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Microfluidic Plant-on-a-Chip Platforms for High-Resolution Real-Time Imaging of Root–Pathogen Dynamics

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ABSTRACT

The rhizosphere constitutes one of the most complex and biologically dense microenvironments, where plant roots continuously interact with diverse soil biota ranging from beneficial rhizobacteria to destructive phytopathogens. Deciphering the spatiotemporal mechanisms governing root–pathogen dynamics is essential for developing targeted crop protection strategies. Traditional methods such as agar plates and hydroponic systems fail to recreate the physical geometry, chemical gradients, and fluidic dynamics of natural soils while imposing severe optical limitations that prevent high-resolution imaging. Plant-on-a-Chip (PoC) and Root-on-a-Chip (RoC) platforms, adapted from mammalian organ-on-chip engineering, have emerged as transformative tools that overcome these methodological bottlenecks. Fabricated primarily via soft lithography using optically transparent polydimethylsiloxane (PDMS), these microfluidic devices manipulate picoliter fluid volumes within sub-millimeter channels to mimic soil-like microenvironments. When integrated with advanced live-cell microscopy including confocal, differential interference contrast, and atomic force microscopy these platforms enable non-destructive, real-time visualization of root–pathogen interactions at single-cell resolution. This review systematically examines microfluidic design paradigms, fabrication methodologies, and imaging techniques while presenting mechanistic insights into oomycete chemotaxis and infection kinetics,

bacterial wilt pathogenesis, nematode feeding site induction, and competitive exclusion by beneficial rhizobacteria. Key pathosystem dynamics revealed include *Phytophthora* zoospore klinokinesis within 300 μm of host roots, *Ralstonia* biofilm-driven xylem hydraulic failure, and *Bacillus subtilis* protective barrier formation. Despite current technical challenges including PDMS hydrophobicity, root growth constraints, and device delamination emerging innovations in thermoplastic materials, 3D soil-mimetic architectures, multi-species synthetic communities, and standardized EcoFAB protocols promise to advance plant microfluidics toward high-throughput screening of agricultural chemicals and pathogen-resistant cultivars.

Keywords—Plant-on-a-Chip, Root-on-a-Chip, microfluidics, rhizosphere, root–pathogen interactions, live-cell imaging, *Phytophthora*, *Ralstonia*, biocontrol, rhizobacteria

1. Introduction

The rhizosphere represents one of the most structurally complex, dynamically variable, and biologically dense microenvironments on Earth. Within this subterranean interface, plant root systems interact continuously with a mega-diverse community of soil biota, ranging from beneficial plant-growth-promoting rhizobacteria (PGPR) to highly destructive phytopathogens, including fungi, oomycetes, bacteria, and plant-parasitic nematodes (Hinsinger et al., 2009). Deciphering the precise spatiotemporal mechanisms that govern host–pathogen interactions at the root interface is essential for developing targeted crop protection strategies and safeguarding global food security against emerging agricultural threats (Tsai et al., 2023).

Historically, investigating root–microbe dynamics *in situ* has been hindered by the inherent opacity, structural heterogeneity, and physical anisotropy of natural soils. Traditional laboratory assays such as growing seedlings on vertical agar plates, in gel matrices, or within static hydroponic channels fail to recreate the microscale physical geometry, localized chemical micro-gradients, and fluidic dynamics of the natural rhizosphere. Furthermore, conventional agar systems impose severe optical limitations (Mehra et al., 2025). The optical thickness and light-scattering properties of agar degrade spatial resolution, while the lack of physical confinement allows roots and root hairs to drift along the vertical optical plane (z -axis) during growth or chemical treatment. This spatial drift continuously shifts target cellular and subcellular structures out of focus during time-lapse microscopy, preventing high-magnification tracking of dynamic infection events (Hammer, 2001).

To overcome these methodological bottlenecks, the plant science community has adapted microfluidic and organ-on-a-chip (OoC) engineering paradigms originally developed for mammalian tissue modeling and biomedical diagnostics. Over the past two decades, Plant-on-a-Chip (PoC) and Root-on-a-Chip (RoC) devices have evolved into transformative research tools. By manipulating picoliter to nanoliter fluid volumes within optically transparent, sub-millimeter channels, these platforms recreate controlled microenvironments that mimic the geometrical, physical, and chemical features of natural soil matrices (Dadhich et al., 2026).

