Invasive processes in the life cycle of plants and fungi

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Abstract

Invasive growth is a common characteristic of a variety of cell types in all kingdoms ranging from animals to plants, fungi and bacteria. Invasion in a biological context can be commonly defined as penetration of a substrate by an actively elongating 'invader' (single cell or multicellular structure). Invasion requires force which in the case of single cells is produced by cell mechanical features such as turgor pressure or the cytoskeleton. Invasion is often facilitated by agents employed to soften the invaded matrix such as lytic enzymes. This review provides an overview of experimental strategies that have been developed to characterize this particular cellular behavior and to measure the invasive forces generated by tip growing cells in plants and fungi.

Introduction

The cells of multicellular organisms generally occupy specific locations within tissues and organs to serve specialized metabolic or structural functions. In animal bodies, there are numerous exceptions to this spatial constancy: Blood and lymph cells are transported through the entire body by the respective circulatory systems, neurons elongate their axons through other tissues to innervate them, and cancer cells migrate through tissues and change location via the circulatory system. In plants, no circulation of cells occurs although the position of individual nuclei may change across substantial distances, in particular in large, multinucleate syncytia or coenocytes. The nuclear migration is similar to that occurring in many filamentous fungi and slime molds where nuclei can move over large distances within multinucleate cells[1]. Because of the absence of cell migration proper, however, spatial constancy of cells within the plant body is more pronounced than in animals. This is even more so since plant cells are encased in an extracellular matrix and glued to each other by the middle lamella, dramatically limiting movement of cells relative to each other[2]. Collectively, the middle lamella and the encasing cell walls in the plant body are called the apoplast, a scaffold that confers structural stability to plant organs and cements cells in space. However, even within the plant body, certain cells produce extensions to reach either distant regions in the same organism or explore the external environment. Just like in animal

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organisms, these motile activities in plants often require the cells to invade or squeeze between their neighbors and through narrow spaces.

Invasive cell types in plants include root hairs, pollen tubes, sclerenchyma fibers, and laticifers. The invasive lifestyle and associated elongated cell shape can serve a variety of purposes. Elongated cells can provide structural stability to the organ, analogous to the steel rods reinforcing a concrete structure (sclerenchyma fibers), facilitate procurement of nutrients and water from distant sources (root hairs)[3], or transport cargo (pollen tubes). A second type of invasion to which plant bodies are subjected is that by symbionts and pathogens of fungal and bacterial origin. Some of the invasive activities performed by other organisms exploit structural openings in the plant body such as stomata or intercellular air spaces, whereas others see the invader drill into the apoplast or even into the lumen of individual cells. As long as cellular extension or invasion exploits openings in the tissue traversing gas or liquid spaces, the invading cell does not encounter any mechanical obstacle. However, when invasion or extension occurs against or through a solid or viscous matrix, typically the apoplast, the invading cell has to overcome mechanical obstacles.

The forces required for the invasive and migratory behavior of animal cells such as neurons, cancer cells and fibroblasts are generated by the cytoskeletal system and have been covered in numerous reviews[4,5]. In the following we focus on the invasive lifestyle of walled cells in plants, fungi, and oomycetes. We elaborate on the key structural parameters involved in maintaining an intrusive activity in walled cells—the turgor pressure and the regulation of cell wall mechanical properties[6]. The cytoskeleton in these cells is important in its role as regulator of intracellular trafficking and cell wall assembly.[7] We discuss how invasive cells may facilitate their activities by secreting agents that soften the invaded substrate and we review experimental techniques and numerical methods developed to measure the invasive force.

Invasive growth serves a diverse range of functions

Elongated cells can confer structural stability

Among the longest cells in the plant body are fibers with lengths up to 120 mm in *Boehmeria nivea*, 33 mm in *Linum usitatissimum*[8]. These sclerenchymatous cells (cells with thick lignified secondary cell wall and typically dead at maturity) serve to stabilize the plant body against mechanical stress[6,9]. This mechanical role depends on structural and geometrical parameters that involve the accumulation of cellulose and lignin in significant amounts at mechanically critical positions within organs[6]. Fibers are formed in various plant organs including roots, shoots, leaves[10,11] and are particularly abundant in the phloem or secondary xylem of eudicotyledon plants and surrounding the vascular bundles in the leaves of monocotyledons[6,12]. The structural function of fibers relies on their resistance to compressive, bending and tensile forces all of which may occur in the same organ. Fibers have also been considered to act akin to animal muscles as in some situations they are thought to have contractile properties[11,13,14]. This action is displayed, for instance, during gravitropic responses, climbing and underground positioning of geophytes[11]. Because of their mechanical, structural and biochemical properties, sclerenchyma fibers are key

for the mechanical properties of timber-based construction material and constitute an energy-rich component for the fuel wood industry. The flexibility combined with tensile resistance of bast fibers in flax, ramie, hemp, jute, kenaf are also exploited in the textile industry[10,11,15,16].

The extreme length of sclerenchyma fibers is generated starting from relatively short precursor cells formed in meristems—the stem cell niches in plants. Fiber differentiation and morphogenesis, therefore, require the cells to expand in highly anisotropic manner and through surrounding, slower-growing tissues (Fig. 1a). This occurs through intrusive growth that penetrates the apoplast connecting neighboring cells[6,10,11,17]. Plant fibers are thus, in principle, an excellent model to study plant invasive growth, cell wall formation and cell wall mechanics. However, despite their structural properties and economic importance the mechanics underlying their intrusive behavior is still poorly understood. This is mainly due to the fact that fibers are formed within the depth of

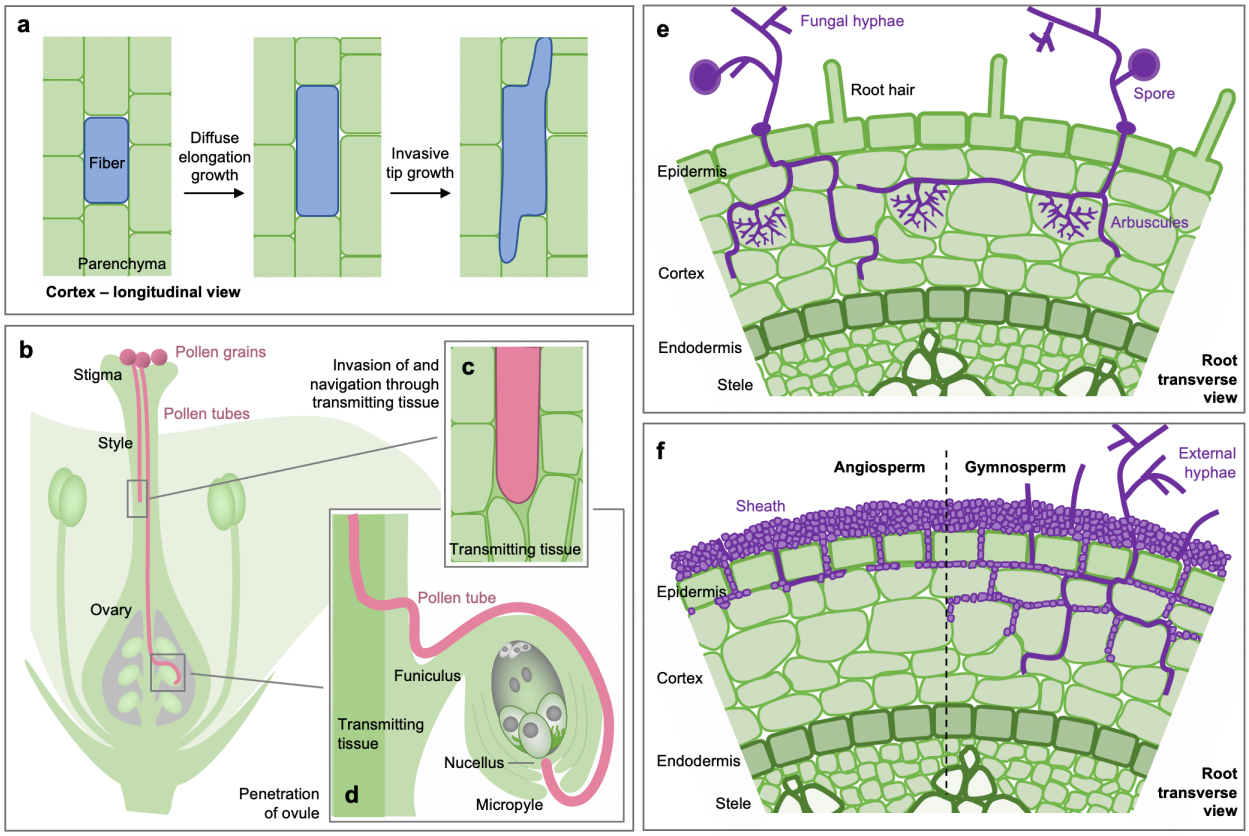

Figure 1. Various types of invasive cells: a) Development of sclerenchyma fiber cell from meristem precursor. Initial elongation occurs by diffuse growth in lockstep with surrounding parenchyma cells, but once these cease elongation the continuation of fiber growth occurs by tip growth. b) Mechanical obstacles in the pathway of pollen tube towards the ovary include the stigmatic cuticle, the apoplast of the transmitting tissue, the micropyle and the nucellus. c) Pollen tube making its way through the apoplast of the stylar transmitting tract. d) Pollen tube emerging from the transmitting tissue elongating on the surface of the funiculus, turning into the micropyle of the ovule. e) Fungal hyphae of arbuscular mycorrhiza, a type of endomycorrhiza, invade the epidermis and cortex of the plant root by penetrating the apoplast and building branched structures inside the lumen of root cortex cells. d) Fungal hyphae of ectomycorrhiza cover the root surface forming a sheath and penetrate the root epidermis forming a Hartig net. Occasionally, in particular in contact with gymnosperms, the hyphae of ectomycorrhiza reach external cortex layers.

