Effects of *Trichoderma harzianum* Fertilizer on the Soil Environment of *Malus hupehensis* Rehd. Seedlings under Replant Conditions

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Additional index words. apple replant disease, biocontrol fungi, real-time fluorescence–quantitative polymerase chain reaction, soil phenolic acids

Abstract. A pot experiment was performed to investigate the effects of *Trichoderma harzianum* on the root morphology of *Malus hupehensis* Rehd. seedlings and their soil environment under replant conditions. The experiment consisted of four treatments: continuously cropped soil (CK1), methyl bromide fumigation (CK2), carrier substrate control (T1), and *T. harzianum* fertilizer (T2). Plant growth parameters, soil phenolic acid content, abundance of soil microorganisms, and root respiration rate were measured. Compared with CK1, plant height, basal diameter, and fresh weight were 34.58%, 27.55%, and 32.91% greater in T2; 11.35%, 12.10%, and 18.33% greater in T1; and 54.34%, 57.64%, and 45.74% greater in CK2. These metrics were significantly higher in the CK2 treatment than in the other treatments. The second highest values were recorded in the T2 treatment. Differences in root architecture were consistent with differences in biomass. Application of *T. harzianum* fertilizer was associated with increases of 45.45%, 120.06%, 86.44%, and 268.29% in the activities of the antioxidant enzymes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX), respectively, and there was little difference between T2 and CK2. The contents of phlorizin and phloretin were 39.39% and 51.70% less in T2, respectively, and 17.85% and 18.14% less in T1, respectively, compared with CK1. *Trichoderma harzianum* fertilizer increased the abundance of bacteria and actinomycetes while decreasing that of fungi. The gene copy numbers of *Fusarium oxysporum* and *Fusarium moniliforme* were 64.30% and 49.35% less, respectively, in the T2 treatment. The fungus population and the gene copy number of *Fusarium oxysporum* and *Fusarium moniliforme* was the least in CK2 because of the good sterilization effect. The *T. harzianum* fertilizer showed satisfactory effects in promoting the root growth of *M. hupehensis*, increasing the root resistance, decreasing the soil phenolic acid content, and significantly reducing the gene copy number of *F. oxysporum* and *F. moniliforme*. In summary, *T. harzianum* fertilizer is an effective and green alternative for the prevention and control of apple replant disease (ARD).

China now ranks first in global apple cultivation area, total output, per-capita share, and export volume; it leads the world in apple

production and consumption (Chen et al., 2010). However, most apple orchards in traditionally dominant cultivation areas are now entering an aging period, and this includes Qixia City in Shandong Province. Nearly 40,000 hm² of apple orchard have been planted in Qixia on 53,000 hm² of arable land. Orchards more than 25 years old account for 67.5% of this area and are now facing the problem of ARD (Mao et al., 2017). When renewing old orchards, fruit growers typically remove old trees and plant new trees in the same soil, leading to apple replant obstacles such as dwarfing and even tree death, which cause huge economic losses for the growers (St. Laurent et al., 2010; Yin et al., 2017).

Rotation, chemical fumigation, and biological control are the primary measures currently used to prevent ARD (Koron et al., 2014; Liu et al., 2014). Preplant soil methyl bromide fumigation to eliminate soil-borne pathogens is recognized as the best means of preventing and controlling ARD in China and

abroad. However, its use has been prohibited because it causes pollution and environmental harm (Koron et al., 2014; Manici et al., 2013). With increased environmental awareness, rotations, chemical fumigation, and other measures are gradually being replaced with biological control measures to save time and minimize environmental pollution (Gaśtoł and Domagała-Świątkiewicz, 2015; Hyakumachi et al., 2013; Ju et al., 2014; Yuan et al., 2016). Biocontrol is an environmentally protective control method that uses beneficial microorganisms to inhibit the number of pathogens in the soil or interfere with infection by plant pathogens (Dubey et al., 2007; Gerbore et al., 2014).