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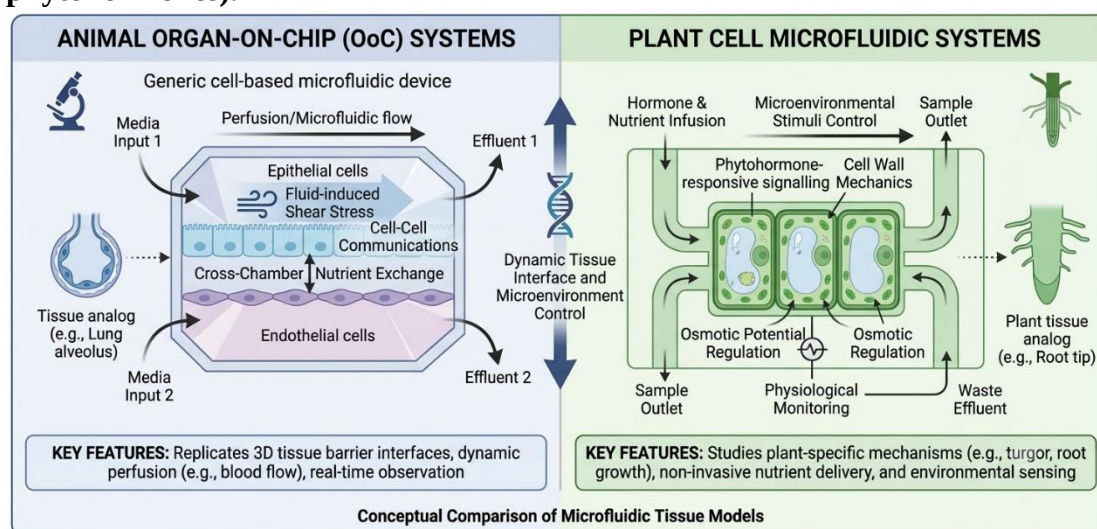
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When integrated with advanced live-cell microscopy, microfluidic root platforms enable non-destructive, high-resolution visualization of living plant tissues over extended timeframes ranging from minutes to weeks. These systems empower researchers to quantitatively track the complete kinetic sequence of root–pathogen dynamics: from initial pathogen chemotaxis and surface aggregation to appressorium differentiation, cell wall penetration, host defense activation, and competitive rhizosphere colonization at single-cell and subcellular resolutions (Yanagisawa et al., 2001).

Figure 1: Conceptual framework contrasting animal OoC (barrier interfaces, shear stress) and plant microfluidic models (turgor, wall mechanics, phytohormones).



2. Microfluidic Design Paradigms and Fabrication Methodologies

2.1 Lithographic Fabrication and Material Selection

The manufacture of plant microfluidic chips relies predominantly on soft lithography techniques adapted from microelectronics. Master molds carrying precise microchannel topographies are fabricated on silicon wafers using high-aspect-ratio negative photoresists (such as SU-8) via UV photolithography (Rose et al., 2019). Liquid prepolymer mixtures of polydimethylsiloxane (PDMS) and its corresponding curing agent are poured over the silicon master, degassed under vacuum to remove entrapped microbubbles, and thermally cured to form flexible elastomeric replicas. After punching fluidic inlet and outlet ports, the patterned PDMS slabs are covalently bonded to glass coverslips or microscope slides using oxygen plasma surface activation (Xu et al., 2025).

PDMS remains the standard material for RoC applications due to its optical transparency across visible wavelengths, non-toxicity, gas permeability (which ensures adequate root oxygenation), and low elastic modulus. However, PDMS exhibits physical drawbacks, notably the non-specific hydrophobic absorption of small lipophilic molecules, phytohormones (such as auxin, jasmonic acid, and salicylic acid), and synthetic agricultural chemicals. To mitigate molecular absorption and accommodate specialized detection modalities, researchers are evaluating alternative

materials, including rigid thermoplastics (poly (methyl methacrylate) [PMMA], cyclic olefin copolymers [COC]), paper-based microfluidics, and functionalized hydrogel matrices (Ariati et al., 2021).