and surrounded by other tissues which renders their isolation or *in situ* live cell observation difficult.[6,11]

Invasion for cargo delivery across tissues

Elongated cells provide an excellent catheter-like system that enables the transport of cargo from one region of the organ to another, even across tissue boundaries. One such trans-tissue transfer is required for successful fertilization in flowering plants. The sperm cells must be shuttled from the pollen grains upon their arrival on the landing platform of the pistil—the stigma—through the stylar transmitting tissue to the female gametophyte located in the ovary. This transfer is accomplished by a cellular protuberance formed by the germinating pollen grain, the pollen tube. This protuberance emerges from an opening in the hard outer wall of the pollen grain and undergoes localized elongation at the very apex. This tip growth process is reflected in the extremely polar organization of the cytoplasm[18,19] and the spatially confined expansion of the cell surface.[20,21] Depending on plant species, the diameter of a pollen tube varies between 5 μm and 20 μm and the length can extend up to tens of centimeters depending on the length of the pistil. While the entire pollen tube consists of a single vegetative cell, the active portion of the pollen tube cytoplasm is restricted to the apical portion of the cell only, and the distal region is plugged off and eventually degenerates.[22] When invading the stigma and transmitting tissue of the style, pollen tubes have to overcome multiple mechanical constraints (Figures 1b-d). The elongating tip of the tube, therefore, has to exert sufficient penetration force in order to withstand the external compressive stress generated by transmitting tissue while maintaining the direction towards its target, the ovule.[23,24]

Spreading out for procurement of nutrients and water

Cells elongating beyond the perimeter of the organism, into the external substrate, confer the ability to explore a larger space on the search for nutrients or water. In order to fulfill their nutritional needs from diverse biological and synthetic substrates, filamentous fungi extend colonies by forming branched hyphae that have the ability to penetrate solid substrates of considerable mechanical stiffness, including rocks. Hyphae are commonly formed by yeast, mushroom forming fungi, and also by oomycete water molds.[6,25,26] Hyphae are tube shaped cells with a diameter ranging from 2-20 μm, elongating at the tip region, similar to pollen tubes. The hyphae of a fungal organism often grow in a direction centrifugal with regards to the center of the mycelium to ensure most efficient exploration of the substrate.[6] Although hypha usually elongate individually, they sometimes aggregate in parallel to form a structure called rhizomorph. These rhizomorphs can have a diameter of several mm and can elongate several meters in length.[6] They have the invading capacity to penetrate through soil or wood in search of nutrients which are transferred to developing fruiting bodies.[27,28] When penetrating and elongating, fungal hyphae secrete enzymes that digest polymers to sugars and other molecules that can be taken up through the plasma membrane.[25] The enzymatic digestion is also thought to soften the physical impedance of the surrounding substrate.[25]

Certain fungal hyphae establish a symbiotic relationship with plant root tissues, where the plant provides organic molecules such as sugar to the fungus and the fungal partner provides water and minerals absorbed from the soil to the plant.[29] This association is highly intimate since it involves the invasion of the fungal hyphae into the apoplast of the root epidermal layer (ectomycorrhiza) or the root cortex (endomycorrhiza)[6,29] (Figures 1e,f). Endomycorrhiza invade not only the root apoplast but actually grow into the root cell lumen where they form arbuscular structures that augment the interaction surface between the fungus and the protoplast of the plant cell.

The plant root is not only subject to invasion, it is an active invader and exerts this action at different scales. At cell scale, roots produce their own extensions that explore the substrate beyond the surface of the organ. This is done by root hairs—tube shaped, tip growing extensions emerging from the epidermal cells of the root that range from 5-17 μm in diameter and about 0.1-1.5 mm in length[30,31]. Similar to fungal symbionts, root hairs serve to increase the interaction surface between the absorbant region of the root and the soil thus facilitating nutrient and water uptake.[3,32,33]

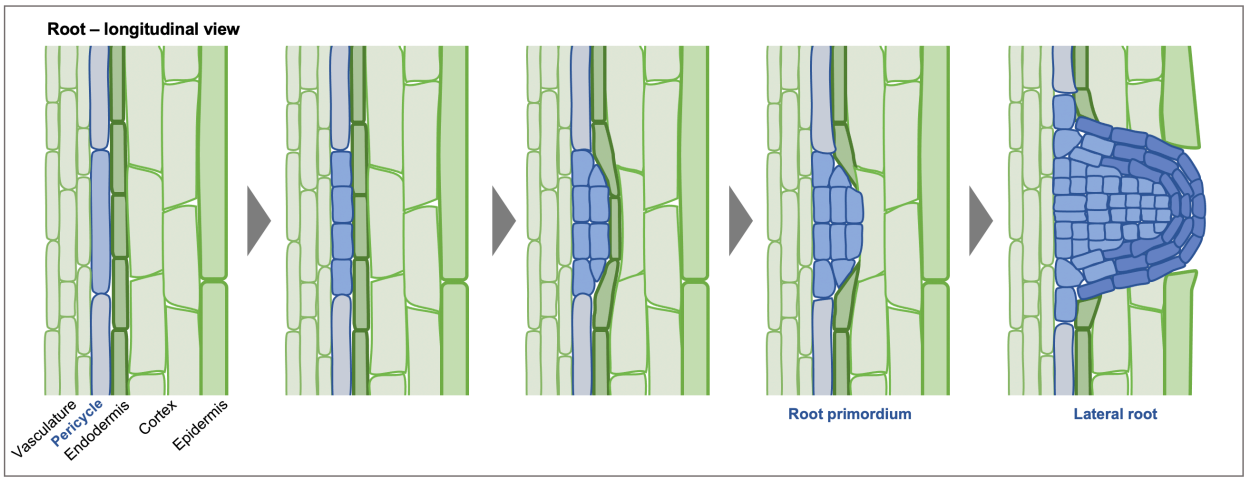

Figure 2. Lateral root formation initiates with cell divisions in a spatially confined region of the pericycle located in the central steele of the primary root. The resulting lateral root primordium develops a new root apical meristem whose continuous cell divisions produce the elongating new root which in turn breaks through the cortex and epidermis of the primary root to continue growing through the soil.

Root invasion also occurs at the scale of the organ. In their interaction with the growth substrate, roots grow against and interact mechanically with soil particles. The soil is not the first or only obstacle for root growth, as newly forming roots (radicles) encounter mechanical impedance in form of the seed coat[34] or, in the case of lateral roots, the outer layers of the primary root.[35] Since lateral roots are formed from the pericycle—a cell layer in the central stele of the primary root—reaching the outside requires the exertion of invasive forces for the young lateral root primordium to break through the outer tissue layers of the primary root (Figure 2). The mechanical interaction of the root with an external matrix influences distinct morphological and developmental changes in the root system.[36] Studies done on the roots of cereal crop species have revealed irregular cortical cell growth, increased root diameter and bending and buckling of the root tip as a result of mechanical resistance from the soil particles.[36,37] This mechanical impedence created as a result of

compacted soil layers or soil drying can be a major limiting factor to root elongation and hence nutrient uptake for the plant. Increased soil strength requires roots to exert higher forces to ensure successful soil penetration.[38] To minimize the effect of soil mechanical impedence, roots of maize (*Zea mays)* and soybean (*Glycine max*) use natural or artificial macropores in the soil or growing matrix. Invading these openings in the otherwise compact material allows following the path of least resistance.[38-40] Another common response to increased soil strength is the thickening of roots which decreases penetrative stresses and stabilizes the root.[41,42] Root hairs have been suggested to play a supportive role during root penetration by anchoring the root to the surrounding soil.[33,43]

Given the fundamental importance of root growth through various soil types for yield and drought resistance, numerous biomechanical frameworks have been established for soil penetration mechanics. Root growth forces have been quantified with the help of technologies such as photoelastic discs[44], or cantilever sensors[45] and 3D living imaging of the roots in transparent soil[46] as well as x-ray based imaging[47] have greatly advanced the field. Since roots are multicellular tissues of macroscopic size, we refer to excellent reviews on the topic of invasive root growth[36,48] and focus on the single-celled growth of root hairs in the present chapter.

