm 0.0054 | 0.0275 \pm 0.0067 |
| Vanillin | 0.0032 \pm 0.0005 | 0.0027 \pm 0.0004 | 0.0040 \pm 0.0007 | 0.0026 \pm 0.0004 |
| Vanillic acid | 0.0222 \pm 0.0025 | 0.0266 \pm 0.0024 | 0.0296 \pm 0.0043 | 0.0316 \pm 0.0058 |
| Syringaldehyde | 0.0012 \pm 0.0003 | 0.0009 \pm 0.0002 | 0.0008 \pm 0.0002 | 0.0009 \pm 0.0002 |
| Esculin | 0.0004 \pm 0.0000 | 0.0007 \pm 0.0003 | 0.0018 \pm 0.0010 | 0.0004 \pm 0.0000 |
| P-coumaric acid | 0.0028 \pm 0.0008 | 0.0020 \pm 0.0003 | 0.0041 \pm 0.0005 | 0.0033 \pm 0.0005 |
| Ferulic acid | 0.0020 \pm 0.0005 | 0.0012 \pm 0.0001 | 0.0023 \pm 0.0005 | 0.0012 \pm 0.0002 |
| Phloretin | 0.0054 \pm 0.0020 | 0.0042 \pm 0.0029 | 0.0105 \pm 0.0023 | 0.0064 \pm 0.0029 |
| Phlorizin | 1.2200 \pm 0.3982 | 1.1454 \pm 0.3057 | 1.4584 \pm 0.6136 | 2.0692 \pm 0.9222 |
| Taxifolin | 0.0073 \pm 0.0029 | 0.0069 \pm 0.0025 | 0.0081 \pm 0.0030 | 0.0073 \pm 0.0030 |
| Dihydrokaempferol | 0.0049 \pm 0.0008 | 0.0040 \pm 0.0003 | 0.0057 \pm 0.0021 | 0.0141 \pm 0.0062 |

Literature cited

- Bhat R.G., and G.T. Browne, 2010. Specific detection of *Phytophthora cactorum* in diseased strawberry plants using nested polymerase chain reaction. *Plant Pathology* 59, 121–129.
- Börner H., 1959. The apple replant problem. I. The excretion of phloridzin from apple root residues. *Contributions of the Boyce Thompson Institute* 20, 39–56.
- Gosch C., H. Halbwirth and K. Stich, 2010. Phloridzin: Biosynthesis, distribution and physiological relevance in plants. *Phytochemistry* 71, 838–843.
- Hammer O., D.A.T. Harper and P.D. Ryan, 2001. PAST: paleontological statistics software package for education and data analysis. *Palaeontologia Electronica* 4.
- Hofmann A., L. Wittenmayer, G. Arnold, A. Schieber and W. Merbach, 2009. Root exudation of phloridzin by apple seedlings (*Malus x domestica* Borkh.) with symptoms of apple replant disease. *Journal of Applied Botany and Food Quality* 82, 193–198.
- Huang L.F., L.X. Song, X.J. Xia, W.H. Mao, K. Shi, Y.H. Zhou and J.Q. Yu, 2013. Plant-Soil Feedbacks and Soil Sickness: From Mechanisms to Application in Agriculture. *Journal of Chemical Ecology* 39, 232–242.
- Inderjit A. and K.M.M. Dakshini, 1995. Quercetin and quercitrin from *Pluchea lanceolata* and their effect on growth of asparagus bean. *Allelopathy: Organisms, Processes, and Applications* 582, 86–93.
- Kong C.H., H.B. Li, F. Hu, X.H. Xu and P. Wang, 2006. Allelochemicals released by rice roots and residues in soil. *Plant and Soil* 288, 47–56.
- Lochman J., O. Sery and V. Mikes, 2004. The rapid identification of European *Armillaria* species from soil samples by nested PCR. *Fems Microbiology Letters* 237, 105–110.
- Mazzola M. and L.M. Manici, 2012. Apple Replant Disease: Role of Microbial Ecology in Cause and Control. *Annual Review of Phytopathology* 50, 45–65.
- Pasini L., D. Prodorutti, S. Pastorelli and I. Pertot, 2016. Genetic Diversity and Biocontrol of *Rosellinia necatrix* Infecting Apple in Northern Italy. *Plant Disease* 100, 444–452.
- Politycka B. and D. Adamska, 2003. Release of phenolic compounds from apple residues decomposing in soil and the influence of temperature on their degradation. *Polish Journal of Environmental Studies* 12, 95–98.
- Verbovšek T., 2011. A comparison of parameters below the limit of detection in geochemical analyses by substitution methods. *RMZ – Materials and Geoenvironment* 58, 393–404.
- Vrhovsek U., D. Masuero, M. Gasperotti, P. Franceschi, L. Caputi, R. Viola and F. Mattivi, 2012. A versatile targeted metabolomics method for the rapid quantification of multiple classes of phenolics in fruits and beverages. *Journal of Agricultural and Food Chemistry* 60, 8831–8840.
- Wang Q., Y. Hu, H. Zhou, X. Zhan, Z. Mao and S. Zhu, 2012. Effects of phloridzin on the tricarboxylic acid cycle enzymes of roots of *Malus hupehensis* Rehd. seedlings. *Scientia Agricultura Sinica* 45, 3108–3114.
- Yin C.M., Y.L. Hu, G.S. Wang, X.F. Zhang, H. Zhou, X. Shen, X.S. Chen and Z.Q. Mao, 2016. Effect of main phenolic acids of the apple replanted soil on the roots of *Malus hupehensis* Rehd. seedlings. *Scientia Agricultura Sinica* 49, 961–969.