2.2 Device Geometry and Spatial Confinement Architectures

Designing a microfluidic architecture for plant roots requires balancing physical space for tissue extension with the strict optical constraints of high-magnification microscopy. Microfluidic platforms have progressed from simple linear channels to multi-layered, multi-chambered systems tailored to specific root architectures and experimental requirements (Agarwal et al., 2026).

Single-layer straight channels represent the foundational layout for platforms such as the *Plant-in-Chip* and the *Tracking Root Interactions System (TRIS)*. These geometries feature elongated rectangular microchannels (typically 100--160 μm in height and 350--800 μm in width) that guide primary taproots along a single axis while providing dedicated ports for media perfusion and microbial inoculation (Cohen et al., 2026).

To observe delicate fine structures such as root hairs, planar confinement devices utilize a two-layer channel geometry. By mechanically confining both the main root and its emerging root hairs within a narrow vertical plane, these devices eliminate z-axis spatial drift during fluid exchange or gravistimulation, keeping subcellular organelles continually within the focal plane of high-numerical-aperture objective lenses (Aufrecht et al., 2017).

For plants with complex or branching root architectures, such as monocot crops (*Oryza sativa*) or woody perennial seedlings (*Populus tremuloides*), specialized multi-chamber radial geometries have been engineered. Platforms like the *Root-Microbe Interaction chip (RMI-chip)* feature expanded growth chambers (36 mm in length by 800 μm in width) that provide physical space for primary root elongation and lateral root branching over multi-week cultivation periods (Bellini et al., 2014).

Furthermore, "Soil-on-a-Chip" architectures incorporate arrays of elastomeric micropillars, artificial pore networks, and physical barriers into the channel floor. These structured microenvironments mimic the physical tortuosity, spatial confinement, and mechanical impedance of natural soil pore networks, enabling studies on root mechanosensing, chemotropism, and microbial navigation (Richter et al., 2022).

2.3 Fluidic Actuation and Microflow Control

Precise control of fluid mechanics inside microchannels allows researchers to deliver nutrients, establish stable chemical micro-gradients, and introduce microbial inoculants without subjecting plant tissues to destructive shear stress. Three primary fluidic actuation mechanisms are utilized in plant microfluidics:

1. **Pressure-Driven Perfusion:** External syringe pumps, peristaltic pumps, or pneumatic pressure regulators drive continuous or pulsatile liquid flow through the chip. Pressure-driven flow enables rapid switching of growth media or environmental stressors (e.g., transitioning from baseline nutrients to high-salinity or pathogen-containing solutions) via multiplexed media delivery systems (Iakovlev et al., 2022).
2. **Capillary-Driven Flow:** Hydrophilic channel surface treatments and capillary forces draw liquid through microchannels passively, eliminating the need for external power sources or mechanical pumps. This self-actuated approach

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simplifies platform operation and facilitates high-throughput screening setups (Khanjani et al., 2025).

3. **Electro-Osmotic Flow:** Applying electric fields across microchannels generates electro-osmotic fluid movement, enabling non-mechanical manipulation of charged molecules and fluid layers in space and time (Alizadeh et al., 2021).

Table 1: Engineering Specifications and Structural Architectures of Representative Root-on-a-Chip Platforms.

Platform Name	Host Species	Channel Dimensions (L×W×H)	Layer Architecture	Actuation Mechanism	Primary Target / Analytical Capability
Plant-in-Chip	<i>Arabidopsis thaliana</i>	1 cm × 350 μm × 80 μm	Single-layer parallel channels	Pressure-driven perfusion	High-throughput root growth tracking; oomycete and nematode infection kinetics
Two-Layer Hair-Chip	<i>Arabidopsis thaliana</i>	Customized fine micro-channels	Two-layer vertical confinement	Passive capillary / hydrostatic pressure	Subcellular organelle tracking in root hairs; elimination of vertical z-axis drift
TRIS (Tracking Root Interactions System)	<i>Arabidopsis thaliana</i>	13 mm × 4 mm × 160 μm (Volume: ~ 6.4 μL)	9-channel independent array	Syringe pump / manual micro-injection	Quantitative bacterial chemotaxis; real-time biofilm tracking; competitive pathogen exclusion
RMI-chip (Root-Microbe Interaction Chip)	<i>Populus tremuloides</i> , <i>Oryza sativa</i>	36 mm × 800 μm × 100 μm	Single-layer with on-chip filters	Continuous pressure-driven micro-perfusion	Long-term woody plant culture (>1 month); rhizobacterial biofilm assembly;

