Exploring Root-mediated Erwinia amylovora Invasion Mechanism in Korla fragrant pear (Pyrus sinkiangensis)

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Abstract

Erwinia amylovora, a Gram-negative bacterium in the genus *Erwinia*, responsible for fire blight in rosaceous plants, has long been studied for its aerial infection pathways. However, the root-mediated transmission of *E. amylovora* remains enigmatic. This study bridges this critical knowledge gap through integrated greenhouse and field experiments on two model systems, i.e. seed-propagated potted birch-leaf pear (*Pyrus betulifolia*) sapling in a greenhouse and grafted Korla fragrant pear (*Pyrus sinkiangensis* YÜ) trees using birch-leaf pears as the rootstocks in orchards located in Xinjiang, China. By combining controlled root wounding regimes (fibrous roots/lateral roots/taproots) and soil drenching or embedded *E. amylovora*-infected branches, we found that taproot-wounded specimens exhibited higher disease incidence (57.4±3.1%) than lateral root- (34.4±2.1%) and fibrous root-wounded (12.3±0.6%) specimens with 10^8 CFU/mL *E. amylovora* suspension at 15 dpi. Field trenching trials demonstrated depth-dependent epidemiology, and 40-cm deep tillage compromising taproots (30–40 cm) caused significant disease incidence versus 0% disease incidence in 20-cm trenching. Based on the findings, we proposed a root-aware tillage protocol, including controlling restrict depth (shallow depth ≤ 20 cm to protect taproots, or deep depth > 40 cm using vertical-shank subsoilers), and implementing dormancy-phase operations to leverage accelerated wound healing. Overall, this study not only first provided robust evidence demonstrating that *E. amylovora* can infect pear plants through the root system in China but also offered practical guidance for establishing fire blight-free cultivation practices in fragrant pears.

Introduction

The gram-negative bacterium *Erwinia amylovora* can cause serious fire blight, which is a devastating bacterial disease affecting rosaceous plants including pears (*Pyrus* spp.), apples (*Malus domestica*), hawthorn (*Crataegus*) and quince (*Cydonia oblonga*) (Vanderzwet et al. 2012; Vanneste 2000a). This pathogen was first documented in New York in 1780, followed by a report in New Zealand in 1910. Transcontinental dissemination occurred after its emergence in the United Kingdom in 1958, from where it subsequently spread across Europe, the Mediterranean basin, and Asia (Gaganidze et al. 2021). *E. amylovora* invades host tissues through multiple infection strategies, including floral stigmas, hypanthium nectarthodes, leaf hydathodes, stomata, and mechanical wounds (Buban et al. 2003; Thomson 2000). Long distance transmission occurs via biotic vectors (insects, birds) and abiotic media (such as rain, wind, human activities) (Momol et al. 1998; Slack et al. 2017; Zhao et al. 2019), resulting in more severe fire blight epidemics. Post-infection characteristic symptoms manifest as water-soaked tissues, necrosis and wilting of flowers, leaves, fruits, and terminal shoots (Vanneste 2000b; Vrancken et al. 2013; Pique et al. 2015), accompanied by exudate secretion and canker development in blossoms, shoots, leaves, fruitlets, limbs, trunks, collars, and rootstocks (Billing 2000; Vanneste and Eden-Green 2000).

Notably, *E. amylovora* exhibits pan-seasonal infectivity across all host tissues, with disease development accelerated under warm and highly humid conditions (Billing 2000; Santander and Biosca 2017). Conventional understanding posits fire blight as an aerial phytopathology (Buban et al. 2003; Thomson

2000; Vanderzwet et al. 2012), while recent epidemiological evidence challenges this paradigm. Field observations of root infections remain rare, primarily due to the widespread use of resistant rootstocks in commercial orchards, such as birch-leaf pear (*Pyrus betulaefolia*) as for pear scion cultivars (Bogs et al. 1998) or *Cydomalus* as rootstocks (Şahin 2024). However, a 2020 fire blight outbreak in a 10-acre pear orchard in Xinjiang, China, revealed atypical disease patterns following deep burial of pruned *E. amylovora*-infected branches. This observation aligns with ecological studies, demonstrating *E. amylovora* survival in soil matrices, aquatic environments, and soil-inhabiting insects and nematodes (Chantanao and Jensen 1969; Hildebrand et al. 2000; Hildebrand et al. 2001; Santander and Biosca 2017). Experimental confirmation of root-mediated infection was provided by Santander et al. (Santander et al. 2020), who demonstrated systemic colonization from inoculated pear roots to aerial tissues, indicating that *E. amylovora* can invade root systems and migrate to aboveground plant organs. Nevertheless, the ecological relevance of root infection in wild and native Chinese pear varieties remains uncharacterized.

The agricultural practice of rotary tillage, extensively implemented in Chinese pear cultivation, presents potential epidemiological risks. In 2020, rotary tillage was applied 2–3 times per season across approximately 44,200 hectares of Korla fragrant pear orchards in Bazhou, Xinjiang, China, to facilitate weed control and soil loosening. Although rotary tillage effectively improved soil structure (Zhang et al. 2018; Li et al. 2020), this practice inadvertently incorporates infected plant debris (such as leaves, small branches) into the soil, thereby significantly increasing the risk of *E. amylovora* transmission to other pear plants via soil and roots.

The resultant soil-pathogen interactions create persistent inoculum reservoirs, yet critical knowledge gaps remain regarding the effects of tillage depth on disease transmission dynamics. Here, we hypothesized that Xinjiang's outbreak originated from pathogen introduction via tillage-mediated incorporation of infected plant tissues, exacerbated by regional cultivation practices. This study investigated the soilborne infection mechanisms of *E. amylovora* in pear root systems using two complementary approaches: (1) greenhouse experiments with potted birch-leaf pear trees exposed to different inoculum sources, and (2) field trials with Korla fragrant pear trees (*Pyrus sinkiangensis* YU) subjected to various rotary tillage depths. Our findings provide practical guidance for reducing fire blight disease in commercial orchards.

Materials and Methods

Bacterial strain and inoculum preparation

E. amylovora strain E.aYZ003 (provided by Prof. Baishi Hu, Nanjing University, China) was revitalized on nutrient agar (NA) plates at 28°C for 24 h. A single colony was transferred in nutrient broth (10.0 g peptone, 3.0 g beef extract, 5.0 g NaCl per liter of distilled water; pH 7.4; autoclaved at 120°C for 15 min) and cultured under orbital shaking (150 rpm, 28°C) for 24 h to prepare standardized inoculum suspensions.

Greenhouse experiments

Greenhouse experiments were performed in a fully automatic greenhouse (25–28°C, 75–80% relative humidity) at the Bazhou Academy of Agricultural Sciences, Korla, Xinjiang, China. Two-year-old *P. betulaefolia* seedlings (grown from seed) were cultivated individually in 24×26 cm pots containing sterilized soil and vermiculite (3:1, v/v). Due to spatial constraints, root systems exhibited limited growth (3–10 cm root length) at the time of inoculation, with plants reaching 35–40 cm in height.

Field experiments

Field experiments were performed in a commercial orchard at the No. 3 Branch of Awati Farm (Topu Agricultural Development Import and Export Co., Ltd., Korla, Xinjiang, China: 41°34'40"N, 86°4'33"E, 901 m elevation). Twelve-year-old Korla fragrant pear trees (*Pyrus sinkiangensis*) grafted onto *P. betulaefolia* were used in this study. PCR-confirmed *E. amylovora* infected branches served as natural inoculum sources.