Accepted for publication: September 19, 2016

Published online: January 9, 2017

Phytopathologia Mediterranea

Contents of Volume 55

Number 1, April, 2016

REVIEW

Apple mosaic virus

L. Grimová, L. Winkowska, M. Konrady and P. Ryšánek 1

Phytophthora nicotianae diseases worldwide: new knowledge of a long-recognised pathogen

F. Panabières, G.S. Ali, M.B. Allagui, R.J.D. Dalio, N.C. Gudmestad, M.-L. Kuhn, S. Guha Roy, L. Schena and A. Zampounis 20

RESEARCH PAPERS

Phenotypic and molecular characterization of *Rhizobium vitis* strains from vineyards in Turkey

D. Canik Orel, A. Karagoz, R. Durmaz and F. Ertunc 41

Biochemical analysis of induced resistance in chickpea against broomrape (*Orobanche foetida*) by rhizobia inoculation

Y. Mabrouk, S. Mejri, I. Hemissi and O. Belhadj 54

Potential distribution of *Xylella fastidiosa* in Italy: a maximum entropy model

L. Bosso, D. Russo, M. Di Febbraro, G. Cristinzio and A. Zoina 62

Genetic variants of *Grapevine leafroll-associated virus 2* infecting Portuguese grapevine cultivars

F. Fonseca, F. Esteves, M. Teixeira Santos, J. Brazão and J.E. Eiras-Dias 73

Identification of QoI fungicide-resistant genotypes of the wheat pathogen *Zymoseptoria tritici* in Algeria

N. Alliou, A. Siah, L. Brinis, P. Reignault and P. Halama 89

Mating system and role of pycnidiospores in biology of *Polystigma amygdalinum*, the causal agent of almond red leaf blotch

A. Habibi and Z. Banihashemi 98

Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and *Bacillus* spp.

A.M. Pastrana, M.J. Basallote-Ureba, A. Aguado, K. Akdi and N. Capote 109

Alternative control of early blight of tomato using plant extracts from *Acacia nilotica*, *Achillea fragrantissima* and *Calotropis procera*

Z.A.M. Baka and Y.M. Rashad 121

NEW OR UNUSUAL DISEASE REPORTS

First report of olive leaf scorch in Brazil, associated with *Xylella fastidiosa* subsp. *pauca*

H.D. Coletta-Filho, C.S. Francisco, J.R.S. Lopes, A.F. de Oliveira and L.F. de Oliveira da Silva 130

Cryptostroma corticale in the northern Apennines (Italy)

C.M. Oliveira Longa, N. Vai and G. Maresi 136

SHORT NOTES

Molecular characteristics of a strain (Salento-1) of *Xylella fastidiosa* isolated in Apulia (Italy) from an olive plant with the quick decline syndrome

G. Bleve, G. Marchi, F. Ranaldi, A. Gallo, F. Cimaglia, A.F. Logrieco, G. Mita, J. Ristori and G. Surico 139

Number 2, August, 2016

REVIEW

Trail of decryption of molecular research on Botryosphaeriaceae in woody plants

K.W.T. Chethana, X. Li, W. Zhang, K.D. Hyde and J. Yan 147

Combined expression of p20 and p23 proteins from *Citrus tristeza virus* show enhanced local silencing suppressor activity

Â.A. Costa, T.R. Martins, N.T. Marques and G. Nolasco 172

RESEARCH PAPERS

Bacterial endophytes of weeds are effective biocontrol agents of *Agrobacterium* spp., *Pectobacterium* spp., and promote growth of tomato plants

Z. Krini, D. Alim, H. Djellout, L. Tafjet, F. Mohamed-Mahmoud and A. Raio 184

Naphthalenone polyketides produced by *Neofusicoccum parvum*, a fungus associated with grapevine Botryosphaeria dieback