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					biosensor profiling
bi-dfRC (Bi-directional Dual-Flow RootChip)	<i>Arabidopsis thaliana</i> , <i>Nicotiana benthamiana</i>	Variable multi-channel matrix	Dual-flow matrix with flexible micropillars	Multiplexed media delivery system (MMDS)	Dynamic stress-switching; directional Ca^{2+} and ROS signaling waves; mechanical force sensing

3. High-Resolution Real-Time Imaging Techniques and Sensor Integration

3.1 Microscopy Modalities and Optical Optimization

The primary analytical advantage of microfluidic plant platforms lies in their seamless compatibility with modern inverted light microscopy. Mounting PDMS chips onto thin glass coverslips (No. 1.5, 170 μm thickness) matches the optical working distance of high-numerical-aperture (NA) immersion objectives, enabling multi-modal live-cell imaging (Alves et al., 2025):

Brightfield and Differential Interference Contrast (DIC) microscopy operating at low magnification ($4\times$ -- $20\times$) allow continuous quantification of organ-level developmental parameters, such as primary root elongation rates, root tip tropisms, and lateral root initiation. When switched to high magnification ($20\times$ -- $63\times$ oil/water immersion objectives), DIC microscopy resolves intracellular phenomena, including cytoplasmic streaming, organelle motility, and host cell wall structural deformations without requiring exogenous fluorescent dyes (Davidson & Abramowitz, 2002).