Cell mechanics of intrusive growth

Highly polarized cell extension directs force generation

The growth pattern of invasive cells is quite unique as the cells are highly polarized and typically extend exclusively at the very apex of the cell.[18,20,21,49] The morphogenetic process generating these cylindrical cells has been subject to multiple efforts to characterize the cell-mechanical underpinnings through modeling.[50,51] The apical cytoplasm of tip growing cells is often densely populated by vesicles, both exocytotic and endocytotic (Figure 3). Exocytotic vesicles deliver the material required for cell expansion, notably cell wall precursors and membrane material. In pollen tubes, the apical vesicle population forms an inverted cone-shaped region that is fed by Golgi-derived vesicles delivered to the apex by an array of actin filaments[52] (Figure 3b), whereas fungal hyphae typically feature a structured vesicle aggregate called Spitzenkörper organized by microtubules[53] (Figure 3a). Bigger organelles such as mitochondria and endoplasmic reticulum sometimes share the apical space but more typically remain in more distal regions of the tubular cell. Vesicle movement in hyphae occurs towards the Spitzenkörper and from there radiates to the apical plasma membrane, whereas in pollen tubes, vesicles are delivered through a circular movement which in angiosperm pollen tubes occurs in an inverse fountain-shaped pattern controlled by the actin cytoskeleton.[54-56] In all tip growing cell types, the spatial organization of the cytoplasm ensures that both delivery of new cell wall material and the expansion of the existing cell wall are confined to the apical region of the elongating cell. This extreme polarization of tip growing cells is very different from the growth behavior of most other plant cells which display more global deformations across the cell surface, also termed diffuse growth.[57] The continuous addition of new cell wall material is controlled by cellular feedback mechanisms[51] and involves breaking and forming crosslinks between newly added and existing cell wall polymers.[58,59] Once the cell wall material at the tip is excreted it starts to stiffen which locks in the cylindrical diameter.

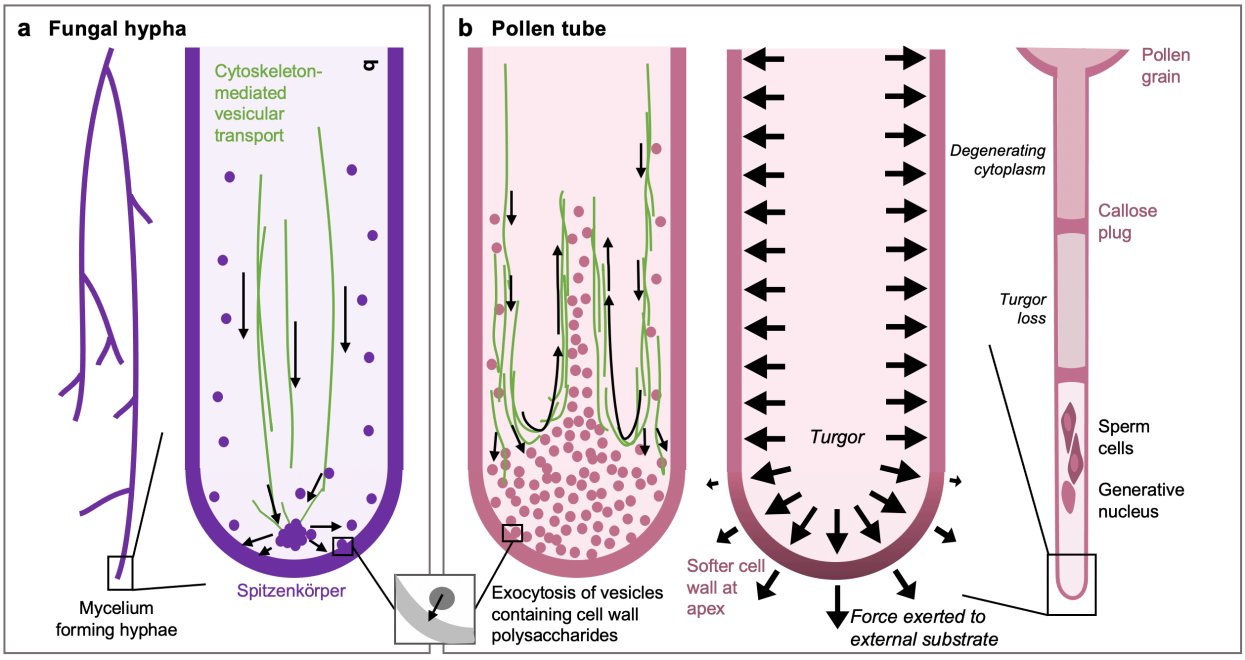

Figure 3. Cellular tip growth is fuelled by a continuous supply of secretory vesicles delivered by cytoskeleton-mediated transport. a) Many fungal hyphae feature a distinct vesicle aggregate, the Spitzenkörper, which regulates vesicle transport to the apical plasma membrane. b) In angiosperm pollen tubes, vesicles and other organelles move in a reverse fountain shaped pattern. The apical cell wall is softer than the wall in the distal region. Yielding of the cell wall to the turgor pressure in the apical region enables the cell to exert forces onto an external substrate. The cytoplasm of the pollen tube is segmented by callose plugs into the apical, viable region containing the sperm cells and generative nucleus, and distal regions that lose turgor and degenerate.

In pollen tubes, this stiffening is caused by the gelation of pectin polymers whose secreted methyl esterified configuration becomes de-esterified *in muro* (in the wall) through the action of pectin methylesterase.[60,61] In fungal hyphae, the main wall components are chitin and β-glucans and the distal stiffening involves hydrogen and covalent bonds.[62,63] The tip-focused maintenance of the cylindrical geometry is a self-similar morphogenetic process that is regulated through a finetuning between internal turgor pressure and biochemical cell wall properties at the apical growing region.[6,21]

While pollen tubes, fungal hyphae and root hairs display a clearly discernable pattern of tip growth, the growth pattern of sclerenchyma fibers may be more complex. In secondary xylem fibers, true tip growth seems to prevail as these fibers develop in the portions of the stem in which the tissues surrounding the fibers ceased elongation.[11] The initial elongation of primary flax phloem fibers on the other hand, seems to occur through diffuse growth as the neighboring cells continue elongating in lockstep at least during the initial developmental phase of the organ. During this early developmental phase, the entire cell surface enlarges,[64] followed by an intrusive elongation of the fiber tips once growth in the neighboring cells has ceased (Figure 1a). Distinguishing the different growth patterns is aided by monitoring strain patterns of the cell surface and by profiling the fiber cell wall and fiber tips via biochemical and mechanical parameters.[14,65,66]

Turgor pressure generates the invasive force in walled cells

Cell growth in plants involves the expansion of the existing cell wall driven by the turgor pressure,[67] but whether the exertion of invasive forces is equally dependent on turgor or whether other cellular features are involved has been a matter of discussion. Extension of sclerenchyma fibers has been proposed to rely on elevated turgor based on the assumption that the soft and thin walled growing fibers would be squished if they were less turgescent than the adjacent cells but experimental quantification of turgor is elusive.[9] The increase in turgor pressure at the initiation of intrusive growth and the maintenance of turgor during the fiber elongation require the movement of water into the developing fiber. Gene expression patterns suggest that regulation of this water movement during fiber growth involves aquaporins—protein channels facilitating water movements across membranes[9,68,69].

The turgor pressure of growing pollen tubes has been measured in lily and was found to range between 0.1 and 0.4 MPa[70]. Consistent with this, the maximum force that a pollen tube can produce when overcoming a mechanical barrier has been measured to be approximately 10 μN for lily[71] for 1.5 μN for *Camellia japonica.*[72] Since force is the product of the pressure and the interaction surface between the pressurized vessel and the substrate[6], the latter value was calculated to correspond to a pressure of 0.19 MPa, consistent with the magnitude of turgor. While the measured invasive forces in pollen tubes are consistent with the notion that turgor is the driving force of their invasive activity, this does not necessarily mean that the turgor pressure is also the parameter that is tuned to regulate the magnitude of growth speed or invasive force. In fact, different growth rates do not seem to be correlated with different turgor values, and even non-growing pollen tubes can have a turgor similar to that of growing pollen tubes.[70] Variations in growth rate seem to rely instead on a modulation of the biomechanical properties of the cell wall which in turn can oscillate through the effect of exocytosis of new cell wall material and cell wall modifying enzymes.[73] This modulation takes place at the apical cell wall which is substantially more compliant than the cylindrical portion[21] thus enabling the exertion of forces against an outside substrate at this site of the cell surface (Figure 3b).[6] Even if the apical wall is relatively compliant, a threshold turgor is necessary for pollen tube elongation and invasion as the plasmolyzed pollen tubes are unable to grow.[58] On the other hand, excessive turgor pressure can result in tube bursting[74] and turgor clearly must be carefully calibrated to be within a particular range.[75]

The maintenance and rapid regulation of turgor in pollen tubes is likely facilitated by the segmentation of the protoplast that separates the continuously elongating growing region from degenerating distal regions where turgor is gradually lost. This segmentation is achieved by the deposition of plugs made through localized centripetal invagination of the cell wall built from callosic wall material (Figure 3b). These plugs are produced repeatedly once the male germ unit has moved forward through a particular tubular segment and ascertains that the volume of the living portion of the cytoplasm remains within a relatively constant range.[74,76]