Root wounding treatments

Through all experiments, trees were subjected to one of four standardized root treatments prior to inoculation: intact roots (non-wounded control), wounded taproots, wounded lateral roots, and wounded fibrous roots. The incidence and severity of *E. amylovora* infection symptoms were monitored at multiple time points post-inoculation (dpi).

For greenhouse-grown potted birch-leaf pear trees (25–28° temperatures, 75%–80% relative humidity), plants were gently removed from their pots and shaken to remove adhering soil. Taproots and lateral roots were mechanically wounded by carefully scratching the root bark with a sterilized scalpel, whereas fibrous roots were trimmed with sterilized scissors. Intact roots with no damage were used as negative controls (NCs). After wounding, plants were repotted into pots containing sterile soil and allowed to recover under greenhouse conditions. For field-grown Korla fragrant pear trees, roots were wounded in situ at defined soil depths. Taproots located at 30–40 cm depth and lateral roots at 20–30 cm depth were wounded by scraping off the root bark with sterile dissecting knives. Fibrous roots located at 5–20 cm depth were excised with sterile scissors. No additional soil management practices were altered during the experiment to minimize confounding effects.

Experimental design and inoculation protocols

To systematically investigate the root infection mechanism of *E. amylovora*, three distinct experimental setups were designed for bacterial inoculation. (1) Experiment A (Fig. 1A): Greenhouse-grown potted birch-leaf pear trees were root-inoculated with *E. amylovora* suspension prepared in sterile water at concentrations ranging from 10^4 to 10^9 CFU/mL. (2) Experiment B (Fig. 1B): Potted birch-leaf pear trees

in the greenhouse were challenged by burying *E. amylovora*-infected branches into the rhizosphere. (3) Experiment C (Fig. 1C): Field-grown Korla fragrant pear trees in an orchard-setting were inoculated by burying *E. amylovora*-infected branches in trenches dug to varying depths (20–40 cm) around the root zone.

Experiment A: root irrigation with E. amylovora suspension in the greenhouse (Fig. 1A)

In the greenhouse inoculation experiment using bacterial suspensions, a total of 168 potted birch-leaf pear trees (as described in the plant materials section) were used. The inoculum consisted of *E. amylovora* strain YZ003 (hereafter E.aYZ003) suspended in sterile distilled water at six concentration levels (10^4–10^9 CFU/mL). Each plant received 300 mL of bacterial suspension (300 mL/plant). All combinations of inoculum concentration and root treatment (wounded taproots, wounded lateral roots, wounded fibrous roots, and intact roots) were established, with six biological replicates per combination.

The experiments were independently conducted in three consecutive years (2019, 2020, and 2021), initiated on 11 May 2019, 29 May 2020, and 20 May 2021, respectively. Inoculated plants were irrigated either with the *E. amylovora* suspension or with sterile water (as NCs), and then maintained in a fully automated greenhouse (25–28°C, 75–80% relative humidity). Fire blight symptom development was monitored and recorded daily after inoculation.

Experiment B: rhizosphere exposure to E. amylovora-infected branches in the greenhouse (Fig. 2B)

In the greenhouse experiment using *E. amylovora*-infected branch segments as inoculum, 48 potted birch-leaf pear trees were maintained in a fully automatic greenhouse. Plants were divided into 2 main groups, an inoculated group and a non-inoculated control group. In the inoculated group, each pot received 300 g of *E. amylovora*-infected branch segments (approximately 1 cm in diameter and 10 cm in length) that were buried along the inner wall of the pot at a depth of approximately 10 cm. Control plants did not receive infected branch segments.

Within each inoculation group, trees were further assigned to the four root treatments (wounded taproots, wounded lateral roots, wounded fibrous roots, and intact roots), with 6 biological replicates per treatment (n = 6). This experiment was independently repeated in 2019, 2020, and 2021, initiated on 11 May 2019, 1 June 2020, and 19 May 2021, respectively. Plants were grown in a greenhouse equipped with an automatic sprinkler system, and fire blight symptom development was monitored daily after inoculation.

Experiment C: field inoculation of Korla fragrant pear trees with buried E. amylovora-infected branches (Fig. 1C)

For the field validation experiment, thirty 12-year-old Korla fragrant pear trees with comparable trunk diameters (23–26 cm) and standardized spacing (6 m × 4 m) were selected in commercial orchards. A randomized block design was implemented. Trenches measuring 80 cm in length and 40 cm in width were excavated at 150 cm from tree trunks, with two trench depths (20 cm and 40 cm). Symptomatic *E.*

amylovora-infected branches (50 cm length, ~ 1 cm diameter) were placed in the trenches to ensure close contact with the root system.

Eight inoculation treatments were established (Table 1), corresponding to four root wounding modes at each trench depth (designed 20 − 1 to 20 − 4 and 40 − 1 to 40 − 4). NC groups (20-NC and 40-NC, Table 1) involved mechanical wounding of lateral and fibrous roots at the corresponding depths without the addition of *E. amylovora*-infected branches. Each treatment was replicated three times, with uniform root dimensions (diameter and length) across experimental trees. After placement of infected branches, trenches were backfilled to ensure intimate contact between soil and inoculum. The experiments were conducted across distinct orchards and repeated in three consecutive years, initiated on 17 June 2019, 13 June 2020, and 17 June 2021, respectively. Disease development was monitored over a four-month observation period.

Epidemiological survey

An ancillary epidemiological survey was conducted from June to July 2021 in multiple agricultural communities in Korla City, Xinjiang, to assess fire blight prevalence under contrasting tillage regimes. Disease incidence and severity were quantified in orchards subjected with shallow (20 cm) versus deep (40cm) rotary tillage. Standardized diagnostic criteria and scoring protocols were employed of disease assessments among different sampling sites.

Pathological assessment and molecular diagnostics

Disease progression was systematically monitored throughout the experiments. In the greenhouse, fire blight severity on potted plants was evaluated using the categorical severity index described by Xu (Xu 2001) (Table S1). This index integrated visual parameters, including the extent of necrotic lesion advancement, the presence of bacterial ooze droplets, and tissue hydration status. In the field, disease severity on Korla fragrant pear tress was assessed based on the number of branches exhibiting blossom and fruit rot, and the proportion of diseased branches or necrosis area relative to the whole plant. Disease severity ratings for these field assessments followed the scales presented in Table S2 and Table S3, respectively.

Disease incidence (%) was calculated as:

$$\text{Incidence } (\%) = \frac{\mathrm{N_d}}{\mathrm{Nt}} \times 100 \text{ (Eq. 1)}$$

where N_d refers to the number of diseased plants and N_t refers to the total number of plants examined.

Disease severity index (%) was calculated as:

$$\text{Disease severity index } (\%) = \frac{\sum (\mathrm{n_i} \times \mathrm{v_i})}{\mathrm{N} \times \mathrm{V_{max}}} \times 100 \text{ (Eq. 2)}$$

where n_i refers to the number of symptomatic plant tissues in severity category i, v_i is the corresponding rating value, N is the total number of plant tissues evaluated, and V_{max} is the highest rating value on the severity scale.