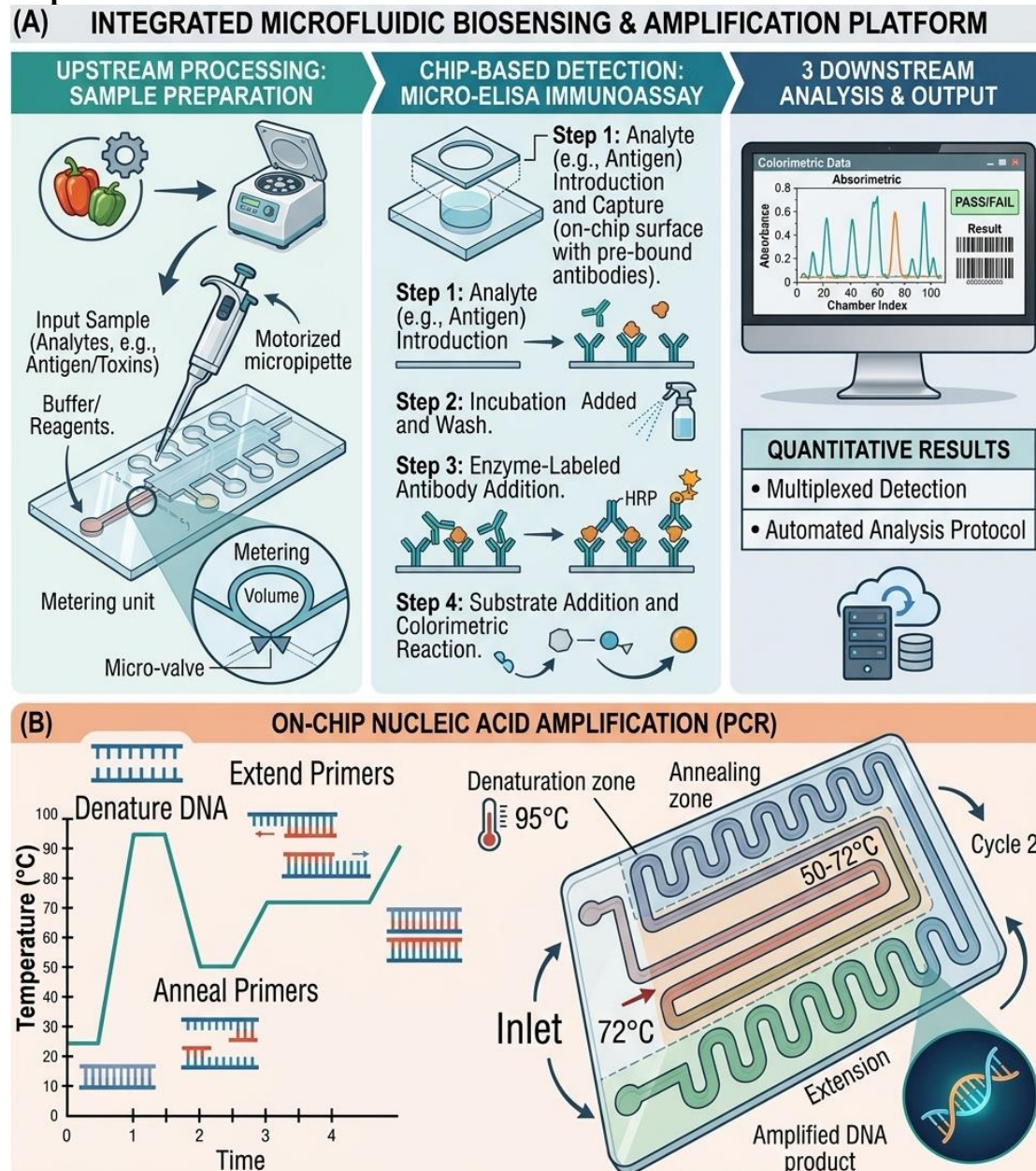
Laser scanning confocal microscopy (LSCM) and spinning-disk confocal microscopy facilitate 3D spatial reconstruction of host–pathogen interfaces. By utilizing multi-channel fluorescence, researchers can simultaneously monitor differentially tagged host plant structures (e.g., cell wall counterstains or fluorescent tissue markers) and microbial pathogens expressing green fluorescent protein (GFP), mCherry, or mKate (Zhu et al., 2021).

Furthermore, specialized platforms integrate Atomic Force Microscopy (AFM) directly with microfluidic chips. By positioning the PDMS device root-side up on an AFM stage and maintaining tissue hydration within an open liquid reservoir, an AFM cantilever can physically probe the surface of living root epidermal cells. This enables quantitative, sub-nanometer mapping of local cell wall stiffness, elasticity, and cell wall degradation during active pathogen invasion (Deliorman et al., 2020).

To optimize optical performance, microfluidic protocols actively mitigate refractive index mismatches between the liquid growth medium ($n \approx 1.33$), PDMS ($n \approx 1.41$), glass ($n \approx 1.52$), and plant cell walls. Confining root tissues directly against the glass coverslip minimizes light scattering through aqueous media layers, drastically reducing background autofluorescence and maximizing signal-to-noise ratios during faint

fluorescence reporter tracking (Kolle & Lee, 2018).

Figure 2: Technical workflow detailing upstream sample processing, micro-ELISA colorimetric detection, and microchannel spatial thermal PCR amplification.



4. Spatiotemporal Dynamics of Root-Pathogen Interactions

4.1 Oomycete Chemotaxis, Aggregation, and Infection Mechanics

Soilborne oomycetes of the genus *Phytophthora* (*P. parasitica*, *P. sojae*, *P. capsici*) rank among the most economically damaging plant pathogens, causing widespread root rot in agricultural crops. Infection is initiated by biflagellate, waterborne zoospores that swim through water-filled soil pores to locate susceptible host roots. Microfluidic platforms have provided unprecedented insights into the kinematic strategies that

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govern early host localization by zoospores (Kamoun et al., 2015).