The values of maximum force generated by pollen tubes in *in vitro* setups does not necessarily allow to deduce the actual penetration force exerted inside the pistil as this force is dependent on

both growth rate and the stiffness and texture of the surrounding matrix.[6,24] The use of differently stiffened growth matrices allows determining whether the penetrative ability in a given cell type is influenced by the substrate and what the optimal stiffness is.[23,24,77] Pollen tube species such as *Arabidopsis* actually grow better in a stiffened medium compared to a liquid medium, in line with the tissue architecture of the pistil in this species.[24] A systematic comparison of plant species with solid style (transmitting tissue consisting of densily packed cells) or hollow style (transmitting tissue lining a cell-free canal filled with a viscous extracellular matrix) revealed a consistently different behavior of the respective pollen tubes when confronted with a stiff artificial matrix *in vitro*.[24] Tubes adapted to a solid style do not only display greater ability to penetrate a stiffer matrix when compared with the pollen tubes from species with hollow style, but also prefer a stiffer medium when presented with a choice, a phenomenon termed durotropism.[24]

Fungal hyphae are exposed to highly variable substrates and, therefore, have to be able to efficiently adjust to changing external osmolyte concentration. Certain substrates that hyphae are able to penetrate are phenomally stiff such as rock. Some fungal species can create specialized structures able to produce the pressures that are required to invade particularly resistant surfaces. One such example is the plant pathogen *Magnaporthe grisea* which produces infection pegs from flattened, enlarged hyphal tips called appressoria. Appressoria can establish a turgor pressure of up to 8 MPa that enable the invasive structure to penetrate the plant epidermis.[78] A tight control of the turgor pressure therefore seems to be crucial for fungal organisms. Upon hyperosmotic shock, fungal hyphae of *Neurospora crassa* display reduced turgor pressure, hyphal growth and decrease in hyphal volume, but all parameters were found to rapidly return to their original values through regulatory mechanisms.[79] The second crucial element in addition to high turgor is the capacity of the appressorium to firmly adhere to the surface of the structure to be invaded. This adherence is key to prevent pushback caused by the invading infection peg. While the invasion angle of the *Magnaporthe grisea* infection peg is normal to the plant leaf surface, a different strategy is employed by *Phytophthora*, a plant pathogenic oomycete. *Phytophthora* hyphae do not form appressoria, but they do adhere to the surface of the plant orgen to then assume an oblique angle to breach the surface. The angled approach has been likened to the slicing principle of single-beveled Japanese kitchen knifes and was accordingly named 'naifu-mechanism'.[80] Whatever the angle of attack, it is generally assumed that the infection peg or invading hypha requires substantial turgor pressure, although whether this truly applies to all types of hyphae and under all conditions remains unclear since certain oomycete hyphae seemingly grow even in the absence of any measurable turgor pressure.[81,82]

Cytoskeletal elements regulate tip growth and invasion through cell wall assembly

Force generation in animal cells involves cytoskeleton-based actions such as polymerization of cytoskeletal arrays and contractile mechanisms. Similar principles have been proposed for some walled cells[83,84] since they were observed to elongate under low or absent turgor pressure.[84] In pollen tubes, pharmacological interference with actin polymerization reduces the cell's ability to invade and penetrate a stiffened medium,[22,23] however, whether this effect can be ascribed directly

to any force generation by the cytoskeletal arrays is unclear. Interestingly, moderate interference with the actin cytoskeleton also abolishes growth oscillations in these tubes.[85] This indicates that the role of actin in regulating the invasive force of pollen tube growth may be mediated by the delivery of cell wall material to the expanding apex and thus the dynamics of the supply of the building material that is required for the cell to elongate.[22,60,86,87]

Studies in oomycetes *Achlya bisexualis* and *Phytophthora cinnamomi* provide similar evidence for such an indirect role of actin. Comparing hyphae growing either invasively (through agar) or non-invasively (on the agar surface) revealed an 'actin-free zone' at the tip region of the former but not the latter.[88] The authors suggest that the depletion of actin at the tip region of invasive cells would ensure a greater yielding capacity of the cell wall thus allowing for higher invasive force to be exerted.[88,89] Similar evidence for actin functioning as a 'restraint' for tip expansion has been found in zygomycete *Gilbertella persicaria*[90] and ascomycete *Geotrichum candidum.*[91] In other words, actin is suggested to be involved in tip yielding and thus the invasive growth in fungal hyphae, through its effect on the cell wall.

Chemical and enzymatic tools facilitating invasion

The pollen tube encounters multiple types of obstacles on its way through the stigmatic and stylar tissues, starting with the stigmatic cuticle layer (if present), followed by the transmitting tissue, the micropyle of the ovule, the nucellus enveloping the female gametophyte and the synergid cells adjacent to the egg cell. In species with solid transmitting tissue, the intercellular spaces are typically narrower than the tube diameter,[92] several additional mechanisms such as enzymatic lysis and chemical digestion are thought to be employed by the tube to facilitate invasion. Enzymes can help break down the molecules involved in cell adhesion or can degrade the invading tissue completely. Some pollen tubes, for example those of *Brassica napus* L., produce cutinase, an enzyme that digests the cuticle of the stigmatic papillae.[93] Agents with potential to affect the apoplast are expansins,[94,95] polygalacturonase, glucanase, endoxylanase,[96] and pectin esterase.[97] While gene expression profiles show that these proteins are expressed by pollen tubes, it has been difficult to tease apart whether they serve to modulate the pollen tube's own wall or are targeted at the transmitting tissue. Another elegant way to soften the solid matrix is autodigestion via programmed cell death[98]. This has been shown to occur upon pollination in plant species such as petunia,[99] where the transmitting tissue undergoes cell death leading to turgor loss and tissue softening. This both softens the pollen tube's path and may also provide additional nutrients for the elongating cell.

Enzymatic lysis is a crucial tool for fungal species to enable infection of other organisms. Plants use their external cuticle layer and the polysaccharide rich cell wall as the 'first line of defense' against invaders.[100] Fungi, therefore, produce a wide variety of enzymes that have the ability to depolymerize the plant cell wall polysaccharides.[100] Among these are pectinase,[101,102] cellulase,[102] arabinase,[103] xylanase,[104] and galactanase.[105] Enzyme deficient fungal mutants display reduced ability to cause infection.[106,107] Cutinase is formed in particular by fungal species that do not form appressoria and hence are unable to generate similarly high physical forces.[77,108] The secondary

cell walls of wood tissues are more resistant to degradation both physically and chemically, but some fungal species such as white rot and brown rot produce enzymes that degrade hemicellulose, lignin and cellulose, core polysaccharides forming the compound middle lamella in wood.[109] Enzymatic digestion of cell walls is also often employed by invasive organisms that trigger the development of plant tumors[110]. For instance, in the case of corn smut disease, the infection by the biotrophic fungus *Ustilago maydis* alters the level of hemicelluloses in the infected plant cell wall, modifying the abundance of xylose and arabinose, a process that presumably facilitates piercing the organ surface[111]. Subsequent tumor formation results from a local plant cell enlargement and cell division triggered by the fungus. Contrary to animals, tumors are rarely fatal for plants since metastasis is impossible due to the lack of circulatory system[110].

Biomechanical approaches to quantify invasive forces

Invasive growth clearly relies on cell mechanical features and the cells' ability to generate forces. To better understand invasive growth, it is, therefore, essential to quantitatively characterize these features. Significant effort has been put into designing experimental devices that are able to determine the mechanics of the cell wall, to quantify turgor, and to measure the invasive force of individual cells. Advancement in micro-measurement technology in recent years has been instrumental, notably microfluidics (microdevices with controlled fluid flow) and microelectromechanical system (MEMS)- based platforms.[112-116] These Lab-on-Chip (LoC) devices allow creating micro-environments that mimic aspects of the natural growth environment such as varying degrees of mechanical resistance, chemical gradients, or patterned physical obstacles (Figures 4a-c). Importantly, LoC allow observation and manipulation of individual cells confining their growth to a single focus plane thus enabling high resolution microscopy and extended time-lapse imaging.[117] The fabrication of microfluidic and MEMS devices allows for micrometer precision but requires engineering expertise (e.g. direct-write lithography and cleanroom facilities)[118,119], but technically simpler and more affordable alternatives can be used if spatial resolution of the design is less critical.[120] Exploiting LoC technology, a platform called the TipChip was developed to study the growth pattern of pollen tubes, for example to characterize their chemotropic behavior and response to electrical fields[113,121] (Figure 4a). These studies are conducted with the aim to understand how the pollen tube orients its growth in the maze of the female tissues.[122] The electric LoC is fabricated from two separate bondable modules: a PDMS-based microfluidic module for accommodating the suspension of cells in liquid medium and a micro electrode module with a metallic layer that serves to apply the electric field[113]. As in all variations of the TipChip, the height of the microfluidic channel network is determined by the size of the pollen grains (80 µm for *Camellia japonica*)[113], whereas other microfluidic platforms place the grains outside of the microchannel network proper allowing for a smaller vertical dimension to fit the growing pollen tubes more snugly[116]. Depending on the experimental design and the needs for continuous fluid flow, the channel design must allow fluid-flow mediated placement of pollen grains to locations or traps where they are immobilized and from where the tubes grow into

or towards the testing setup.[123,124] The microchannel architecture must also be designed to avoid clogging to allow for effective liquid exchange.