Symptomatic tissues (leaves, branches, and trunks) exhibiting bacterial exudates were collected from inoculated potted birch-leaf pear trees in the greenhouse and from Korla fragrant pear trees in orchards for pathogen isolation. Samples were surface-sterilized by sequential immersion in 75% (v/v) ethanol for 30 s, 1% (v/v) sodium hypochlorite for 1 min, and three rinses in sterile water, followed by blotting dry on sterile filter paper. Tissues were then homogenized in sterile water using a mortar-pestle grinding to obtain bacterial suspensions, which were serially diluted in 10-fold steps, plated onto NA medium via an inoculation looping, and incubated at 28°C for 48 h (Ivanovic et al. 2013). Presumptive *Erwinia amylovora* colonies, characterized by smooth, convex, white colonies, were sub-cultured onto fresh NA plates to obtain pure cultures.

Genomic DNA was extracted from purified bacterial colonies using the Bacterial Genome DNA Extraction Kit (DP302, Tiangen, China), and detected by nested PCR amplification using primer pairs P29A/P29B (Bereswill et al. 1992) and PEANT1/ PEANT2 (Llop et al. 2000). Primary PCR was performed in a 20 μL reaction mixture containing 1×Taq PCR buffer and 0.1 μM of each primer (P29A: 5'-CGGTTTTTAACGCTGGG-3', P29B: 5'-GGGCAAATACTCGGATT-3'), with 1 μL of genomic DNA from pure bacterial cultures as the template. Amplification was carried out in a Veriti thermal cycler (Applied Biosystems) under the following conditions: initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 30 s, and extension at 72°C for 55 s, followed by a final extension at 72°C for 45 s. Secondary PCR was conducted in a 50 μL reaction mixture containing 1×Taq PCR buffer and 0.2 μM of each primer (PEANT1: 5'-TATCCCTAAAAACCTCAGTGC-3', PEANT2: 5'-GCAACCTTGTGCCCTTTA-3'), using 1 μL of the primary PCR product as a template (Llop et al. 2000). Cycling conditions were as follows: initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 56°C for 30 s, and extension at 72°C for 25 s, and a final extension at 72°C for 10 min. The expected 391-bp DNA fragments were separated by electrophoresis on 1% (w/v) agarose gels, purified from the gels, and then sequenced (Beijing Dingguo Co. Ltd., China). Sequences were compared with reference sequences in the NCBI databases using BLASTn to confirm the *E. amylovora* identity.

Statistical analysis

Treatment effects on disease incidence and severity in *P. betulaefolia* and *P. sinkiangensis* cv. Korla fragrant pear were analyzed through one-way analysis of variance (ANOVA) in Data Processing System (DPS) v15.0 (http://www.dpsw.cn/dps_eng/) (Tang and Zhang 2013), with statistical significance set at $p < 0.05$. All analysis included biological replicates to ensure statistical robustness.

Results

Effects of wounding and concentration of E. amylovora bacterial suspension on infection and disease progress in potted birth-leaf pear trees

Representative symptoms of potted birch-leaf trees following soil inoculation with *E. amylovora* suspension or *E. amylovora*-infected branch fragrants were shown in Fig. 2. NC groups (Fig. 2A) contrasted with progressive symptomatology in taproot-wounded plants post inoculation with *E. amylovora* suspension (10^7 CFU/mL): newly expanded leaves began to wither at 15 dpi (initial stage), progressing to extensive leaf necrosis at 40 dpi (middle-late infection stage) (Fig. 2C), culminating in plant mortality with characteristic shepherd's crook deformities at 70 dpi (final disease stage) (Fig. 2D). Histological analysis of wounded roots revealed concentration-dependent vascular discoloration (reddish-brown xylem/phloem), which increased in intensity with higher inoculum loads (10^6–10^9 CFU/mL) (Fig. 2E-F). Moreover, comparative sections demonstrated wound-dependency for lateral root infections when pots were inoculated with *E. amylovora* infected branches (Fig. 2G).

Table 2 showed the mean disease incidence and severity across four root-wounding treatments and six *E. amylovora* inoculum concentrations (10^4–10^9 CFU/mL) monitored at 78-, 84- and 97-days post-inoculation (dpi) during 2019–2021 greenhouse trials. The analysis of disease incidence and severity revealed that 86.2% of taproot- and lateral root-wounded trees were infected (severity index > 56.5) when exposed to inoculum concentration ≥ 1×10^7 CFU/mL by 78 dpi, in stark contrast to only 18% infection in intact-root control trees under equivalent inoculum loads. The analysis further demonstrated dose-dependent infection patterns across all four root-wounding treatments. Temporal progression data from Tables S4-S6 encompassed four distinct phases including 15–16 dpi (early phase), 32–33 dpi (mid-phase), 48–67 dpi (advanced phase), and 79–96 dpi (terminal phase) observations (Fig. 3), which confirmed positive correlations between inoculum concentrations and both incidence and severity. Fibrous roots wounds exhibited delayed susceptibility, showing symptoms at 30 dpi when inoculated with 10^4 CFU/mL *E. amylovora* suspension, whereas taproot and lateral root wounds demonstrated early colonization, with symptoms evident at 15 dpi at the same inoculum concentration (Table S4-S6). Infection velocity followed a strict hierarchy pattern: taproot wounds > lateral root wounds > fibrous root wounds, establishing root architecture as a critical determinant of *E. amylovora* invasion efficiency.

Symptomatic leaves and branches exhibiting characteristic hook-shaped dieback were collected from all experimental groups at 30 dpi for bacterial isolation. Pathogen detection was performed using a nested PCR protocol with specific primers (Llop et al. 2000). Representative agarose gel electrophoresis profiles spanning 2019–2021 (Fig. S3–S5) revealed consistent amplification of a distinct 391 bp band corresponding to *E. amylovora* in root-wounded potted trees, while non-inoculated controls showed no target amplification. The PCR detection results were summarized in Table 3. Samples were considered positive if ≥ 1/6 technical replicates showed amplification products of 391 bp, and negative if all replicates showed no amplification products of 391 bp. As detailed in Table 3, all NC plants consistently tested negative across six biological replicates, whereas most root-wounded trees tested positive for *E. amylovora* (> 72.2%), with successful pathogen identification confirmed in symptomatic tissues.

Effects of root-wounding and buried E. amylovora-infected branches on E. amylovora infection and disease progress in potted birch-leaf pear trees

The synergistic effects of root wounding and buried *E. amylovora*-infected branches on disease progression in potted birch-leaf pear trees were systematically investigated across three growing seasons (2019–2021). Characteristic symptoms, including leaf wilt, necrosis, and hook-shaped dieback, were documented in Fig. S6. Terminal disease incidence, disease severity, and ANOVA results from each annual trial were compiled in Table 4, while Fig. 4 synthesized temporal progression patterns using averaged values from Tables S7-S9 across four critical phases: early infection (15–17 dpi), mid-phase (32–33 dpi), advanced phase (48–67 dpi), and terminal stage (79–96 dpi). Control groups with wounded roots but no pathogen exposure remained asymptomatic throughout the study period, whereas those receiving *E. amylovora*-infected branches exhibited wound-dependent susceptibility gradients: taproot-wounded > lateral root-wounded > fibrous root-wounded ($p < 0.05$). Notably, intact-root specimens exposed to *E. amylovora*-infected branches demonstrated delayed symptom onset (32 dpi) with significantly lower disease severity (16.9 ± 2.7% incidence vs. 67.5 ± 2.3% in taproot-wounded plants, $p < 0.05$), establishing mechanical root injury as a critical predisposing factor for fire blight establishment.