High-speed video tracking within microfluidic root channels reveals that *P. parasitica* zoospores swim at velocities reaching 250 $\mu\text{m/s}$ through aqueous media. Quantitative trajectory analysis demonstrates that zoospores do not utilize long-range directional chemotropic steering over millimeter distances. Instead, zoospore swimming kinetics remain unbiased until the swimming cells navigate within a critical threshold distance of approximately 300 μm from the root surface (Tran et al., 2022).

Upon crossing this 300 μm threshold, zoospores undergo a sharp transition to positive klinokinesis. Their linear velocity decreases significantly while their turning and tumbling frequency increases sharply. This localized change in swimming behavior increases zoospore residence time near the host root, trapping the micro-swimmers within the chemical boundary layer of the rhizosphere. Local chemical cues, including root exudate components and potassium ion (K^+) efflux gradients, subsequently trigger zoospore encystment, flagellar shedding, primary cell wall synthesis, and germ tube emergence (Latijnhouwers, 2003).

Following encystment on *Arabidopsis* roots inside platforms such as the *Plant-in-Chip* and *bi-dfRC*, time-lapse imaging captures germ tube elongation, appressorium differentiation, and host cell wall penetration. At the host interface, early oomycete contact triggers an immediate, highly directional cytosolic Ca^{2+} signal within target epidermal cells. This localized calcium influx precedes a broader, non-directional accumulation of H_2O_2 ROS over the first 30 minutes post-inoculation, culminating in epidermal cell darkening and localized host cell death (Brand & Gow, 2011).

4.2 Bacterial Wilt and Xylem Biofilm Occlusion

Vascular bacterial pathogens, such as the *Ralstonia solanacearum* species complex, enter host roots through natural openings or physical wounds, colonize the vascular xylem vessels, and block water transport. Within the xylem, these bacteria multiply rapidly and secrete massive quantities of high-molecular-weight exopolysaccharides (EPS) (Chachar et al., 20250

Microfluidic devices designed to mimic the cylindrical micro-geometry and hydraulic flow profiles of tomato xylem conduits have shed light on the physical mechanics of bacterial wilt. Live confocal imaging within artificial xylem channels demonstrates that *Ralstonia pseudosolanacearum* cells attach to channel walls under active microfluidic flow, establishing microcolonies that synthesize dense EPS matrices (Chu et al., 2024). As the bacterial population expands, the growing biofilm physically restricts fluid passage through the channel. This biofilm accumulation increases hydraulic resistance, causing a steep drop in volumetric flow rates that mimics the lethal vascular failure and systemic wilting observed in infected host plants (Conrad & Poling-Skutvik, 2018).

Table 2: Pathosystem Dynamics and Mechanistic Insights Elucidated via On-Chip Live Imaging.

Pathogen / Agent	Taxon	Host Plant	Microfluidic System	Key Spatiotemporal Dynamic Insights Elucidated

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<i>Phytophthora parasitica</i>	Oomycete	<i>Arabidopsis thaliana</i>	Custom Root-Chip	Zoospores display positive klinokinesis (reduced speed, elevated turning) within 300 μ m of roots; no long-range attraction detected.
<i>Phytophthora capsici</i>	Oomycete	<i>Arabidopsis thaliana</i>	bi-dfRC	Immediate directional cytosolic Ca^{2+} wave triggered upon host contact, followed by a gradual 30-min non-directional H_2O_2 ROS burst.
<i>Phytophthora sojae</i>	Oomycete	<i>Arabidopsis thaliana</i>	Plant-in-Chip	High-throughput tracking of zoospore attachment, appressorium differentiation, and host epidermal cell death.
<i>Ralstonia pseudosolanacearum</i>	Bacterium	<i>Solanum lycopersicum</i> (Tomato)	Xylem-Mimicking Chip	<i>In situ</i> tracking of vascular biofilm assembly and EPS matrix secretion driving xylem hydraulic failure and wilting.
<i>Heterodera schachtii</i>	Nematode	<i>Arabidopsis thaliana</i>	Plant-in-Chip	Real-time tracking of nematode navigation (1–2

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				h), stylet probing, cell penetration, and syncytial feeding site induction (2–3 d).
<i>Bacillus subtilis</i>	PGPR Bacterium	<i>Arabidopsis thaliana</i>	TRIS	Rapid chemotaxis specifically targeting the root elongation zone within 30 min; forms protective biofilms that competitively exclude pathogens.