Trichoderma spp. is a common biocontrol fungus with strong environmental adaptability and rapid growth that is widely found in rhizosphere soils (Bae et al., 2016). In recent years, researchers have focused on the use of antagonistic microorganisms to prevent plant disease, and *Trichoderma* is considered to be among the most promising biological control agents (Contreras-Cornejo et al., 2014; Harman, 2011; Shores et al., 2010; Tucci et al., 2011). *Trichoderma harzianum* has been widely used in various agricultural systems because of its excellent biocontrol characteristics. Continuous cropping constitutes a type of stress for crop roots, which can be attributed to the overall deterioration of the soil environment (Hofmann et al., 2012; Tewoldemedhin et al., 2011a, 2011b). The damage sustained by plants under stress conditions is related to the degree of stress, the duration of stress, and the tolerance of the plants. The greater the stress degree and duration, and the weaker the plant tolerance, the less likely plants are to recover from damage (Ahmad et al., 2021). Roots, as important plant organs, perform the functions of anchorage, transportation, absorption, synthesis, and distribution. At the same time, the rhizosphere is an important interface for resource exchange between plants and their soil environment (Saravanakumar et al., 2017). Therefore, it is important to study changes in plant roots and their surrounding environment.

As a good biological control agent, *T. harzianum* has been widely used in vegetable production (Singh et al., 2018). After the inoculation with *Trichoderma harzianum*, increased root weight in wheat, root length in beans, and greater yields in lettuce and tomato have been recorded (Al-Hazmi and Tariqjaveed, 2016; Buysens et al., 2016; Hoyos-Carvajal et al., 2009; Kucuk, 2013). Nzanza et al. (2012) demonstrated that the beneficial effect of inoculating seedlings with *T. harzianum* was shown in the earliness of the yield and the percentage of large fruit increased, and *T. harzianum* inoculant had the potential to increase the total yield of tomato. Antagonistic fungal strains are a potential source of novel biological fungicides that lack the side effects of chemical fungicides. Yassin et al. (2021) demonstrated that *T. harzianum* had antagonistic activity against *Fusarium proliferatum* and *Fusarium*

Received for publication 4 May 2021. Accepted for publication 14 June 2021.

Published online 9 August 2021.

The research was supported by the earmarked fund for National Modern Agro-industry Technology Research System of China (CARS-27), Qingchuang Science and Technology Support Project of Shandong Colleges and Universities (2019KJF020), the Shandong Agricultural Major Applied Technology Innovation Project (SD2019ZZ008), Taishan Scholars (NO.ts20190923), the Natural Science Foundation of Shandong Province (ZR2020MC131), the National Natural Science Foundation of China (32072510), and the Fruit Innovation Team in Shandong Province, China (SDAIT-06-07).

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verticillioides, with mycelial inhibition rates of 68.38% and 60.64%, respectively. Culture filtrates suppressive rates of *T. harzianum* strains exhibited antifungal activity against an *F. verticillioides* strain (32.2%) that was stronger than mycelium (23.50%). On the other hand, *T. harzianum* can also affect plants. *Trichoderma harzianum*-induced enhanced resistance to fusarium wilt is associated with an increase in defense-related enzymes in cucumber plants (Tan et al., 2012). Ainhua et al. (2010) found that *T. harzianum* could control fusarium wilt in melon plants by inducing plant basal resistance and attenuating the hormonal disruption caused by *F. oxysporum*. Yan et al. (2021) showed that *T. harzianum* could effectively suppress root knot nematode (*Meloidogyne incognita*) infestation by increasing secondary metabolite synthesis and defense-related enzyme activity, and root knot nematode infestation was reduced by 61.88% in tomato plants (*Solanum lycopersicum* L.).

There have been few studies on the application of *T. harzianum* to fruit trees, especially in the context of ARD. In our experiment, *T. harzianum* was used to make a microbial fertilizer. *Malus hupehensis* Rehd., a common apple rootstock, was used as the test material, and methyl bromide fumigation was used as a high standard control. The effects of *T. harzianum* on plant root growth and the soil/rhizosphere environment under replant conditions were studied to assess the biocontrol effect of *T. harzianum* on ARD. The objective of our study was to explore the biocontrol effect of *T. harzianum* on ARD and provide a scientific theoretical basis for it.