Furthermore, symptomatic leaves and branches with hook-shaped dieback were collected from inoculated potted birch-leaf pear trees for bacterial isolation and pathogen detection via nested PCR (Llop et al. 2000). Representative agarose gel electrophoresis profiles spanning 2019–2021 (Fig. S7–S9) revealed consistent amplification of a 391 bp *E. amylovora*-specific amplicon in root-wounded specimens, while NC groups produced no target amplification. As presented in Table 5, taproot-wounded showed the highest detection rate (75.0%, 27/36 samples), followed by lateral root-wounded trees (63.9%, 23/36 samples), while fibrous root-wounded trees exhibited the lowest rate (55.6%, 20/36 samples). Notably, NC plants remained PCR–negative across all tissues (0/36 sample), confirming that mechanical root wounding severity is a critical determinant of systemic *E. amylovora* colonization.

Burial depth and root wounding mediate soil-borne E. amylovora transmission in Korla fragrant pear trees

The influence of *E. amylovora*-infected branch burial depth on root-mediated fire blight transmission in Korla fragrant pear trees was investigated through field trenching trials (Fig. S10). Pathognomonic bacterial ooze emerged at 9 dpi in taproot-wounded trees buried at 40 cm depth (11.8% disease incidence) (Fig. S10a), whereas the shallower 20 cm trench treatment and NC group maintained 0% disease incidence throughout the observation period (Table S10). Among 40 cm trench treatments, cohort 40 − 1 (with 3 taproots and 22 lateral roots wounded) exhibited the earliest symptom onset (11.8 ± 0.8% at 9 dpi), progressing to 49.7 ± 1.4% incidence by 15 dpi, significantly exceeding cohorts 40 − 4 (38.0 ± 1.3%), 40 − 2 (39.1 ± 1.7%), and 40 − 3 (38.7 ± 2.7%) by 15 dpi. Longitudinal analysis of disease incidence and severity from 2019 to 2021 (Table S10) confirmed that all 40-cm trench cohorts exceeded 22.6% incidence by 15 dpi, with the root wouding hierarchy persisting beyond 15 dpi in the following order: 40 − 1 > 40 − 4 > 40 − 2 > 40 − 3 (Fig. 5). Disease severity progression paralleled incidence trends,

demonstrating a positive correlation between the number of wounded taproots and lateral roots and symptom escalation rate. Temporal accumulation of infected trees across all treatments confirmed roots as the primary transmission, with mechanical injuries enabling soil stratum penetration of *E. amylovora*.

Symptomatic tissues (leaves, branches, and cankered trunk bark) from *E. amylovora*-infected Korla fragrant pear plants across all experimental groups (2019–2021) were collected for bacterial isolation, and pathogen detection via nested PCR (Llop et al. 2000). Agarose gel electrophoresis profiles (Fig. S11–S13) consistently revealed a 391 bp amplicon characteristic of *E. amylovora*, with S anger sequencing of representative isolates demonstrating 99.87% sequence identity to *E. amylovora* reference strains via BLASTn alignment (Table 6). Notably, roots and trunk base tissues were the predominant sites of *E. amylovora* localization compared to leaf tissues, with an overall pathogen detection rate of approximately 56.48% across all the specimens. Field monitoring of the effects of rotary tillage depth on fire blight epidemiology revealed that shallow rotary tillage (20 cm depth) resulted in no disease occurrence, whereas deep tillage (40 cm depth) resulted in disease incidence of 5.6–8.9% (Table 7).

Discussion

The emergence of fire blight in Korla fragrant pear trees was first documented in Yili, Xinjiang, China during 2016 epiphytotic surveys (Sun et al. 2023). Subsequent epidemiological investigations (2017 – 2018) across Korla pear orchards demonstrated higher canker incidence in deep-tillage orchards (≥ 40 cm depth) compared to shallow–tillage (≤ 20 cm depth) or no–tillage controls. To mechanically replicate tillage impacts, we established a stratified trenching system: 20 cm trenches targeting fibrous roots (10–20 cm stratum) and 40 cm trenches damaging both lateral roots (20–30 cm stratum) and taproots (30–40 cm stratum). In our study, deep tillage (40 cm) exposed taproots and lateral roots to mechanical injuries, facilitating rapid systemic colonization through direct access to high-density vascular tissues (xylem vessels) (Baer et al. 2021), whereas shallow tillage (20 cm) primarily damaged fibrous roots with less developed vascular connections, impeding pathogen establishment. This difference in root morphology and vascular development explained the observed disparity in field disease incidence (Sun et al. 2023; Tsunoda and van Dam 2017).

Since Korla fragrant pear trees (*P. sinkiangensis* YÜ) are grafted onto birch-leaf pear (*P. betulaefolia*) rootstock, we further investigated *E. amylovora* susceptibility in birch-leaf pear trees through controlled greenhouse trials using two inoculation approaches: (1) mechanical root wounding followed by soil drenching with *E. amylovora* suspensions, and (2) rhizosphere embedding of *E. amylovora*-infected branches. Taproot-wounded specimens exhibited the fastest infection and the highest disease incidence at 15 dpi, significantly exceeding fibrous root-wounded and intact root specimens (Fig. 4), confirming the critical role of mechanical root wounding in facilitating *E. amylovora* root colonization. Taproots serve as the primary hydraulic and structural conduits, orchestrating resource allocation through centralized vascular networks (Tsunoda and van Dam 2017), thereby facilitating rapid apoplastic transport and enabling *E. amylovora* colonization through wound sites.

Field epidemiological investigations quantified fire blight management risks associated with rotary tillage depths in Korla fragrant pear orchards. Shallow tillage (20 cm depth) resulted in no disease occurrence, while deep tillage (40 cm depth) increased disease incidence to 5.6–8.9% within one month (Table 7), correlating with root architecture damage severity. These findings aligned with controlled trenching trials (Table S10). Therefore, to mitigate *E. amylovora* transmission through mechanized tillage, we proposed three complementary management strategies: depth-zoned tillage, sanitation synchronization, and temporal optimization. Depth-zoned tillage employs precision shallow tillage (< 20 cm) to protect taproots for year-round management, or alternatively, strategic deep tillage (> 40 cm) using a vertical-shank subsoiler to avoid the critical 20–40 cm root zone where major root wounding occurs. Timely removal of fallen leaves and small branches using vacuum-assisted debris harvesters may reduce *E. amylovora* inoculum accumulation in the soil, thereby decreasing transmission risk during rotary tillage operations. Moreover, scheduling tillage operations during the dormancy period may promote wound healing and reduce *E. amylovora* colonization through wound sites.

Controlled inoculation trials conducted on greenhouse-grown birch-leaf pear (*P. betulaefolia*) and field-grown Korla fragrant pear trees revealed that mechanical root wounding, particularly of taproots, plays a critical role in facilitating *E. amylovora* root infection and systemic colonization. Rotary tillage depth significantly affected *E. amylovora* spread in the orchard. Therefore, standardized tillage protocols must be implemented to control soil disturbance depth and maintain safe distance from trees, thereby preventing taproot wounding and reducing disease transmission risk. Our study provided strong evidence for the *E. amylovora* root transmission pathway and practical management recommendations for disease control in pear cultivation.