5. Rhizosphere Microbiome Dynamics, Biocontrol, and Competitive Exclusion

5.1 Spatiotemporal Colonization Niche Mapping via TRIS

Microfluidic platforms have proved critical in revealing how beneficial plant-growth-promoting rhizobacteria (PGPR) locate, colonize, and protect host roots. A prominent system designed for this purpose is the *Tracking Root Interactions System (TRIS)*, which incorporates nine independent microchannels on a single chip, enabling parallel, high-throughput confocal monitoring of distinct microbial treatments (Aufrecht et al., 2018).

Experiments in the TRIS platform using fluorescently labeled *Bacillus subtilis* and *Arabidopsis thaliana* seedlings demonstrate that bacterial colonization is highly spatially organized. Within 30 minutes of inoculation, *B. subtilis* cells exhibit strong chemotaxis directed specifically toward the **root elongation zone**. Time-lapse confocal microscopy confirms that bacteria rapidly concentrate along this specific tissue region, attracted by high localized fluxes of root exudates. Over the subsequent 6 hours, these founder cells transition from motile planktonic states to sessile microbial assemblies, secreting exopolysaccharides and proteinaceous matrices to construct robust biofilms along the root surface (Massalha et al., 2017).

5.2 Mechanisms of Competitive Pathogen Exclusion

The biocontrol capacity of beneficial rhizobacteria relies heavily on competitive niche exclusion occupying physical root surfaces and consuming exudate nutrients before pathogenic invaders can establish. Microfluidic platforms allow direct, real-time visualization of these competitive microbial interactions (Compant et al., 2005).

Dual-inoculation assays in TRIS microchannels demonstrate that pre-colonization of *Arabidopsis* roots by *B. subtilis* forms a continuous protective biofilm barrier along the root surface. When secondary bacterial invaders (such as *Escherichia coli*) are

subsequently introduced into the channel, the established *B. subtilis* biofilm physically shields the host tissue, actively excluding the secondary cells from contacting or adhering to the root surface (Sowmiya et al., 2024).

In contrast, mutant *B. subtilis* strains defective in matrix production (Δeps mutants) still undergo chemotaxis toward the elongation zone but fail to construct structural biofilms. As a result, these mutants fail to establish protective barriers, allowing secondary bacterial competitors to readily attach to the host root (Dragoš et al., 2018). Furthermore, multi-channel microfluidic setups featuring dual root channels enable host-preference choice assays. By positioning wild-type and mutant plant roots in adjacent microchannels within a single chip, researchers can track bacterial swimming trajectories in real time to evaluate host selection driven by distinct exudate profiles (Marczakiewicz-Perera et al., 2026).

5.3 Host-Microbe Specificity and Woody Plant Interactions

Expanding microfluidic studies from model herbaceous plants (*Arabidopsis*) to perennial woody species has highlighted the importance of host-symbiont evolutionary matching. The *Root-Microbe Interaction chip (RMI-chip)* was specifically engineered to accommodate seedlings of *Populus tremuloides* (trembling aspen) over extended culture periods exceeding one month (Li et al., 2026).

In the RMI-chip, colonization of aspen roots by native *Pseudomonas fluorescens* strains involves dynamic, highly coordinated cycles of biofilm assembly, matrix expansion, and regulated dispersal under continuous media micro-perfusion. Conversely, when whole-cell biosensors derived from non-adapted soil bacteria (*Bacillus subtilis*) were introduced into the aspen RMI-chip, the cells failed to adhere stably or establish persistent colonies on aspen root surfaces. Under active fluid flow, non-adapted strains were rapidly washed out of the growth chamber (Noirot-Gros et al., 2026).

This contrast demonstrates that persistent rhizosphere colonization within microfluidic microenvironments requires precise evolutionary alignment between host root exudation profiles and microbial surface adhesion mechanisms (Aufrecht et al., 2022).