Materials and Methods

Experimental materials and design

The pot experiment was performed at the Apple Engineering Experimental Center of Shandong Agricultural University (lat. 36.16°N, long. 117.15°E) located in Tai'an City, Shandong Province, China. The soil for the pot experiment was obtained from a 31-year-old apple orchard in Manzhuang Town, Tai'an City, Shandong Province. The sandy soil had a pH of 5.65, with 10.24 g·kg⁻¹ organic matter, 28.19 mg·kg⁻¹ rapid available nitrogen, 137.55 mg·kg⁻¹ potassium, and 9.79 mg·kg⁻¹ phosphorus.

Malus hupehensis, a common apple rootstock variety, was used in the study. The seeds and sand were mixed and stored at 4 °C for 30 d. After the seeds had germinated and turned white, they were sown in seedling trays in Mar. 2018. In the middle of April, when the seedlings had grown six true leaves, they were transplanted into clay pots with an outer diameter of 29 cm and an inner diameter of 25 cm.

Trichoderma harzianum was obtained from stocks maintained in our laboratory and was formulated into a microorganism fertilizer by Dezhou Chuangdi Microbial Resources Co., Ltd. The fertilizer was a black-gray powder, and the viable biomass was 2.1 × 10⁹ cfu/g. Four treatments were applied: continuously cropped soil only (CK1), methyl bromide fumigated soil (CK2), carrier substrate

control (T1), and *T. harzianum* fertilizer (T2). *Trichoderma harzianum* fertilizer was applied at a rate of 1% relative to the mass of the replant soil, and the soil and fertilizer were well mixed. The carrier substrate was applied in the same manner. Each treatment was applied to 20 pots, for a total of 80 pots in the experiment. Two seedlings of similar size were transplanted to each pot. The experimental plants were harvested and soil was collected on Aug. 15, 2018, 4 months after transplant. Three plants were selected randomly from each treatment and were washed with water. The fresh weight of their root systems was then determined. When collecting soil samples, the topsoil and the soil around the basin were removed, fresh rhizosphere soil was obtained, and the soil samples were sieved and placed into sealed bags for transport back to the laboratory.

Experimental methods

The professional version of the WinRHIZO root analysis system (version 2007; Regent Instruments, Quebec, Canada) was used to scan and analyze images of the root samples. Root length, total root volume, total root surface area, and other root architectural parameters were measured.

Measurement of root respiration rate. To measure root respiration rate, 0.05 g of fresh white roots was obtained from the seedlings, and their oxygen consumption rate was measured using an Oxytherm oxygen electrode (Hansatech, Norfolk, UK). The formula for calculating root respiration rate (R), measured in nanomoles of oxygen per gram fresh weight per minute, was

$$R = U \times V / m_{\text{test root}},$$

where *U* is the oxygen consumption rate, *V* is the total volume of the liquid in the reaction vessel, and *m_{test root}* is the root fresh weight.

Measurement of antioxidant enzyme activity. To measure antioxidant enzyme activity, 1 g of fresh white roots was obtained from the seedlings, mixed with 8 mL phosphoric acid buffer (0.05 mol·L⁻¹; pH, 8), ground in an ice bath, and centrifuged at 10,500 rpm for 20 min. The supernatant was kept cold at 4 °C and was used to measure antioxidant enzyme activity. SOD activity was measured by the nitroblue tetrazole method, POD activity was measured by the guaiacol method, CAT activity was measured by the ultraviolet absorption method, and APX activity was measured by the colorimetric method. The unit of measure for antioxidant enzyme activity was units per minute per gram fresh weight. The formulas for SOD, POD, CAT, and APX activities were

$$\text{Total activity of SOD} = (A_{CK} - A_E) \times V / (0.5 \times A_{CK} \times Vt \times W),$$

$$\text{Total activity of POD} = \Delta OD_{470} \times V / (Vt \times W \times T),$$

$$\text{Total activity of CAT} = \Delta OD_{240} \times V / (Vt \times W \times T), \text{ and}$$

$$\text{Total activity of APX} = OD_{290} \times V / (Vt \times W \times T),$$

where *A_{CK}* is the absorbance value of the control, *A_E* is the absorbance value of the supernatant, *pOD* is the value of *A_E* minus *A_{CK}* at a specific wavelength, *V* is the total volume of the reaction solution, *Vt* is the volume of the supernatant used in the measurement, *W* is the fresh weight of the root sample, and *T* is the measurement interval.