Declarations

Author Contributions

WMC and LLH conceived and directed the study, design and perform the infection experiments; RL and LLH analyzed the data; RL, LLH, YS, JHW, FZ and XD wrote the manuscript.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article and its additional files. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. Baer, A. B., Fickle, J. C., Medina, J., Robles, C., Pratt, R. B., & Jacobsen, A. L. (2021). Xylem biomechanics, water storage, and density within roots and shoots of an angiosperm tree species. J Exp Bot. 72: 7984–7997. https://doi.org/10.1093/jxb/erab384%J Journal of Experimental Botany.
2. Bereswill, S., Pahl, A., Bellemann, P., Zeller, W., & Geider, K. (1992). Sensitive and species-specific detection of *Erwinia amylovora* by polymerase chain reaction analysis. *Applied And Environment Microbiology*, *58*, 3522–3526. https://doi.org/10.1128/aem.58.11.3522-3526.1992
3. Billing, E. (2000). Fire blight risk assessment systems and models. In J. L. Vanneste (Ed.), *Fire Blight: The Disease and its Causative Agent, Erwinia Amylovora* (pp. 1–6). CABI Publishing. https://doi.org/10.1079/9780851992945.0293
4. Bogs, J., Bruchmuller, I., Erbar, C., & Geider, K. (1998). Colonization of host plants by the fire blight pathogen *Erwinia amylovora* marked with genes for bioluminescence and fluorescence. *Phytopathology*, *88*, 416–421. https://doi.org/10.1094/phyto.1998.88.5.416
5. Buban, T., Orosz-Kovacs, Z., & Farkas, A. (2003). The nectary as the primary site of infection by *Erwinia amylovora* (Burr.) Winslow et al.: a mini review. Plant Syst Evol. 238: 183–194. https://doi.org/10.1007/s00606-002-0266-1
6. Chantanao, A., & Jensen, H. J. (1969). Saprozoic nematodes as carriers and disseminators of plant pathogenic bacteria. *J Nematol*, *1*, 216–218.
7. Gaganidze, D., Sadunishvili, T., Aznarashvili, M., Abashidze, E., Gurielidze, M., Carnal, S., Rezzonico, F., & Zubadalashvili, M. (2021). Fire blight distribution in Georgia and characterization of selected *Erwinia amylovora* isolates. *J Plant Pathol*, *103*, 121–129. https://doi.org/10.1007/s42161-020-00700-5
8. Hildebrand, M., Dickler, E., & Geider, K. (2000). Occurrence of *Erwinia amylovora* on insects in a fire blight orchard. *J Phytopathol-Phytopathol Z*, *148*, 251–256. https://doi.org/10.1046/j.1439-0434.2000.00504.x
9. Hildebrand, M., Tebbe, C. C., & Geider, K. (2001). Survival studies with the fire blight pathogen *Erwinia amylovora* in soil and in a soil-inhabiting insect. *J Phytopathol-Phytopathol Z*, *149*, 635–639. https://doi.org/10.1046/j.1439-0434.2001.00685.x
10. Ivanovic, M., Kuzmanovic, N., Prokic, A., Blagojevic, N., Obradovic, A., & Gasic, K. (2014). Evaluation of Three Extraction Methods for Detection of *Erwinia amylovora* from Pear Leaves by Real-Time PCR. In: 13th International Workshop on Fire Blight, Zurich, SWITZERLAND, Jul 02–05 2013. Acta Horticulturae. pp 81–84 https://doi.org/DOI.
11. Li, X. Z., Wei, B. H., Xu, X. L., & Zhou, J. (2020). Effect of Deep Vertical Rotary Tillage on Soil Properties and Sugarcane Biomass in Rainfed Dry-Land Regions of Southern China. *Sustainability*, *12*, 10199. https://doi.org/10.3390/su122310199
12. Llop, P., Bonaterra, A., Peñalver, J., & López, M. M. (2000). Development of a highly sensitive nested-PCR procedure using a single closed tube for detection of *Erwinia amylovora* in asymptomatic plant

material. *Applied And Environment Microbiology*, *66*, 2071–2078. https://doi.org/10.1128/aem.66.5.2071-2078.2000

13. Momol, M. T., Norelli, J. L., Piccioni, D. E., Momol, E. A., Gustafson, H. L., Cummins, J. N., & Aldwinckle, H. S. (1998). Internal movement of *Erwinia amylovora* through symptomless apple scion tissues into the rootstock. *Plant Disease*, *82*, 646–650. https://doi.org/10.1094/pdis.1998.82.6.646
14. Pique, N., Minana-Galbis, D., Merino, S., & Tomas, J. M. (2015). Virulence Factors of *Erwinia amylovora*: A Review. *International Journal Of Molecular Sciences*, *16*, 12836–12854. https://doi.org/10.3390/ijms160612836
15. Şahin, M. (2024). Determination of tolerance to Erwinia amylovora of intergeneric hybrid genus: Cydomalus. *J Plant Pathol*, *106*, 1039–1044. https://doi.org/10.1007/s42161-024-01692-2
16. Santander, R. D., & Biosca, E. G. (2017). *Erwinia amylovora* psychrotrophic adaptations: evidence of pathogenic potential and survival at temperate and low environmental temperatures. *PeerJ*, *5*, e3931. https://doi.org/10.7717/peerj.3931
17. Santander, R. D., Catala-Senent, J. F., Figas-Segura, A., & Biosca, E. G. (2020). From the roots to the stem: unveiling pear root colonization and infection pathways by *Erwinia amylovora*. *Fems Microbiology Ecology*, *96*, fiaa210. https://doi.org/10.1093/femsec/fiaa210
18. Slack, S. M., Zeng, Q., Outwater, C. A., & Sundin, G. W. (2017). Microbiological Examination of *Erwinia amylovora* Exopolysaccharide Ooze. *Phytopathology*, *107*, 403–411. https://doi.org/10.1094/phyto-09-16-0352-r
19. Sun, W. B., Gong, P. J., Zhao, Y. C., Ming, L., Zeng, Q., & Liu, F. Q. (2023). Current Situation of Fire Blight in China. *Phytopathology*, *113*, 2143–2151. https://doi.org/10.1094/phyto-05-23-0170-rvw
20. Tang, Q. Y., & Zhang, C. X. (2013). Data Processing System (DPS) software with experimental design, statistical analysis and data mining developed for use in entomological research. *Insect Sci*, *20*, 254–260. https://doi.org/10.1111/j.1744-7917.2012.01519.x
21. Thomson, S. (2000). Epidemiology of fire blight. In J. L. Vanneste (Ed.), *The Disease and its Causative agent, Erwinia Amylovora* (pp. 9–36). CABI Publishing. https://doi.org/10.1079/9780851992945.000
22. Tsunoda, T., & van Dam, N. M. (2017). Root chemical traits and their roles in belowground biotic interactions. *Pedobiologia*, *65*, 58–67. https://doi.org/https://doi.org/10.1016/j.pedobi.2017.05.007
23. Vanderzwet, T., Orolaza-Halbrendt, N., & Zeller, W. (2012). *Fire Blight: History, Biology, and Managemen*. APS. https://doi.org/10.1094/9780890544839
24. Vanneste, J. L. (2000a). Genetic diversity and host range of *Erwinia amylovora*? In J. L. Vanneste (Ed.), *Fire Blight: The Disease and Its Causative Agent, Erwinia Amylovora* (pp. 55–86). CABI Publishing. https://doi.org/10.1079/9780851992945.005
25. Vanneste, J. L. (2000b). What is fire blight? Who is *Erwinia amylovora*? How to control it? In J. L. Vanneste (Ed.), *Fire Blight: The Disease and its Causative Agent, Erwinia Amylovora* (pp. 1–6). CABI Publishing. https://doi.org/10.1079/9780851992945.0001