6. Technical Bottlenecks and Future Perspectives

6.1 Current Biological and Material Constraints

Despite significant engineering advances, current Plant-on-a-Chip technologies face several technical challenges:

High cell wall turgor pressure (0.2--1.0 MPa) and biomechanical expansion forces generated by growing roots can cause physical deformation or delamination of microfluidic devices. Measuring cell wall strain or applying precise mechanical forces requires robust device bonding and careful material calibration (Bizet et al., 2016).

The tendency of PDMS to absorb small hydrophobic molecules distorts localized chemical micro-gradients and alters the effective concentrations of lipophilic phytohormones (such as auxin, jasmonic acid, and salicylic acid) inside microchannels over multi-day experiments (Taleban et al., 2026).

Primary root tips eventually make physical contact with the end-walls of closed microfluidic chambers. This mechanical boundary contact triggers thigmotropic stress responses, artificial root bending, and altered lateral root initiation, restricting most high-resolution studies to early seedling developmental stages (typically 7 to 30 days) (Chen et al., 2023).

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Accommodating both large host roots and small filamentous pathogens within a single channel introduces design tradeoffs. Microchannels tall enough to house main roots allow smaller micro-swimmers (such as zoospores or bacteria) to drift vertically out of the focal plane, whereas shallow microchannels mechanically crush growing fungal hyphae or restrict root growth (Milletet al., 2019).

6.2 Emerging Innovations and Future Directions

To overcome these limitations, the field of plant microfluidics is integrating several emerging technologies:

The incorporation of non-absorbent thermoplastics (such as PMMA and COC), paper-based microfluidic channels, and functionalized hydrogels is expanding material options beyond standard PDMS. Combining microfluidic outlets with capillary electrophoresis, microfluidic impedance cytometers, or mass spectrometry enables continuous, non-destructive profiling of root exudates and secondary metabolites. Furthermore, integrating spatial transcriptomics with live microfluidic imaging will allow researchers to map host gene expression changes directly onto real-time infection patterns at single-cell resolution (Campbell et al., 2020)

Transitioning from smooth PDMS channels to 3D micro-patterned soil surrogates recreates the complex physical tortuosity, pore size distribution, and fluid mechanics of natural soils. Inoculating these structured devices with multi-species synthetic microbial consortia (SynComs) will facilitate studies on how complex microbiomes suppress soilborne pathogens through quorum sensing, metabolic complementation, and collective biofilm assembly (Wang et al., 2026).

Finally, the adoption of standardized Ecosystem Fabrication (EcoFAB) protocols and automated microfluidic platforms will enhance cross-laboratory data comparability, paving the way for high-throughput screening of novel agricultural chemicals, biological control agents, and pathogen-resistant crop cultivars (Orebaugh et al., 2026).

Conclusion

Plant-on-a-Chip and Root-on-a-Chip platforms have fundamentally transformed the study of root–pathogen dynamics by enabling high-resolution, real-time visualization of host–microbe interactions within controlled microenvironments that closely mimic natural soil conditions. Through precise spatial confinement, microfluidic devices eliminate z-axis drift, maintain subcellular structures within the focal plane, and facilitate quantitative tracking of infection kinetics from initial pathogen chemotaxis to host defense responses and competitive colonization. The mechanistic insights gained ranging from oomycete klinokinesis and calcium signaling to bacterial biofilm-mediated vascular occlusion and PGPR competitive exclusion demonstrate the power of these platforms in elucidating previously inaccessible spatiotemporal dynamics. Nevertheless, several technical limitations persist, including PDMS hydrophobicity causing phytohormone absorption, physical root constraints restricting long-term studies, and challenges in accommodating both host roots and microbial pathogens within optimal optical geometries. Future innovations in non-absorbent thermoplastic materials, 3D soil-mimetic architectures, integrated biosensors, and multi-species synthetic communities will address these bottlenecks. The standardization of EcoFAB protocols and automated platforms will enhance cross-laboratory reproducibility and accelerate high-throughput screening of biocontrol agents, agricultural chemicals, and

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disease-resistant cultivars. As these technologies mature, plant microfluidics will become indispensable for developing sustainable crop protection strategies to safeguard global food security against emerging agricultural threats in an era of climate change.

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