Measurement of soil enzyme activity. Soil urease activity was measured by a colorimetric assay using sodium phenate-sodium hypochlorite. Urease activity was calculated by subtracting the absorbance of the sample from the absorbance of the blank, and the ammonia nitrogen content was calculated from a standard curve. Urease activity was expressed as the ammonia-nitrogen content of 1 g of soil after 24 h. Soil phosphatase activity was determined by a colorimetric assay with disodium phenyl phosphate. A standard curve was prepared using phenolic standards. Phosphatase activity was expressed as micrograms of phenolics per gram of soil. Soil sucrase activity was measured by a colorimetric assay with 3,5-dinitrosalicylic acid. A glucose solution was used as the standard. The activity of sucrase was expressed as the mass (measured in milligrams) of glucose produced in 1 g of soil after 24 h. Soil catalase activity was measured by potassium permanganate titration. Two grams of air-dried soil were placed in a 100-mL triangular bottle, and 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution were added. A control bottle containing 40 mL of distilled water and 5 mL of 0.3% hydrogen peroxide solution was prepared without adding soil. The triangular bottle was shaken for 20 min, and 5 mL of 3 N sulfuric acid was added to stabilize the undecomposed hydrogen peroxide. The suspension was then filtered through filter paper, and 25 mL of filtrate was titrated with 0.1 N potassium permanganate until it became light pink. The formulas for calculating soil enzyme activities were

$$\text{Urease} = (\Delta OD_{578} - 0.00434) \times V \times n / (118.38 \times m),$$

$$\text{Phosphatase} = (\Delta OD_{660} + 0.0098) \times V \times n / (0.6779 \times m),$$

$$\text{Sucrase} = (\Delta OD_{508} + 0.0146) \times V \times n / (9.3125 \times m), \text{ and}$$

$$\text{Catalase} = (V_{ck} - V_E) \times 0.1,$$

where ΔOD is the absorbance value of the supernatant minus the absorbance value of the control at a specific wavelength, *V* is volume of the measured solution, *n* is the dilution factor, *m* is the weight of air-dried soil, *V_{ck}* is the volume of potassium permanganate solution consumed by 25 mL of hydrogen peroxide solution, and *V_E* is the volume of potassium permanganate solution consumed by 25 mL of test soil solution.

Measurement of phenolic acids in the soil. Eighty grams of dry soil were passed through a

12-mesh sieve, 10 g of diatomite were added, and the materials were mixed evenly in a beaker. A cellulose membrane was placed at the bottom of a 100-mL extraction tank, and the mixed sample was loaded into the tank. Extraction was performed under optimized accelerated solvent extraction conditions: anhydrous ethanol was used as the extraction solvent, the temperature was 120 °C, the pressure was 10.3 MPa, the static extraction time was 5 min, the cycle was performed twice, the purge volume was 60%, and the purge time was 90 s. The same sample was extracted again with methanol as the extraction solvent under the same conditions. After extraction, the collected solutions from the two extraction solvents were mixed, concentrated, and dried at 34 °C under reduced pressure, then redissolved with 1 mL methanol and filtered through a 0.22-μm organic phase filter membrane for high-performance liquid chromatographic analysis. The chromatographic column (3 μm, 150 × 3 mm) was an Acclaim 120 C18 (Dionex, Sunnyvale, CA), and the column temperature was 30 °C. Mobile phase A was acetonitrile, mobile phase B was water (adjusted to pH 2.6 with acetic acid), and the flow rate was 0.50 mL·min⁻¹. Automatic sampling was used.

Number of soil microorganisms. The numbers of bacteria, fungi, and actinomycetes in the soil were determined by the dilution plate-counting method, and LB (Luria-Bertani), potato dextrose agar, and Gao's media were used to determine the numbers of bacteria, fungi, and actinomycetes, respectively.