To quantify the pollen tube's invasive forces, LoC have been employed to expose them to a variety of narrow spaces and complex mazes[114] with sophisticated microscopic design features such as elastic cantilevers serving as strain gauges (Figures 4b-c, 5c&d)[72]. Microchannels featuring consecutive narrow gaps were designed to mimic the microarchitecture of the pistillar tissue (Figures 4b, 5b).[114,116,125] An elongating pollen tube deformed the PDMS sidewalls of the gap allowing for the calculation of the force exerted to maintain its diameter against compressive stress.[125] Intriguingly, the pollen tube diameter changed transiently after it made its way through

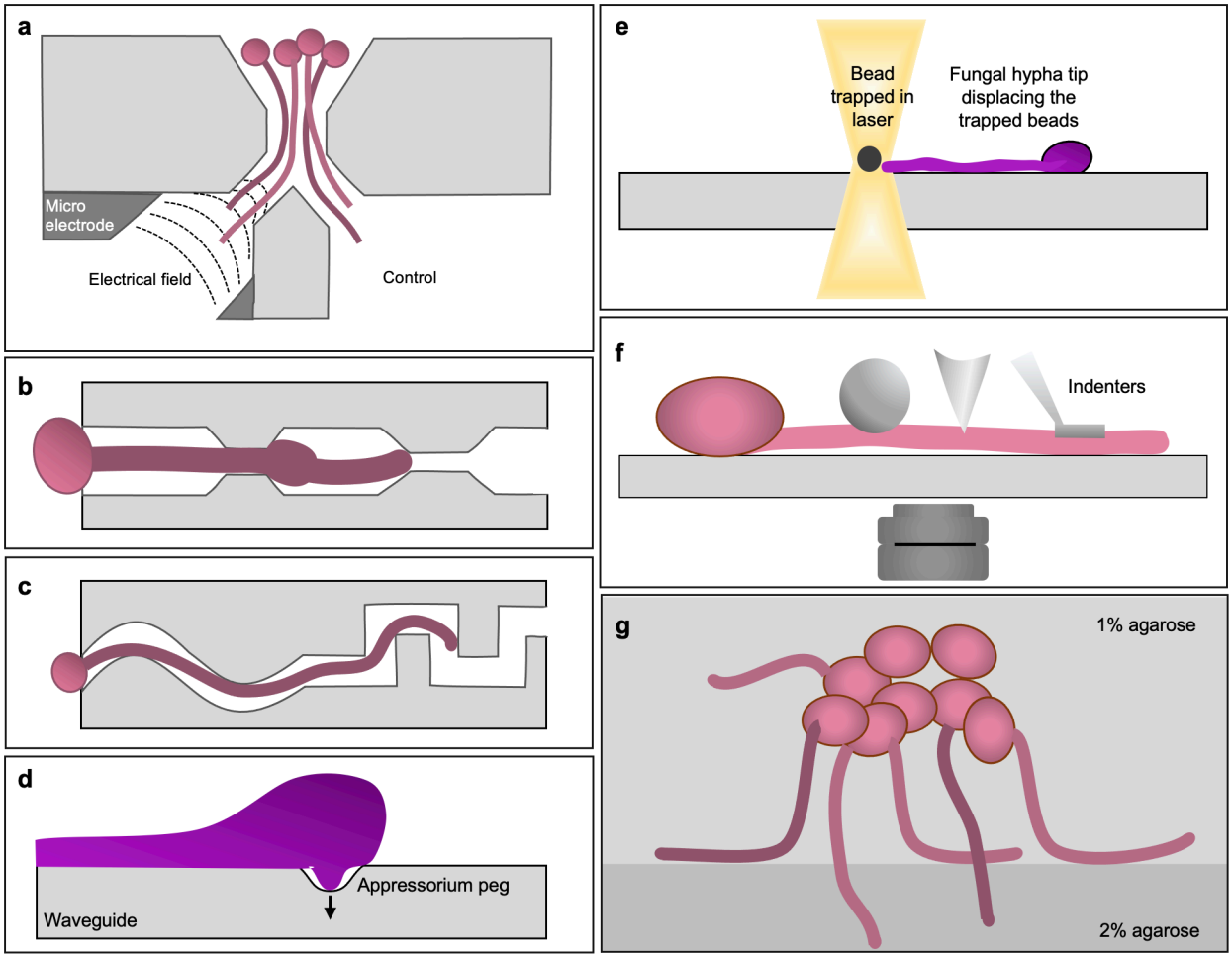

Figure 4. Biomechanical approaches to quantifying invasive and oriented tip growth behavior. a) Lab-on-chip device offering pollen tubes a choice to grow toward or away from an electrical field. b) Microfluidic design to challenge elongating pollen tubes with narrow gaps. The pollen tube diameter transiently widens following gap passage. c) Microchannels with complex geometrical patterns designed to investigate the pollen tube's ability to cope with mechanical obstacles and reorient growth. d) Measuring penetrative forces of fungal appressorium using waveguide deformation. e) Optical tweezers to measure the force exerted by hyphal tips by way of displacement of trapped beads. f) Microindentation using indenters of different shape and size used to determine cellular stiffness. The indenters are attached to cantilevers the deflection of which is monitored by optical sensors. g) Assay exposing growing pollen tubes to media stiffened to different degrees by varying the concentration of agarose.

the gap (Figure 4b), suggesting the existence of a feedback mechanism that calibrates the invasive force through modulation of cell wall mechanical properties.[125] It was also shown that the vegetative nucleus and sperm cells were able to move forward through the tube while significantly constricted by the gap demonstrating substantial elastic deformability (Figure 5b). Similar observations were made for root hairs and moss protonemata.[116]

The strain gauge principle was also used to measure the invasive force exerted by the hyphal apices.[25,126] In both pollen tubes and fungal hyphae, a technical difficulty consisted in stabilizing the longitudinal cell sufficiently to enable measurement of reliable data for the elongation force. Strain gauges and cantilevers have been combined with kinked microchannels (Figure 5c) or agarose to stabilize the base of the tubular cell. This is a critical element of the experimental design as both pushback and buckling must be prevented to enable reliable and reproducible quantitative measurements. Because of their ability to strongly adhere to surfaces, the problem of stabilizing the base of the cell was less of a challenge in the case of the infection pegs formed from fungal appressoria. This allowed the use of a waveguide whose deformation by the emerging peg could be monitored optically and used to calculate the force[78,127] (Figure 4d).

Other attempts to measure the invasive force of tip growing cells used optical tweezers. Fungal hyphae of *N. crassa* were faced with obstacles in form of polystyrene beads trapped in a beam of laser light (Figure 4e). The force required to push a bead from its trapped position is directly proportional to the bead's size implicating that more force is required to dislocate a larger bead.[126] Regular hyphae displaced all the beads, because the strength of the optical trap is limited only to a few piconewton range,[25,126] but the force of a conidial germ tube was determined to be within the dynamic range of this assay revealing that its invasive force is much lower than that of leading fungal hypha tips.[128]

Since the invasive force of tip growing walled cells is the turgor pressure, establishing values for this parameter is an important component when characterizing the cell mechanical underpinnings of invasion. If the cell wall is completely pliable, the 'entire' internal pressure generated can be exerted to an outside substrate, but if the apical cell wall poses substantial resistance to deformation, the invasive force exerted by the tip growing cell can be expected to be lower than its turgor.[25,126] The invasive force therefore does not equate turgor. In order to measure the turgor pressure, several techniques have been employed such as incipient plasmolysis or the pressure probe—an oil filled microcapillary that is injected into the cell.[70,129,130] Since these methods are invasive in nature, they cannot be used repeatedly or over longer periods of time.[131] As a minimally invasive method, ball tonometry and other indentation techniques (Figure 4f) have been used to estimate turgor. In ball tonometry, a large spherical probe with a controlled load is applied to the cell and the contact area is measured to deduce turgor. Other indentation techniques are based on pressing a cylindrical probe into the cell and measuring the applied force and the indentation depth. Recently, Burri et al. (2019) coupled a modified ball tonometry approach with microindentation technique to calculate turgor pressure and measure cell wall elasticity. The authors employed a non-invasive microrobotic system based on cellular force microscopy (CFM) in combination with

two force sensors with different geometries and force ranges for simultaneous biomechanical measurements on elongation pollen tubes.[131]

Cell wall mechanical properties are important parameters and atomic force microscopy (AFM), an indentation technique with smaller indentation depth and higher spatial resolution than CFM, has been used to assess plant cells.[132-135] Depending on tip size and indentation depth, the deformation that is applied normal to the cell surface may be influenced by the turgor pressure and/or by the