26. Vanneste, J. L., & Eden-Green, S. (2000). Migration of *Erwinia amylovora* in host plant tissues. In J. L. Vanneste (Ed.), *Fire Blight: The Disease and Its Causative Agent, Erwinia Amylovora* (pp. 73–86). CABI Publishing. https://doi.org/10.1079/9780851992945.007
27. Vrancken, K., Holtappels, M., Schoofs, H., Deckers, T., & Valcke, R. (2013). Pathogenicity and infection strategies of the fire blight pathogen *Erwinia amylovora* in Rosaceae: State of the art. Microbiology-(UK). 159: 823–832. https://doi.org/10.1099/mic.0.064881-0
28. Xu, Z. G. (2001). *Experimental guidance for general plant pathology*. Higher Education Press.
29. Zhang, L., Wang, J., Fu, G., & Zhao, Y. (2018). Rotary tillage in rotation with plowing tillage improves soil properties and crop yield in a wheat-maize cropping system. *PLoS One*, *13*, e0198193. https://doi.org/10.1371/journal.pone.0198193
30. Zhao, Y. Q., Tian, Y. L., Wang, L. M., Geng, G. M., Zhao, W. J., Hu, B. S., & Zhao, Y. F. (2019). Fire blight disease, a fast-approaching threat to apple and pear production in China. *J Integr Agric*, *18*, 815–820. https://doi.org/10.1016/s2095-3119(18)62033-7

Tables

Tables are available in the Supplementary Files section.

Figures

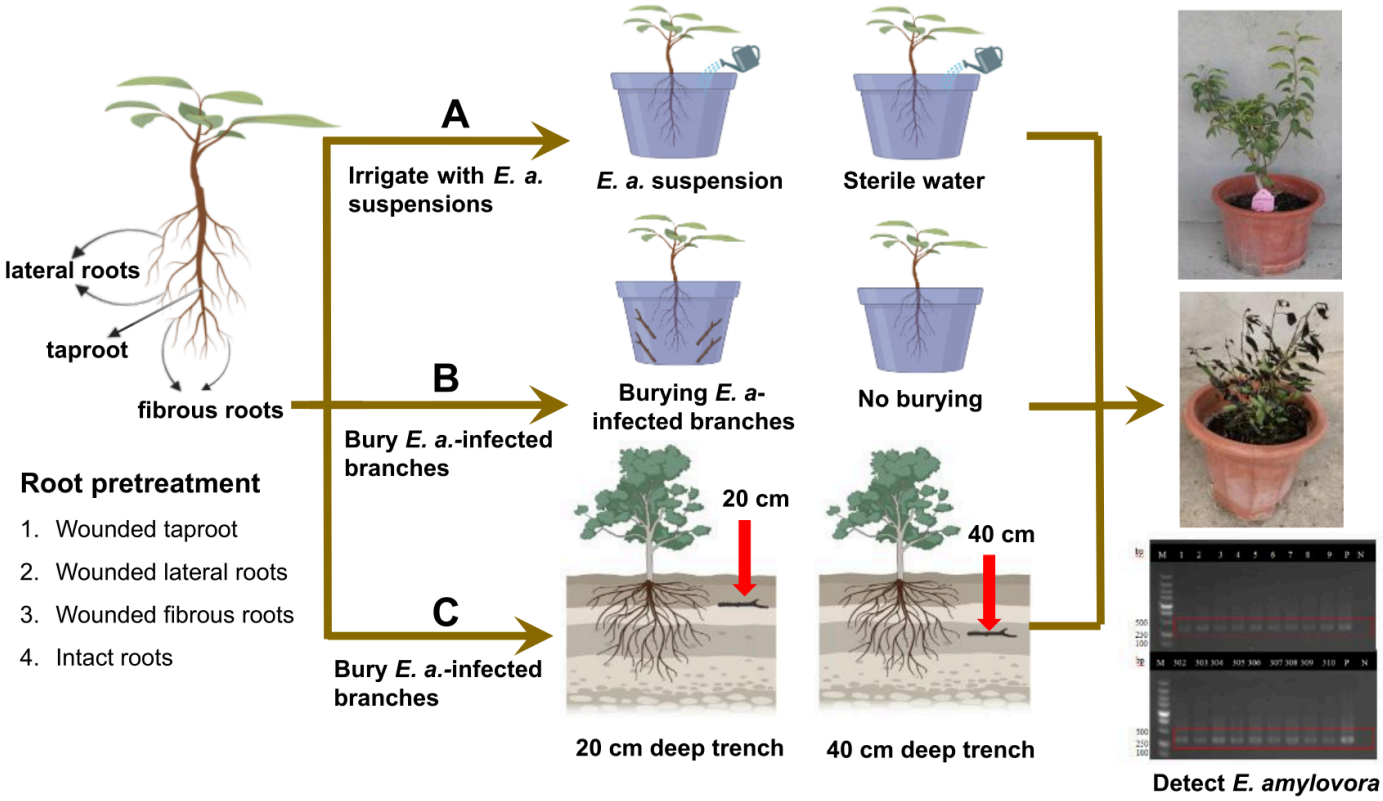

Fig. 1 Experimental framework for investigating the root infection mechanism of *E. amylovora*. **(A)** Greenhouse-grown potted birch-leaf pear trees were root-inoculated by irrigation with an *E. amylovora* suspension (10^4–10^9 CFU/mL in sterile water) or with sterile water as a NC. **(B)** Greenhouse-grown potted birch-leaf pear trees were challenged by burying *E. amylovora*-infected branches in the rhizosphere. **(C)** Field validation experiment on Korla fragrant pear trees in an orchard, in which *E. amylovora*-infected branches were buried in shallow (20 cm) or deep trenches (40 cm) around the root zone. All trees were subjected to four root treatments: wounded taproots, wounded lateral roots, wounded fibrous roots and intact (unwounded) roots.