Real-time fluorescence quantitative polymerase chain reaction. DNA was extracted from 0.5 g fresh soil using the E.Z.N.A. soil DNA extraction kit (Omega Bio-Tek, Norcross, GA). Real-time fluorescence quantitative polymerase chain reaction (RT-qPCR) was performed on the Bio-Rad CFX96 quantitative PCR instrument to determine the gene copy number of *Fusarium oxysporum* and *Fusarium moniliforme*. The 25-μL reaction

system consisted of 12.5 μL SYBR Green PCR Master Mix (TaKaRa Biotech Co., Ltd., Dalian, China), 1 μL upstream- and downstream-specific primers (10 μmol·L⁻¹), 1.5 μL of test DNA, and 9 μL of double-distilled water. The reaction conditions were pre-denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 94 °C for 5 s, annealing at 60 °C for 30 s, and extension at 72 °C for 30 s. Gene copy numbers of *F. oxysporum* and *F. moniliforme* were calculated as follows:

$$F. oxysporum \text{ gene copy number} = \{10^{[ct - 36.396/(-2.291)]} \times 9.1212\} / 2801 \times 10^{11}$$

$$F. moniliforme \text{ gene copy number} = \{10^{[ct - 12.421/(-3.495)]} \times 9.1212\} / 2801 \times 10^{11}$$

Data analysis

Microsoft Excel 2007 (Microsoft Corp., Redmond, WA) was used for calculations and data visualization. SPSS 19.0 software (SPSS Inc., Chicago, IL) was used to perform statistical analyses using one-way analysis of variance and Duncan's mean separation test ($\alpha = 0.05$).

Results

Effects of *T. harzianum* fertilizer on biomass and root growth

As shown in Table 1, plant height, basal diameter, and fresh weight were 34.58%, 11.35%, and 54.34% greater in T2, 27.55%, 12.10%, and 57.64% greater in T1, and 32.91%, 18.33%, and 45.74% greater in CK2 than in CK1. These metrics were significantly higher in CK2 than in the other treatments.

Table 2 shows that the root length, root surface area, and root volume of T2 were 67.48%, 73.86%, and 6.79% greater than those of CK1. By contrast, the root length, root surface area, and root volume of CK2 were 9.84%, 14.70%, and 6.52% greater.

Effects of *T. harzianum* fertilizer on antioxidant enzyme activities

Figure 1 shows that the four antioxidant enzymes of T2 and CK2 tended to exhibit the highest activities, followed by T1 and CK1. Compared with CK1, the activities of SOD, POD, CAT, and APX were 45.45%, 120.06%, 86.44%, and 268.29% greater in T2 and 47.79%, 126.40%, 97.16%, and 264.63% greater in CK2.

Effects of *T. harzianum* fertilizer on soil enzyme activities

The activities of four soil enzymes were greatest in T2 (Fig. 2). The activities of urease, neutral phosphatase, sucrase, and catalase were 195.22%, 152.43%, 227.16%, and 169.48% greater in T2 than in CK1. There was little difference between CK1 and CK2; soil enzyme activities were low in both.

Effects of *T. harzianum* fertilizer on phenolic acid contents of replant soil

As shown in Table 3, compared with CK1, the contents of phloridzin, ferulic acid, catechin, and vanillin were 30.07%, 32.20%, 41.88%, and 17.01% less in CK2; 17.85%, 18.14%, 11.74%, and 11.81% less in T1; and 39.39%, 51.70%, 63.80%, and 43.40% less in T2. The content of benzoic acid was 17.54%, 15.79%, and 36.84% less in CK2, T1, and T2, respectively; T2 had the strongest effect.

Effects of *T. harzianum* fertilizer on rhizosphere microbes

Table 4 shows that, compared with CK1, T2 reduced the number of fungi by 73.33%, but increased the number of bacteria by 100.00%, almost as much as CK2.

Figure 3 demonstrates that the abundance of *F. oxysporum* and *F. moniliforme* showed a similar pattern across the treatments: CK2 < T2 < T1 < CK1. The gene copy numbers of the two *Fusarium* species were 64.30% and 49.35% less in T2 than in CK1, but showed little difference between T1 and CK1.

Discussion

Trichoderma harzianum fertilizer promoted the growth of *M. hupehensis* seedlings

There have been numerous reports on research into the growth-promoting effects of *T. harzianum*. In studies of maize, cotton, and cucumber treated with *Trichoderma*, seed germination and plant growth have been improved to varying degrees, manifested as improvements in seed vigor, germination rate, emergence rate, seedling root activity, plant height, and plant dry quality (Contreras-Cornejo et al., 2016; Harman, 2011; Salas-Marina et al., 2011). In our experiment, the plant height and root dry and fresh weight increased in response to the application of *T. harzianum* fertilizer, and root respiration rate also increased to some extent. The apple root systems grew vigorously after application of the microbial fertilizer. Root vitality generally refers to the absorptive and anabolic

Table 1. Effects of *Trichoderma harzianum* fertilizer on plant biomass of *Malus hupehensis* Rehd. seedlings.