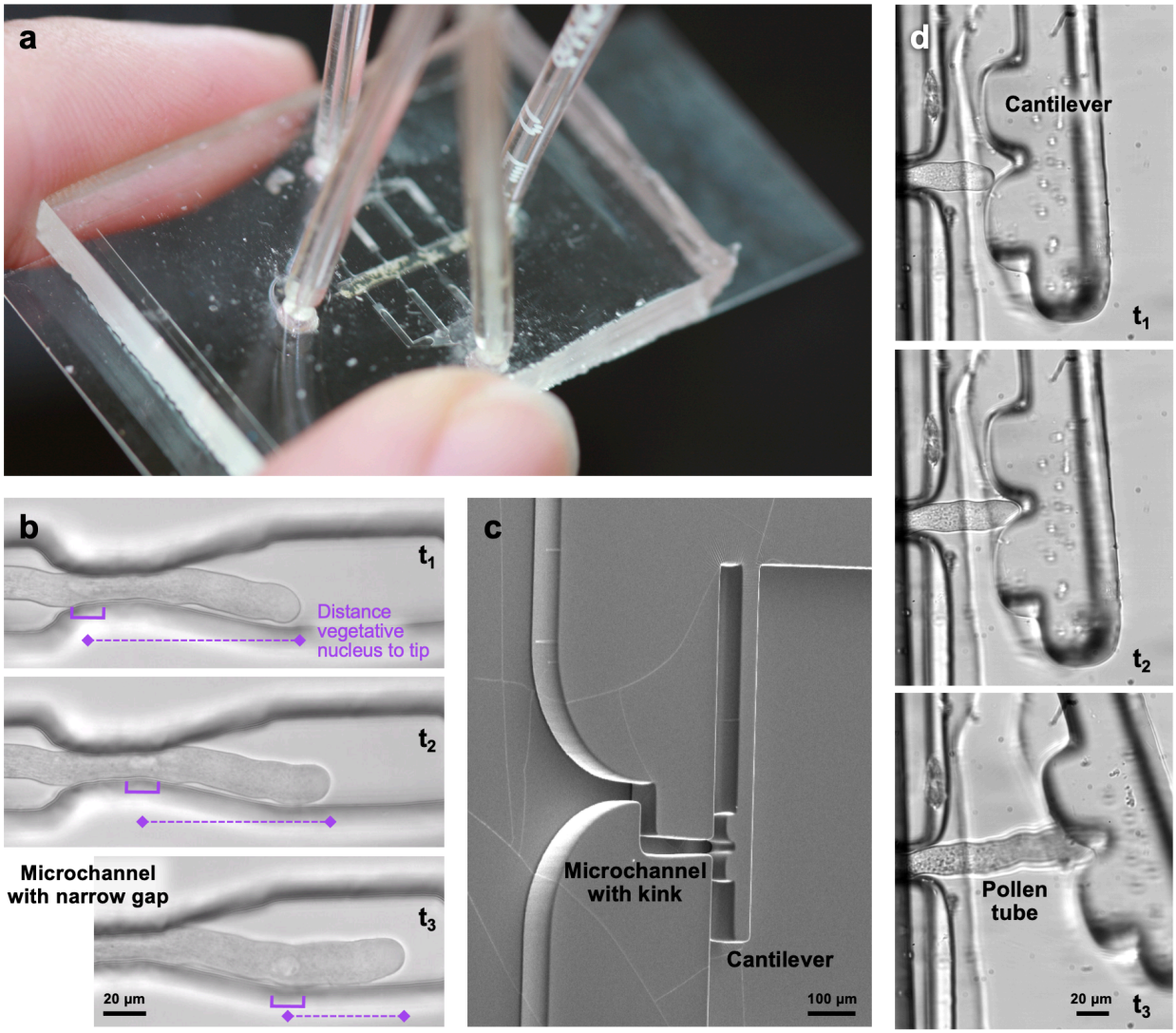

Figure 5. Applications of Lab-on-chip devices for the assessment of invasive growth behavior. a) General design of the TipChip with PDMS layer containing microfluidic network adherent to cover slip. Tubes serve as inlets and outlets for liquids and for injection of pollen suspension. b) *Camellia* pollen tube traversing a narrow gap. At t_1, the vegetative nucleus (purple bracket) has fallen behind its default distance from the growing tip because it got trapped in the narrow passage created by the gap. At t_2, the vegetative nucleus has almost made its way through the gap thanks to its elastic deformability. At t_3, the vegetative nucleus has reached its default distance from the pollen tube tip (60 µm) by accelerating its forward movement following gap passage. Image series provided by Amir Sanati Nezhad. Related data in Sanati Nezhad et al.[125] c) Scanning electron micrograph of PDMS cantilever used to measure the growth force of pollen tubes in Ghanbari et al.[72] d) Brightfield micrographs of *Camellia* pollen tube growing against cantilever shown in c). Images in d) reproduced from Ghanbari et al.[72] with permissions.

geometry of the tissue structure and the extraction of absolute mechanical values from indentation measurements is not trivial,[136,137] but has delivered insights on the viscoelastic nature of the fungal hyphae cell wall.[25,138]

Exposing a tip growing cell to a mechanical cue has the potential to trigger the cell to modulate its invasive force or other cellular parameter—a consideration that must be made when making force measurements. Evidence for this ability of invading cells to modulate their force stems from the observation that pollen tubes growing through increasingly narrow openings continue at a constant speed despite the increasing impedance.[125] This suggests that they may increase their invasive force during the process. This adaptive dynamic behavior was corroborated by the finding that once the obstacle is passed, the tube widens (Figure 4b) indicating that its cell wall had softened while it was pressing against the obstacle.

In addition to the absolute value of the invasive force, a comparative approach is therefore warranted. Pollen tubes exposed to an interface between two different concentrations of agarose were scored for their behavior and ability to penetrate into the stiffer medium thus offering a test assay to assess the effect of pharmacological interference with specific cell features on invasive growth (Figure 4g). To put the measured values for the penetrative behavior of tip growing cells in context, it will be valuable to measure the stiffness of the invaded matrix *in situ*.[24] In case of pollen tubes, it will therefore be crucial to quantify the stiffness of the pistil transmitting tissue. Determining the stiffness of a uniform material is relatively straightforward and can be done by microindentation.[24] Results obtained with larger indenters can be extrapolated to calculate the invasion required by the microscopic invasive cell. This extrapolation is less obvious for matrices with complex micron-scale architecture such as that generated by the cellularity of the transmitting tissue and the presence of the middle lamella. Here, indenter size will matter, and the interpretation of such measurements will require careful consideration of geometrical features.

Conclusion and Perspective

Quantifying physical and mechanical properties at cellular and subcellular levels is a challenge as practical experimentation on these structures is difficult owing to the small size of the specimens. Despite these challenges, the parameters characterizing invasion and penetration events such as turgor driven growth and the dynamic regulation of cell wall mechanical properties have been assessed successfully in a range of cell types. Recent developments in micromechanics technologies such as LoC and MEMS-based force sensors will enhance our ability to directly quantify the role of osmolyte concentration and turgor pressure and will also provide insight into the cellular regulation of biomechanical properties. Quantitative micromanipulation will increasingly be coupled with powerful image analysis software, for example those that enable the detection of the subpixel resolution in order to do quantitative analysis of cell behavior.[139]

One of the major challenges associated with studying cellular invasive growth is the observation of this behavior *in situ*, as the invasive growth events occur several layers deep within the invaded tissues. Two-photon excitation microscopy allows deeper penetration of plant tissues compared to

conventional epi-fluorescence microscopy and confocal laser scanning microscopy and may thus be one of several possible avenues[140]. Novel high-resolution microscopy techniques such as lightsheet fluorescence microscopy are other options for deep tissue imaging and the continuous improvement in spatial resolution is promising. Since lightsheet imaging requires only low doses of light in order to acquire an image in a single plane, this technique will be crucial to allow long-term time-lapse imaging.[141] The combination of micromechanics with powerful microscopy technology will open exciting avenues for single cell analysis.