Figure 1

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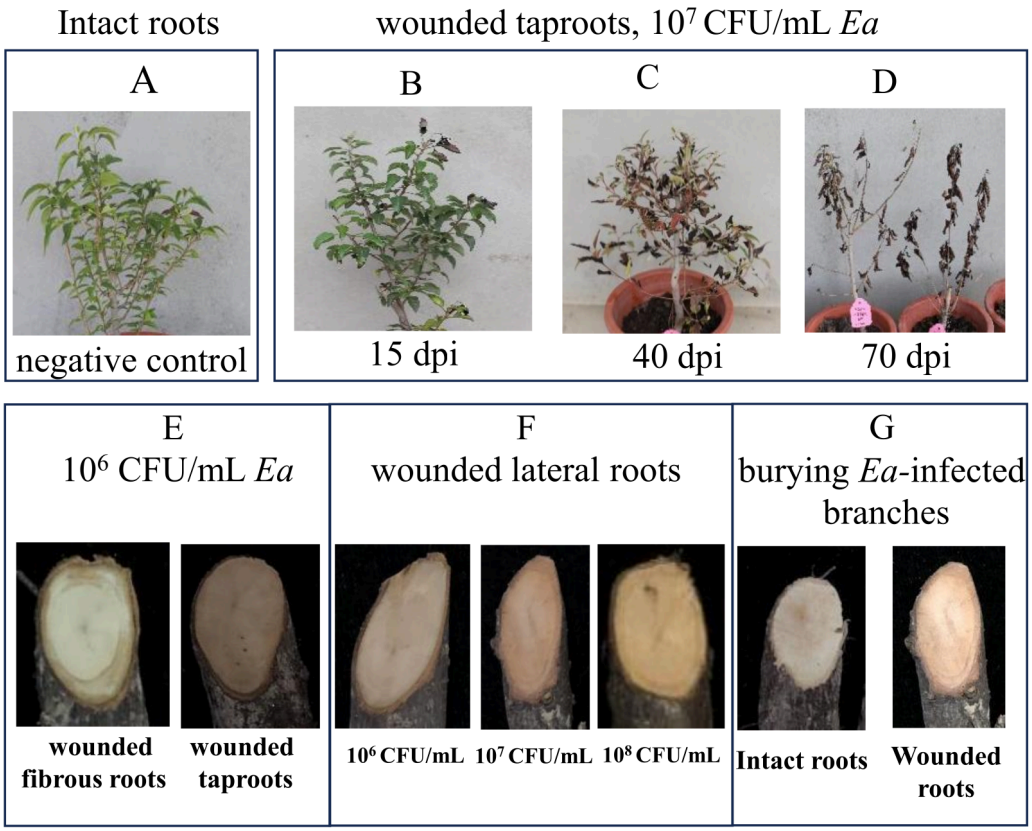

Fig. 2 Symptoms progression in potted birch-leaf trees under different inoculation conditions. **(A)** NC: taproot-wounded plant irrigated with sterile water at 40 dpi. **(B-D)** Temporal disease progression in taproot-wounded plants treated with *E. amylovora* suspension (1×10^7 CFU/mL): initial leaf wilt (B, 15 dpi), progressive necrosis (C, 40 dpi), and systemic collapse with characteristics shepherd's crook deformity (D, 70 dpi). **(E)** Vascular discoloration in wounded fibrous roots and taproots following inoculation with 1×10^6 CFU/mL *E. amylovora* suspension (15 dpi). **(F)** Dose-dependent symptom severity in lateral root-wounded plants exposed to inoculum gradients of 1×10^6–1×10^8 CFU/mL (33 dpi). **(G)** Wound-dependent infectivity: comparison between intact roots and lateral root-wounded plants inoculated with *E. amylovora*-infected branches (33 dpi).

Figure 2

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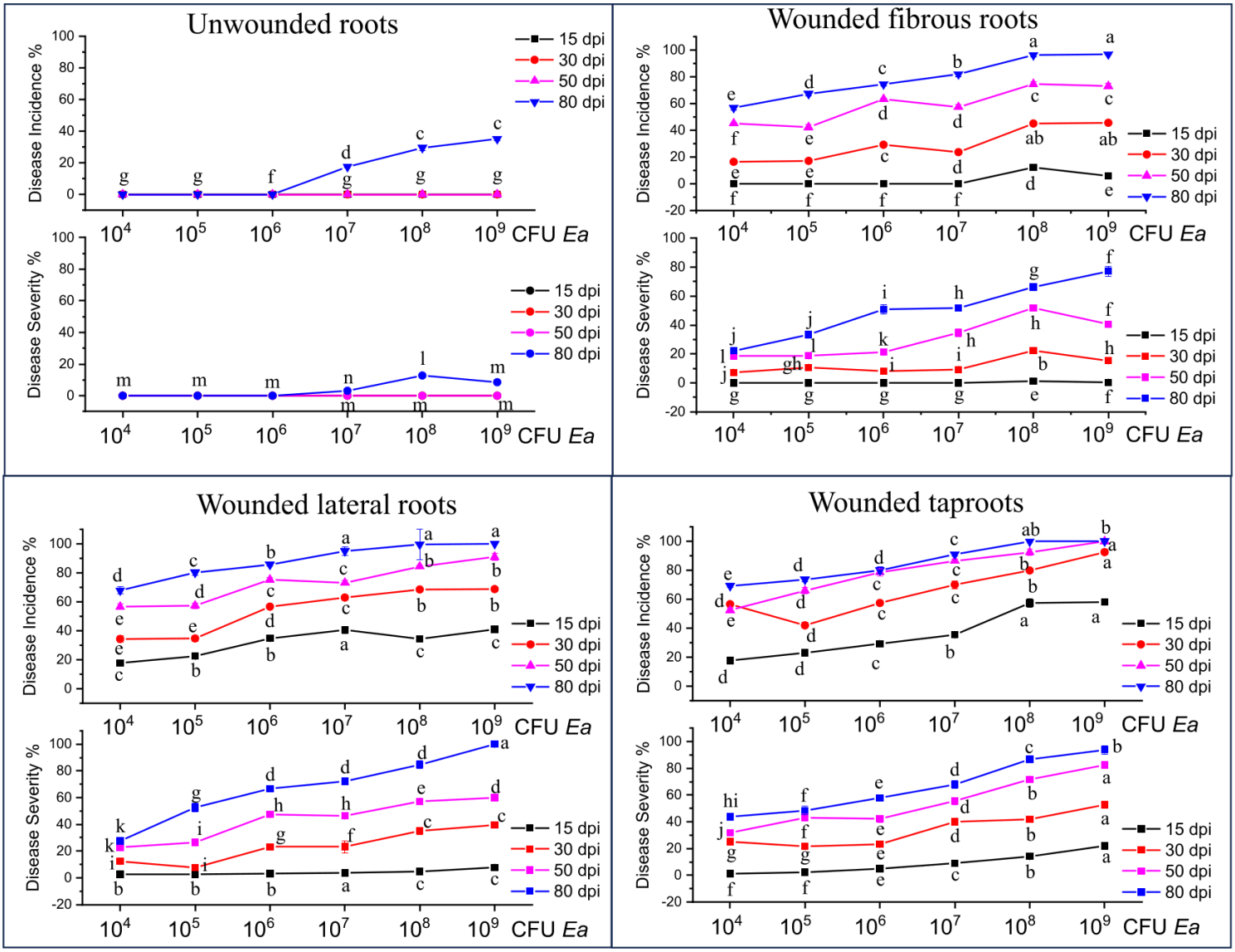

Fig. 3 Analysis of mean disease incidence and severity (2019–2021) in root-wounded potted birch-leaf trees exposed to various concentrations of *E. amylovora* suspension. The lowercase letters indicated statistically significant differences among all treatments (one-way ANOVA, $p<0.05$).