Treatment	Plant ht (cm)	Ground diam (mm)	Root fresh wt (g)	Root dry wt (g)
CK1	49.93 ± 1.52 d	6.28 ± 0.16 d	55.59 ± 0.24 d	19.19 ± 0.65 d
CK2	77.07 ± 1.72 a	9.90 ± 0.28 a	81.02 ± 0.82 a	28.71 ± 0.31 a
T1	55.60 ± 0.78 c	7.04 ± 0.04 c	65.78 ± 0.76 c	24.13 ± 0.33 c
T2	67.20 ± 0.52 b	8.01 ± 0.19 b	73.88 ± 0.25 b	26.03 ± 0.43 b

Values followed by different letters in a column indicate a significant difference at the 0.05 level. CK1 = replanted apple orchard soil; CK2 = methyl bromide fumigation; T1 = carrier substrate control; T2 = *Trichoderma harzianum* fertilizer.

Table 2. Effects of *Trichoderma harzianum* fertilizer on root growth of *Malus hupehensis* Rehd. seedlings.

Treatment	Length (cm)	Surface area (cm ²)	Root volume (cm ³)	Root respiration rate (nmol O ₂ /g/min FW)
CK1	926.38 ± 18.68 d	507.64 ± 8.34 c	9.20 ± 0.29 d	402.35 ± 38.24 b
CK2	1704.10 ± 22.51 a	887.40 ± 5.98 a	17.04 ± 0.20 a	579.90 ± 28.40 a
T1	1316.36 ± 11.92 c	542.10 ± 24.51 c	12.08 ± 0.26 c	447.76 ± 33.46 b
T2	1551.47 ± 34.42 b	773.70 ± 27.06 b	15.99 ± 0.45 b	559.96 ± 36.97 a

Values followed by different letters in a column indicate a significant difference at the 0.05 level. CK1 = replanted apple orchard soil; CK2 = methyl bromide fumigation; FW = fresh weight; T1 = carrier substrate control; T2 = *Trichoderma harzianum* fertilizer.

Fig. 1. Effects of *Trichoderma harzianum* fertilizer on superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) activities in roots of *Malus hupehensis* Rehd. seedlings. CK1, replanted apple orchard soil; CK2, methyl bromide fumigation; FW, fresh weight; T1, carrier substrate control; T2, *Trichoderma harzianum* fertilizer. Values followed by different letters in each column chart indicate a significant difference at the 0.05 level.

Table 3. Effects of *Trichoderma harzianum* fertilizer on phenolic acid contents of replanted soil.

Treatment	Phloridzin (mg·kg ⁻¹)	Phloretin (mg·kg ⁻¹)	Ferulic acid (mg·kg ⁻¹)	Catechins (mg·kg ⁻¹)	Vanillin (mg·kg ⁻¹)	Benzoic acid (mg·kg ⁻¹)
CK1	10.12 ± 0.19 a	5.38 ± 0.05 a	1.70 ± 0.07 a	0.96 ± 0.01 a	0.70 ± 0.02 a	0.57 ± 0.02 a
CK2	7.08 ± 0.05 c	3.65 ± 0.06 c	0.99 ± 0.02 c	0.80 ± 0.01 c	0.56 ± 0.02 b	0.47 ± 0.02 b
T1	8.31 ± 0.14 b	4.41 ± 0.08 b	1.50 ± 0.04 b	0.85 ± 0.01 b	0.60 ± 0.01 b	0.48 ± 0.01 b
T2	6.13 ± 0.04 d	2.60 ± 0.03 c	0.62 ± 0.01 c	0.54 ± 0.01 d	0.48 ± 0.01 c	0.36 ± 0.02 c

Values followed by different letters in a column indicate a significant difference at the 0.05 level.