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References

1. M. Roper, C. Lee, P. C. Hickey and A. S. Gladfelter, *Current Opinion in Microbiology*, 2015, **26**, 116-122.
2. M. Zamil and A. Geitmann, *Physical biology*, 2017, **14**, 015004.
3. A. Carminati, J. B. Passioura, M. Zarebanadkouki, M. A. Ahmed, P. R. Ryan, M. Watt and E. Delhaize, *New Phytologist*, 2017, **216**, 771-781.
4. C. Fife, J. McCarroll and M. Kavallaris, *British Journal of Pharmacology*, 2014, **171**, 5507-5523.
5. M. Lasser, J. Tiber and L. A. Lowery, *Frontiers in Cellular Neuroscience*, 2018, **12**, 165.
6. A. Sanati Nezhad and A. Geitmann, *Journal of Experimental Botany*, 2013, **64**, 4709-4728.
7. Y. Chebli, A. Bidhendi, K. Kapoor and A. Geitmann, *Current Biology, in press*, 2021.
8. J. S. Han, Proceedings of the Korean Society of Wood Science and Technology Annual Meeting, 1998.
9. A. Snegireva, M. Ageeva, S. Amenitskii, T. Chernova, M. Ebskamp and T. Gorshkova, *Russian Journal of Plant Physiology*, 2010, **57**, 342-355.
10. K. Esau, *Plant Anatomy.*, 1965.
11. T. Gorshkova, P. Mikshina, A. Petrova, T. Chernova, N. Mokshina and O. Gorshkov, in *Plant Biomechanics*, Springer, 2018, pp. 141-163.
12. S. Lev-Yadun, *Russian Journal of Plant Physiology*, 2010, **57**, 305-315.
13. B. Clair, T. Alméras, H. Yamamoto, T. Okuyama and J. Sugiyama, *Biophysical Journal*, 2006, **91**, 1128-1135.
14. E. J. Mellerowicz, P. Immerzeel and T. Hayashi, *Annals of Botany*, 2008, **102**, 659-665.
15. A. F. Hill, *Economic Botany*, 1952.
16. H. Hayward, The Structure of Economic Plants (Cramer, L. ed.). New York: Macmillan, 1938, 550-579.
17. A. Fahn, *Secretory Tissues in Plants*, Academic Press London, 1979.
18. A. Geitmann, *Sexual Plant Reproduction*, 2010, **23**, 63-71.
19. Y. Chebli, J. Kroeger and A. Geitmann, *Molecular Plant*, 2013, **6**, 1037-1052.
20. A. Geitmann, in *Mechanical Integration of Plant Cells and Plants*, Springer, 2011, pp. 117-132.
21. P. Fayant, O. Girlanda, Y. Chebli, C.-É. Aubin, I. Villemure and A. Geitmann, *The Plant Cell*, 2010, **22**, 2579-2593.
22. Y. Chebli and A. Geitmann, *Functional Plant Science and Biotechnology*, 2007, **1**, 232-245.
23. O. Gossot and A. Geitmann, *Planta*, 2007, **226**, 405-416.

24. R. Reimann, D. Kah, C. Mark, J. Dettmer, T. M. Reimann, R. C. Gerum, A. Geitmann, B. Fabry, P. Dietrich and B. Kost, *Plant Physiology*, 2020, **183**, 558-569.
25. N. Money, in *Biology of the Fungal Cell*, Springer, 2007, pp. 237-249.
26. R. R. Lew, in *Biology of the Fungal Cell*, Springer, 2019, pp. 83-94.
27. L. Yafetto, D. J. Davis and N. P. Money, *Fungal Genetics and Biology*, 2009, **46**, 688-694.
28. C. G. Shaw III and G. A. Kile, *Armillaria Root Disease* 1991.
29. N. Bolan, *Plant and Soil*, 1991, **134**, 189-207.
30. A. Jungk, Journal of Plant Nutrition and Soil Science, 2001, **164**, 121-129.
31. H. J. Dittmer, *American Journal of Botany*, 1949, 152-155.
32. C. H. Farr, *The Quarterly Review of Biology*, 1928, **3**, 343-376.
33. A. G. Bengough, K. Loades and B. M. McKenzie, *Journal of Experimental Botany*, 2016, **67**, 1071-1078.
34. A. Creff, L. Brocard and G. Ingram, *Nature Communications*, 2015, **6**, 1-8.
35. M. Lucas, K. Kenobi, D. Von Wangenheim, U. Voβ, K. Swarup, I. De Smet, D. Van Damme, T. Lawrence, B. Péret and E. Moscardi, *Proceedings of the National Academy of Sciences*, 2013, **110**, 5229-5234.
36. L. X. Dupuy, M. Mimault, D. Patko, V. Ladmiral, B. Ameduri, M. P. MacDonald and M. Ptashnyk, *Current Opinion in Genetics & Development*, 2018, **51**, 18-25.
37. J. Lipiec, R. Horn, J. Pietrusiewicz and A. Siczek, *Soil and Tillage Research*, 2012, **121**, 74-81.
38. T. Colombi, N. Kirchgessner, A. Walter and T. Keller, *Plant Physiology*, 2017, **174**, 2289-2301.
39. R. G. White and J. A. Kirkegaard, *Plant, Cell & Environment*, 2010, **33**, 133-148.
40. R. Stirzaker, J. Passioura and Y. Wilms, *Plant and Soil*, 1996, **185**, 151-162.
41. S. Materechera, A. Alston, J. Kirby and A. Dexter, *Plant and Soil*, 1992, **144**, 297-303.
42. J. G. Chimungu, K. W. Loades and J. P. Lynch, *Journal of Experimental Botany*, 2015, **66**, 3151-3162.
43. L. Stolzy and K. Barley, *Soil Science*, 1968, **105**, 297-301.
44. E. Kolb, C. Hartmann and P. Genet, *Plant and Soil*, 2012, **360**, 19-35.
45. F. Bizet, A. G. Bengough, I. Hummel, M.-B. Bogeat-Triboulot and L. X. Dupuy, *Journal of Experimental Botany*, 2016, **67**, 5605-5614.
46. F. E. O'Callaghan, R. A. Braga, R. Neilson, S. A. MacFarlane and L. X. Dupuy, *Scientific Reports*, 2018, **8**, 1-17.
47. J. Helliwell, C. J. Sturrock, S. Mairhofer, J. Craigon, R. Ashton, A. Miller, W. Whalley and S. J. Mooney, *Scientific Reports*, 2017, **7**, 1-10.
48. C. J. Stubbs, D. D. Cook and K. J. Niklas, *Journal of Experimental Botany*, 2019, **70**, 3439-3451.
49. A. Geitmann, M. Cresti and I. Heath, Cell biology of plant and fungal tip growth. Proceedings of the NATO Advanced Research Workshop, Siena, Italy, 19-23 June 2000., 2001.
50. A. J. Bidhendi and A. Geitmann, *Plant Physiology*, 2018, **176**, 41-56.
51. J. H. Kroeger and A. Geitmann, *Mechanics Research Communications*, 2012, **42**, 32-39.
52. J. H. Kroeger, F. B. Daher, M. Grant and A. Geitmann, *Biophysical Journal*, 2009, **97**, 1822-1831.
53. S. Bartnicki-Garcia, D. D. Bartnicki, G. Gierz, R. a. López-Franco and C. E. Bracker, *Experimental Mycology*, 1995, **19**, 153-159.
54. P. K. Hepler, L. Vidali and A. Y. Cheung, *Annual Review of Cell and Developmental Biology*, 2001, **17**, 159-187.
55. J. Bove, B. Vaillancourt, J. Kroeger, P. K. Hepler, P. W. Wiseman and A. Geitmann, *Plant Physiology*, 2008, **147**, 1646-1658.
56. A. Geitmann and A. Nebenführ, *Molecular Biology of the Cell*, 2015, **26**, 3373-3378.
57. A. Geitmann and J. K. Ortega, *Trends in Plant Science*, 2009, **14**, 467-478.
58. J. H. Kroeger, R. Zerzour and A. Geitmann, *PLoS One*, 2011, **6**, e18549.
59. A. Geitmann and M. Steer, in *The Pollen Tube*, Springer, 2006, pp. 177-200.
60. C. Y.-h. Chen, A. Y. Cheung and H.-m. Wu, *The Plant Cell*, 2003, **15**, 237-249.
61. Y. Chebli, M. Kaneda, R. Zerzour and A. Geitmann, *Plant Physiology*, 2012, **160**, 1940-1955.
62. P. W. De Groot, A. F. Ram and F. M. Klis, *Fungal Genetics and Biology*, 2005, **42**, 657-675.