S. Burruano, S. Giambra, V. Mondello, M. Dellagrecia, S. Basso, A. Tuzi and A. Andolfi 197

Quantitative detection of four pome fruit viruses in apple trees throughout the year

L. Winkowska, L. Grimova and P. Rysanek 207

First detection of *Grapevine rupestris stem pitting-associated virus* and *Grapevine rupestris vein feathering virus*, and new phylogenetic groups for *Grapevine fleck virus* and *Hop stunt viroid* isolates, revealed from grapevine field surveys in Spain

N. Fiore, A. Zamorano, N. Sánchez-Diana, X. González, V. Pallás and J. Sánchez-Navarro 225

Viruses affecting lentil (*Lens culinaris* Medik.) in Greece; incidence and genetic variability of *Bean leafroll virus* and *Pea enation mosaic virus*

E.K. Chatzivassiliou, A. Giakountis, S.G. Kumari and K.M. Makkouk 239

Impacts of previous crops on Fusarium foot and root rot, and on yields of durum wheat in North West Tunisia

S. Chekali, S. Gargouri, M. Rezgui, T. Paulitz and B. Nasraoui 253

Unmanned Aerial Vehicle (UAV)-based remote sensing to monitor grapevine leaf stripe disease within a vineyard affected by esca complex

S.F. Di Gennaro, E. Battiston, S. Di Marco, O. Facini, A. Matese, M. Nocentini, A. Palliotti and L. Mugnai 262

SHORT NOTES

Cassava starch coatings for postharvest control of papaya anthracnose

B.F. Oliveira, A.F. Cruz and E. Alves 276

Real-time PCR for rapid in planta detection of *Plectosphaerella cucumerina* on wild rocket (*Diplotaxis tenuifolia*)

G. Gilardi, K. Webb, G. Ortu, M.L. Gullino and A. Garibaldi 285

Trichoderma spp. and *Bacillus subtilis* for control of *Dactylonectria macrodidyma* in grapevine
R.F. dos Santos, L.I. Heckler, M. Lazarotto, L. da R. Garrido, C. Rego and E. Blume 293

Number 3, December, 2016

REVIEW

Modes of action for biological control of *Botrytis cinerea* by antagonistic bacteria
R. Haidar, M. Fermaud, C. Calvo-Garrido, J. Roudet and A. Deschamps 301

RESEARCH PAPERS

Chinese medicinal plants: an alternative approach for management of *Verticillium* wilt of cotton
M.I. Khaskheli, J.L. Sun, S.P. He, Z.E. Pan, Y.H. Jia, H.Q. Zhu, A.J. Khaskheli and X.M. Du 323

***Xylella fastidiosa* from almond in Iran: overwinter recovery and effects of antibiotics**
N. Amanifar, M. Taghavi and M. Salehi 337

Antioxidant response in *Chenopodium album* elicited by *Ascochyta caulina* mycoherbicide phytotoxins
C. Paciolla, S. De Leonardis, M.C. Zonno and M. Vurro 346

Suppression of crown and root rot of wheat by the rhizobacterium *Paenibacillus polymyxa*
L. Lounaci, S. Guemouri-Athmani, H. Boureghda, W. Achouak and T. Heulin 355

Biological and molecular characterisation of *Pilidium lythri*, an emerging strawberry pathogen in Iran
K. Karimi, M. Arzanlou, A. Babai-Ahari and I. Pertot 366

Characterization of *Neopestalotiopsis*, *Pestalotiopsis* and *Truncatella* species associated with grapevine trunk diseases in France
S.S.N. Maharachchikumbura, P. Larignon, K.D. Hyde, A.M. Al-Sadi and Z.-Y. Liu 380

Incidence and etiology of postharvest diseases of fresh fruit of date palm (*Phoenix dactylifera* L.) in the grove of Elx (Spain)
Ll. Palou, R. Rosales, V. Taberner and J. Vilella-Esplá 391

Simultaneous detection of mixed '*Candidatus Phytoplasma asteris*' and '*Ca. Liberibacter solanacearum*' infection in carrot
E. Satta, A.S. Ramirez, S. Paltrinieri, N. Contaldo, P. Benito, J. Bismark Poveda and A. Bertaccini 401

Patterns of phytoalexins in the grapevine leaf stripe disease (esca complex)/grapevine pathosystem
F. Calzarano, V. D'Agostino, A. Pepe, F. Osti, F. Della Pelle, M. De Rosso, R. Flamini and S. Di Marco 410