Figure 3

See image above for figure legend

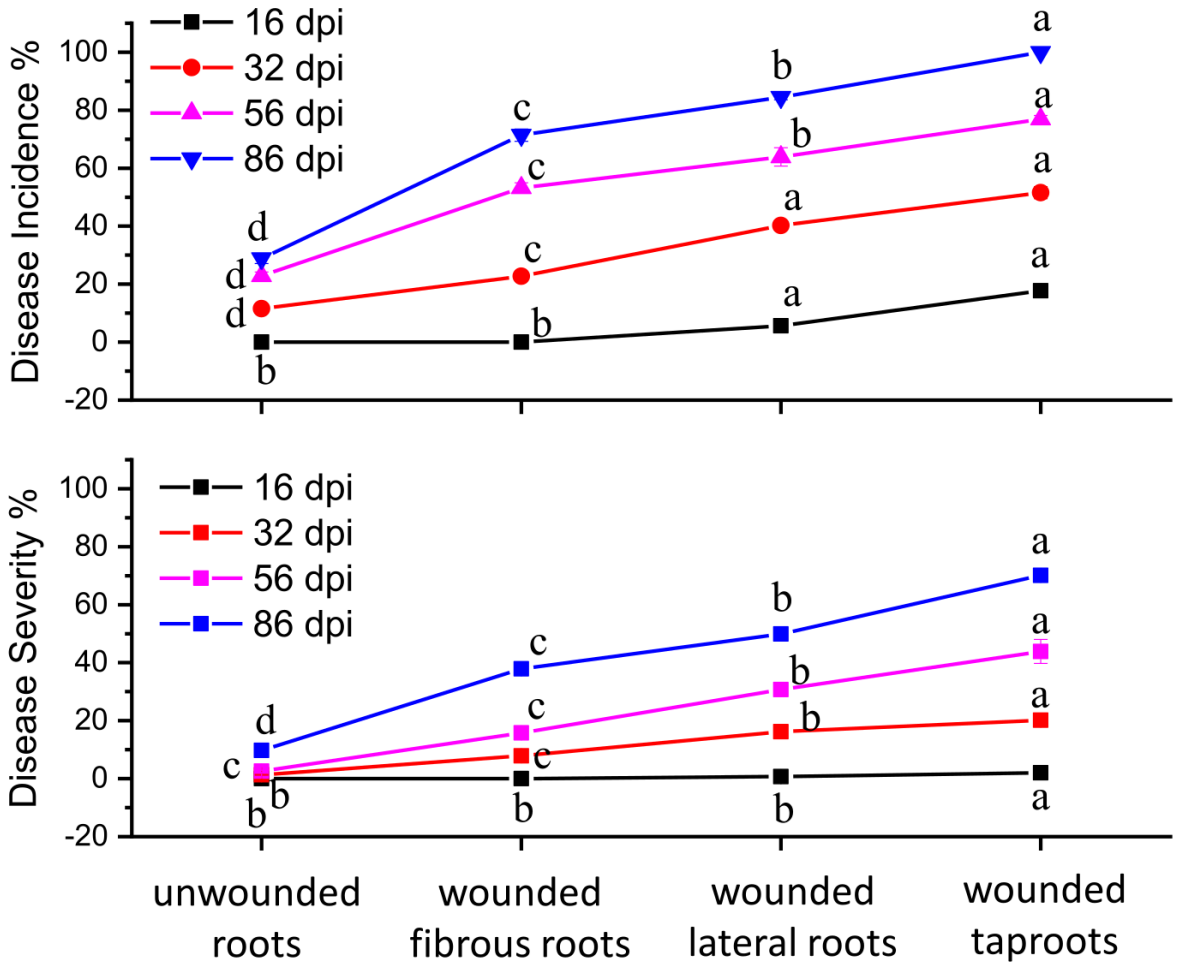

Fig. 4 Analysis of mean disease incidence and severity (2019–2021) in root-wounded potted birch-leaf trees exposed to buried *E. amylovora*-infected branches. The lowercase letters indicated statistically significant differences among all treatments (one-way ANOVA, $p < 0.05$).

Figure 4

See image above for figure legend

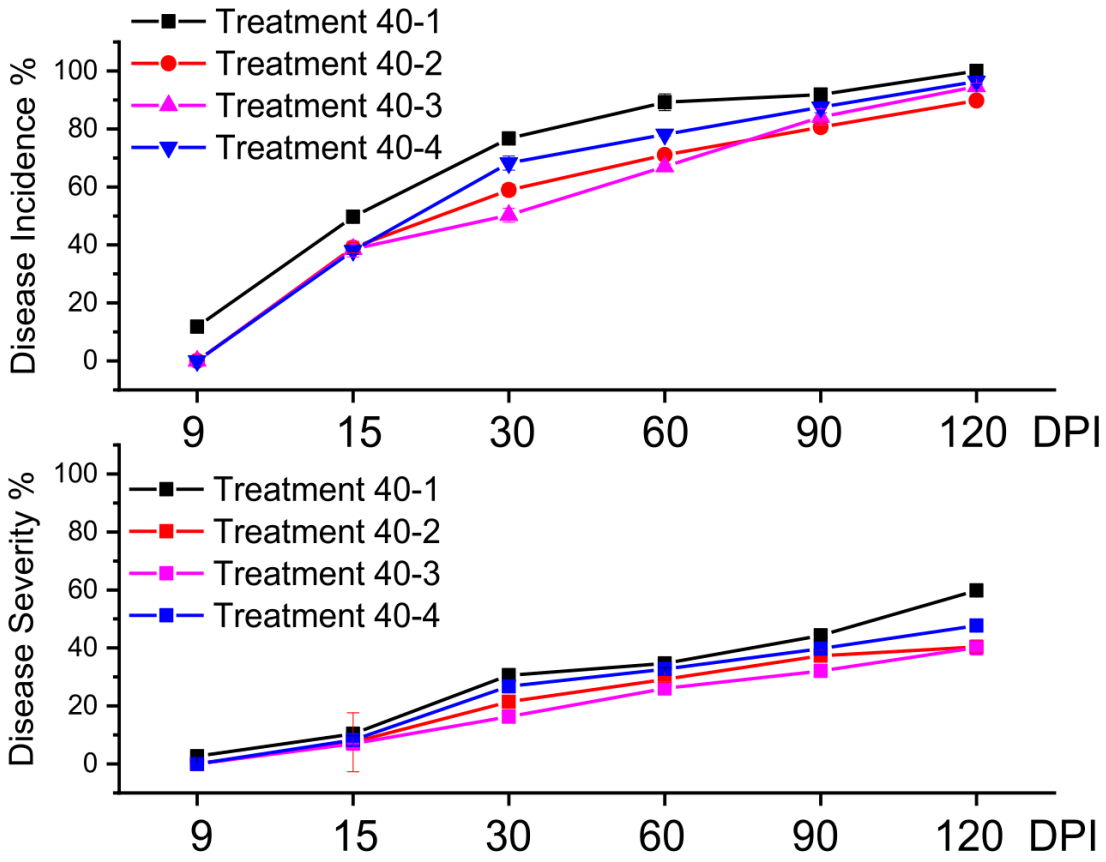

Fig. 5 Analysis of mean disease incidence and severity (2019–2021) for root-wounded Korla fragrant pear trees exposed to buried *E. amylovora*-infected branches in 40 cm deep trenches. The lowercase letter indicated the statistically significant difference (one-way ANOVA, $p < 0.05$).

Figure 5

See image above for figure legend

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Tables.pdf
- ErwiniaamylovoraSupplementaryinformation.docx