CK1 = replanted apple orchard soil; CK2 = methyl bromide fumigation; T1 = carrier substrate control; T2 = *Trichoderma harzianum* fertilizer.

capacity of roots; its magnitude directly affects the water and nutrient absorption needed to support plant growth (Grunewaldt-Stöcker et al., 2019). The growth results presented here suggest that *T. harzianum* can promote the growth of apple seedlings under replant conditions. *Trichoderma* may initially affect root growth and development after transplant to replant soil, enabling plants to absorb more soil nutrients and thereby ensuring good plant growth.

***Trichoderma harzianum* fertilizer enhanced root and soil enzyme activities, and reduced rhizosphere phenolic acid concentrations**

Under normal growth conditions, plant cells maintain a dynamic balance between the

production and removal of reactive oxygen species (ROS). Under stress, the production of free radicals outstrips their elimination, and plants suffer oxidative injury (Mittler et al., 2004). POD, SOD, CAT, and APX are important antioxidant enzymes in plants and can effectively reduce the damage caused by ROS and other peroxide free radicals, thereby enhancing the plant's stress resistance (Huang et al., 2011). Chen et al. (2019) demonstrated that *T. harzianum* played a role in reducing *F. oxysporum*-induced ROS and reactive nitrogen species, potentially alleviating oxidative and nitrostatic stress in cucumber roots. Moreover, inoculation with *T. harzianum* attenuated *F. oxysporum*-induced inhibition of the ascorbate-glutathione cycle and the oxidative pentose phosphate pathway, and improved the

antioxidant capacity of plants. In our experiment, the antioxidant enzyme activities of POD, SOD, CAT, and APX in the root system increased significantly after the application of *T. harzianum* fertilizer compared with their levels in the continuously cropped control soil (CK1). The T2 treatment produced results more similar to those of CK2. *Trichoderma harzianum* alleviated oxidative and nitrifying stress by improving ROS and reactive nitrogen species metabolism during *Fusarium* infection of cucumber roots (Chen et al., 2019). Metabolites from *T. harzianum* may activate the plant's own defense system, enhancing its ability to resist pathogenic fungi and thereby promoting its growth. Research by Li et al. (2017) found that solutions of *T. harzianum* or *Trichoderma viride* could improve plant stress resistance and promote the growth of Chinese fir seedlings, which is consistent with the conclusions of our experiment.

Levels of soil enzyme activity are related to the levels of soil biochemical reactions and the vitality of organisms; at the same time, they affect the availability of soil nutrients (Qi et al., 2016). Previous researchers have found that functional microbial fertilizers can increase the availability of soil nutrients significantly and change the structure of the soil

Table 4. Effect of *Trichoderma harzianum* fertilizer on the population of soil microorganisms.

Treatment	Fungus (×10 ³ cfu/g)	Actinomycetes (×10 ⁴ cfu/g)	Bacteria (×10 ⁵ cfu/g)
CK1	15.00 ± 1.15 a	20.33 ± 0.88 c	9.67 ± 0.88 b
CK2	6.67 ± 0.88 b	35.00 ± 0.58 a	20.00 ± 1.15 a
T1	14.67 ± 0.33 a	24.33 ± 0.88 b	10.67 ± 0.88 b
T2	4.00 ± 0.58 c	37.00 ± 0.58 a	19.33 ± 0.88 a

Values followed by different letters in a column indicate a significant difference at the 0.05 level.

cfu = colony-forming units; CK1 = replanted apple orchard soil; CK2 = methyl bromide fumigation; T1 = carrier substrate control; T2 = *Trichoderma harzianum* fertilizer.

Fig. 2. Effects of *Trichoderma harzianum* fertilizer on soil enzyme activities of replanted soil. CK1, replanted apple orchard soil; CK2, methyl bromide fumigation; T1, carrier substrate control; T2, *Trichoderma harzianum* fertilizer. Values followed by different letters in each column chart indicate a significant difference at the 0.05 level.

microbial community, thereby regulating directly or indirectly the availability and supply capacity of rhizosphere nutrients, and improving soil fertility (Singh et al., 2018). In our experiment, soil enzyme activity was enhanced after the application of *T. harzianum* fertilizer relative to both the control soil (CK1) and the fertilizer carrier substrate (T1). This result allows us to exclude the influence of the carrier substrate and conclude that *T. harzianum* improves soil enzyme activity and replant rhizosphere soil properties, creating beneficial conditions for plant growth.