NEW OR UNUSUAL DISEASE REPORTS

First report of collar rot caused by *Pseudomonas aeruginosa* on calla lily (*Zantedeschia elliottiana*)
V. Shanmugam, H. Thakur, S. Paul, P. Bhadwal, S. Mahajan and K. Kumar Mondal 427

SHORT NOTES

Phlorizin released by apple root debris is related to apple replant disease
L. Nicola, U. Vrhovsek, E. Soini, H. Insam and I. Pertot 432

We warmly thank for their kind cooperation the following referees who have reviewed papers during this year in order to publish this Volume:

Abou-Mansour Eliane, Fribourg, Switzerland
Abouzeid Mohamed, Cairo, Egypt
Abrantes Isabel, Coimbra, Portugal
Afouda Leonard, Gottingen, Germany
Agustí-Brisach Carlos, Cordoba, Spain
Altomare Claudio, Bari, Italy
Alves Eduardo, Lavras, MG, Brasil
Antoniou Polymnia, Athens, Greece
Armengol Forti Josep, Valencia, Spain
Babadoost Mohammad, Urbana, Illinois, USA
Balconi Carlotta, Bergamo, Italy
Balestra Giorgio, Viterbo, Italy
Banihashemi Ziaddin, Shiraz, Iran
Benlioglu Seher, Aydin, Turkey
Bubici Giovanni, Rome, Italy
Cacciola Santa Olga, Catania, Italy
Chaouachi Maher, Evry cedex, France
Cinelli Tamara, Florence, Italy
Cohen Roni, Ranat Yishay, Israel
Coletta-Filho Helvécio, Cordeirópolis, Brazil
Cooke David, Scotland, UK
Datnoff Lawrence, Baton Rouge, LA, USA
Delgado Jose, Sevilla, Spain
Edwards Jacqueline, Victoria, Australia
Elad Yigal, Hazeva, Israel
Faoro Franco, Milan, Italy
Feliziani Erica, Ancona, Italy
Fernandez Myriam, Swift Current, Canada
Firrao Giuseppe, Udine, Italy
Fischer Jochen, Kaiserslautern, Germany
Gamba Fernanda, Montevideo, Uruguay
Gams Walter, Baarn, The Netherlands
Garcia-Pineda Ernesto, Morelia, Mich, Mexico
Genov Nikolay, Pleven, Bulgaria
Huang Lili, Yangling, Shaanxi, China
Iacobellis Nicola, Potenza, Italy
Isakeit Thomas, College Station, TX, USA
Kassemeyer Hanns-Heinz, Freiburg, Germany
Krugner Rodrigo, Parlier, CA, USA
Labbé Frédéric, Bordeaux, France
Lawrence Daniel, Davis, CA, USA
Le May Christophe, Le Rheu Cedex, France
Lee Sims Laura, Berkeley, USA
Lefort Francois, Genève, Switzerland
Liefing Lia, Auckland, New Zealand
Lima Alison, Uberlandia, Brazil
Linaldeddu Benedetto, Sassari, Italy
Martini Marta, Udine, Italy
Masi Marco, Naples, Italy
Massart Sebastien, Liège, Belgium
Mazzola Mark, Wenatchee, Washington, USA
McPhee Kevin, Fargo, ND, USA
Menzies Jim, Winnipeg, Manitoba, Canada
Moragrega Concepcio, Girona, Spain
Mostert Lizelle, Stellenbosch, South Africa
Neshawy Saneya, Giza, Egypt
Palacio-Bielsa Ana, Zaragoza, SPAIN
Palou Lluís, Valencia, Spain
Pantelides Iakovos, Cyprus
Pappu Hanu, Pullman, WA, USA
Parbery Doug, Australia
Parrella Giuseppe, Naples, Italy
Phillips Alan, Lisbon, Portugal
Puglisi Ivana, Catania, Italy
Purcell Alexander, Berkeley, CA, USA
Rubio María Belén, Villamayor, Salamanca, Spain
Ruscic Jelena, Zagreb, Croatia
Sarocco Sabrina, Pisa, Italy
Smith Grant, Auckland, New Zealand
Stefani Emilio, Reggio Emilia, Italy
Surico Giuseppe, Florence, Italy
Tanaka Kazuaki, Hirosaki, Aomori, Japan
Tashiro Nobuya, Nanri, Kawasoe, Saga, Japan
Topalidou Eleni, Vassilika, Greece
Vanneste Joel, Sandringham, New Zealand
Vieira dos Santos Maria Clara, Coimbra, Portugal
Weintraub Phyllis, Negev, Israel
Wim Bert, Ghent, Belgium
van der Wolf Jan, Wageningen, The Netherlands

Corresponding author: H. Özer
E-mail: hayrettin.ozer@mam.gov.tr