The pathogenesis of ARD is extremely complicated. Previous studies have indicated that phenolic acids are an important cause of ARD (Bai et al., 2009). Accumulation of soil phenolic acids above a threshold concentration can cause stress to plant roots. Phenolic acids are autotoxic substances and include phlorizin, phloretin, benzoic acid, phloroglucinol, ferulic acid, p-hydroxybenzoic acid, vanillin, syringic acid, caffeic acid, and others (Yin et al., 2018). Our experiment shows that the contents of phloridin and phloretin were greatest in the continuously

cropped control soil (CK1), and the growth of plant seedlings was weak using this treatment. This result is consistent with the ability of phloridin and phloretin to inhibit plant growth, as reported by Yin et al. (2016). Compared with the control soil and the carrier substrate, the *T. harzianum* treatment had markedly lower levels of phlorizin, phloretin, and other phenolic acid substances. In recent years, microbial degradation has been proposed as an effective means to remove phenolic acids in nature. It can reduce the concentration of phenolic acids in rhizosphere soil to alleviate their autotoxic effects on crops (Chen et al., 2011; Zhang et al., 2010). Sun et al. (2014) isolated and identified a cinnamic acid-degrading fungus from continuously cropped rhizosphere soil in a cucumber greenhouse. This fungus could grow with cinnamic acid as the sole carbon source and exhibited high degradation in liquid fermentation. *Trichoderma harzianum* may secrete certain resistant substances into the soil or use phenolic acids as energy sources, thereby reducing their content, but this possibility requires experimental confirmation.

***T. harzianum* demonstrated antagonism toward *F. oxysporum* and *F. moniliforme* in apple replant soil**

The relationship between plants and soil microbial communities is crucial to plant health (Oldroyd, 2013). In general, beneficial microorganisms in the soil can promote plant growth, whereas harmful microorganisms can inhibit plant growth and even cause death. For example, *Fusarium* causes diseases such as fusarium wilt in more than 100 economically important crops worldwide (Gordon, 2017). *Fusarium* is thought to be the primary pathogen responsible for ARD in the main apple-producing areas of China, and it exhibits strong pathogenicity (Wang et al., 2018a, 2018b). Numerous studies have shown that soil microorganisms are the main reason for apple continuous cropping obstacles (Franke-Whittle et al., 2018; Manici et al., 2013). In our experiment, the abundance of soil fungi was reduced significantly and the abundance of bacteria was increased after application of *T. harzianum* fertilizer. RT-qPCR analyses showed that the application of *T. harzianum* fertilizer greatly reduced the gene copy number

Fig. 3. Effects of *Trichoderma harzianum* fertilizer on real-time fluorescence quantitative polymerase chain reaction analysis of *Fusarium oxysporum* and *Fusarium moniliforme*. CK1, replanted apple orchard soil; CK2, methyl bromide fumigation; T1, carrier substrate control; T2, *Trichoderma harzianum* fertilizer. Values followed by different letters in each column chart indicate a significant difference at the 0.05 level.

of two *Fusarium* species. The fumigation agent methyl bromide has a strong sterilization ability and can kill pests and pathogens in the deep soil layer. Methyl bromide treatment may reduce the number of *Fusarium* by killing some fungi in the soil. Previous studies have found that the *Trichoderma* protein elicitor TVHYDII2 is involved in the establishment of plant root colonization and increases the plant's antagonistic activity toward pathogens (Guzman-Guzman et al., 2017). In addition, *T. harzianum* can reduce the impact of pathogens in the soil and improve the richness of the soil bacterial community (Ganuza et al., 2019). *Trichoderma* can promote the growth of beneficial flora and reduce pathogen invasion through specific interactions with host pathogens (Woo et al., 2014). Our results are consistent with these observations and indicate that *T. harzianum* can exhibit antagonism toward pathogens in apple replant soil, promote the growth of other beneficial microorganisms, and create a better soil microbial environment for plant growth.

Conclusion

Application of *T. harzianum* fertilizer can improve plant root activity, reduce the levels of phenolic acids and pathogenic fungi in replant soil, and minimize apple replant obstacles. It has great potential as an effective, green amendment for the prevention and control of replant obstacles in apple.

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