63. E. Gomes, R. da Silva, J. de Cassia Pereira and G. Ladino-Orjuela, in *Current Developments in Biotechnology and Bioengineering*, Elsevier, 2018, pp. 31-56.
64. M. Ageeva, B. Petrovska, H. Kieft, V. Sal'nikov, A. Snegireva, J. Van Dam, W. Van Veenendaal, A. Emons, T. Gorshkova and A. Van Lammeren, *Planta*, 2005, **222**, 565-574.
65. A. Siedlecka, S. Wiklund, M.-A. Péronne, F. Micheli, J. Leśniewska, I. Sethson, U. Edlund, L. Richard, B. Sundberg and E. J. Mellerowicz, *Plant Physiology*, 2008, **146**, 554-565.
66. T. Gorshkova, N. Brutch, B. Chabbert, M. Deyholos, T. Hayashi, S. Lev-Yadun, E. J. Mellerowicz, C. Morvan, G. Neutelings and G. Pilate, *Critical Reviews in Plant Sciences*, 2012, **31**, 201-228.
67. D. J. Cosgrove, *Plant Physiology and Biochemistry*, 2000, **38**, 109-124.
68. M. Hertzberg, H. Aspeborg, J. Schrader, A. Andersson, R. Erlandsson, K. Blomqvist, R. Bhalerao, M. Uhlén, T. T. Teeri and J. Lundeberg, *Proceedings of the National Academy of Sciences*, 2001, **98**, 14732-14737.
69. M. Israelsson, M. E. Eriksson, M. Hertzberg, H. Aspeborg, P. Nilsson and T. Moritz, *Plant Molecular Biology*, 2003, **52**, 893-903.
70. R. Benkert, G. Obermeyer and F.-W. Bentrup, *Protoplasma*, 1997, **198**, 1-8.
71. J. T. Burri, H. Vogler, N. F. Läubli, C. Hu, U. Grossniklaus and B. J. Nelson, *New Phytologist*, 2018, **220**, 187-195.
72. M. Ghanbari, M. Packirisamy and A. Geitmann, *Technology*, 2018, **6**, 101-109.
73. R. Zerzour, J. Kroeger and A. Geitmann, *Developmental Biology*, 2009, **334**, 437-446.
74. L. Beauzamy, N. Nakayama and A. Boudaoud, *Annals of Botany*, 2014, **114**, 1517-1533.
75. A. E. Hill, B. Shachar-Hill, J. N. Skepper, J. Powell and Y. Shachar-Hill, *PLoS One*, 2012, **7**, e36585.
76. E. Parre and A. Geitmann, *Plant Physiology*, 2005, **137**, 274-286.
77. M. Bastmeyer, H. B. Deising and C. Bechinger, *Annual Review of Biophysics and Biomolecular Structure*, 2002, **31**, 321-341.
78. C. Bechinger, K.-F. Giebel, M. Schnell, P. Leiderer, H. B. Deising and M. Bastmeyer, *Science*, 1999, **285**, 1896-1899.
79. R. R. Lew and S. Nasserifar, *Microbiology*, 2009, **155**, 903-911.
80. J. Bronkhorst, M. Kasteel, S. v. Veen, J. M. Clough, K. Kots, J. Buijs, J. v. d. Gucht, T. Ketelaar, F. Govers and J. Sprakel, *Nature Microbiology, in press*, 2021.
81. R. L. Harold, N. Money and F. Harold, *Protoplasma*, 1996, **191**, 105-114.
82. R. R. Lew, N. N. Levina, S. K. Walker and A. Garrill, *Fungal Genetics and Biology*, 2004, **41**, 1007-1015.
83. J. Gay and A. Greenwood, *The Fungus Spore*, 1966, 95-108.
84. J. Li and I. B. Heath, *Experimental Mycology*, 1994, **18**, 57-69.
85. A. Geitmann, in *Fertilization in Higher Plants*, Springer, 1999, pp. 283-302.
86. B. C. Gibbon, D. R. Kovar and C. J. Staiger, *The Plant Cell*, 1999, **11**, 2349-2363.
87. L. Vidali, S. T. McKenna and P. K. Hepler, *Molecular Biology of the Cell*, 2001, **12**, 2534-2545.
88. S. K. Walker and A. Garrill, *Mycologist*, 2006, **20**, 26-31.
89. S. Suei and A. Garrill, *Protoplasma*, 2008, **232**, 165.
90. S. N. Grove and J. A. Sweigard, *Experimental Mycology*, 1980, **4**, 239-250.
91. I. B. Heath, M. Bonham, A. Akram and G. D. Gupta, *Fungal Genetics and Biology*, 2003, **38**, 85-97.
92. C. Erbar, International Journal of Plant Sciences, 2003, **164**, S265-S277.
93. S. J. Hiscock, F. M. Dewey, J. O. Coleman and H. G. Dickinson, *Planta*, 1994, **193**, 377-384.
94. D. J. Cosgrove, P. Bedinger and D. M. Durachko, *Proceedings of the National Academy of Sciences*, 1997, **94**, 6559-6564.
95. M. Pezzotti, R. Feron and C. Mariani, *Plant Molecular Biology*, 2002, **49**, 187-197.
96. F. Y. Bih, S. S. Wu, C. Ratnayake, L. L. Walling, E. A. Nothnagel and A. H. Huang, *Journal of Biological Chemistry*, 1999, **274**, 22884-22894.
97. R. Konar and R. G. Stanley, *Planta*, 1969, **84**, 304-310.
98. J. T. Greenberg, Proceedings of the National Academy of Sciences, 1996, **93**, 12094-12097.
99. M. Herrero and H. G. Dickinson, *Journal of Cell Science*, 1979, **36**, 1-18.

100. G. De Lorenzo, R. Castoria, D. Bellincampi and F. Cervone, in *Plant Relationships*, Springer, 1997, pp. 61-83.
101. R. J. Linhardt, K. G. Rice, Z. M. Merchant, Y. S. Kim and D. L. Lohse, *Journal of Biological Chemistry*, 1986, **261**, 14448-14454.
102. R. Blanchette, A. Abad, R. Farrell and T. Leathers, *Applied and Environmental Microbiology*, 1989, **55**, 1457-1465.
103. M. J. Flipphi, M. van Heuvel, P. van der Veen, J. Visser and L. H. de Graaff, *Current Genetics*, 1993, **24**, 525-532.
104. P. Apel, D. Panaccione, F. Holden and J. Walton, *Molecular Plant-Microbe Interactions: MPMI*, 1993, **6**, 467-473.
105. H. Urbanek and J. Zalewska-Sobczak, *Biochemie und Physiologie der Pflanzen*, 1986, **181**, 321-329.
106. L. M. Rogers, M. A. Flaishman and P. E. Kolattukudy, *The Plant Cell*, 1994, **6**, 935-945.
107. N. J. Tonukari, J. S. Scott-Craig and J. D. Walton, *The Plant Cell*, 2000, **12**, 237-247.
108. K. Mendgen, H. M and D. Holger, *Annual Review of Phytopathology*, 1996, **34**, 367-386.
109. G. Daniel, in *Secondary Xylem Biology*, Elsevier, 2016, pp. 131-167.
110. C. I. Ullrich, R. Aloni, M. E. Saeed, W. Ullrich and T. Efferth, *Phytomedicine*, 2019, **64**, 153081.
111. A. Matei, C. Ernst, M. Günl, B. Thiele, J. Altmüller, V. Walbot, B. Usadel and G. Doehlemann, *New Phytologist*, 2018, **217**, 1681-1695.
112. M. Horade, M. M. Kanaoka, M. Kuzuya, T. Higashiyama and N. Kaji, *The Royal Society of Chemistry Advances*, 2013, **3**, 22301-22307.
113. C. G. Agudelo, A. Sanati Nezhad, M. Ghanbari, M. Naghavi, M. Packirisamy and A. Geitmann, *The Plant Journal*, 2013, **73**, 1057-1068.
114. A. Sanati Nezhad, *Lab on a Chip*, 2014, **14**, 3262-3274.
115. A. Geitmann, in *Pollen Tip Growth*, Springer, 2017, pp. 87-103.
116. N. Yanagisawa, N. Sugimoto, H. Arata, T. Higashiyama and Y. Sato, *Scientific Reports*, 2017, **7**, 1403.
117. N. Yanagisawa, E. Kozgunova , G. Grossmann and A. Geitmann, *Plant and Cell Physiology*, 2021.
118. C. Agudelo, A. Sanati Nezhad, M. Ghanbari, M. Packirisamy and A. Geitmann, *Journal of Micromechanics and Microengineering*, 2012, **22**, 115009.
119. C. G. Agudelo, M. Packirisamy and A. Geitmann, in *Plant Cell Morphogenesis*, Springer, 2014, pp. 237-248.
120. H. Bertrand-Rakusová, Y. Chebli and A. Geitmann, in *Pollen and Pollen Tube Biology*, Springer, 2020, pp. 211-221.
121. C. Agudelo, M. Packirisamy and A. Geitmann, *Scientific Reports*, 2016, **6**, 19812.
122. A. Geitmann and R. Palanivelu, *Floriculture and Ornamental Biotechnology*, 2007, **1**, 77-89.
123. M. Ghanbari, A. Sanati Nezhad, C. G. Agudelo, M. Packirisamy and A. Geitmann, *Journal of bioscience and bioengineering*, 2014, **117**, 504-511.
124. A. Sanati Nezhad, M. Ghanbari, C. G. Agudelo, M. Naghavi, M. Packirisamy, R. B. Bhat and A. Geitmann, *Biomedical Microdevices*, 2014, **16**, 23-33.
125. A. Sanati Nezhad, M. Naghavi, M. Packirisamy, R. Bhat and A. Geitmann, *Proceedings of the National Academy of Sciences*, 2013, **110**, 8093-8098.
126. G. D. Wright, J. Arlt, W. C. Poon and N. D. Read, Optical Trapping and Optical Micromanipulation II, 2005.
127. A. Geitmann, *American Journal of Botany*, 2006, **93**, 1380-1390.
128. G. D. Wright, J. Arlt, W. C. Poon and N. D. Read, *Mycoscience*, 2007, **48**, 15-19.
129. P. B. Green, *Plant Physiology*, 1968, **43**, 1169-1184.
130. A. D. Tomos and R. A. Leigh, *Annual review of plant biology*, 1999, **50**, 447-472.
131. J. T. Burri, H. Vogler, G. Munglani, N. F. Läubli, U. Grossniklaus and B. J. Nelson, *IEEE Robotics and Automation Letters*, 2019, **4**, 641-646.
132. P. Milani, M. Gholamirad, J. Traas, A. Arnéodo, A. Boudaoud, F. Argoul and O. Hamant, *The Plant Journal*, 2011, **67**, 1116-1123.

133. A. N. Fernandes, X. Chen, C. A. Scotchford, J. Walker, D. M. Wells, C. J. Roberts and N. M. Everitt, *Physical Review E*, 2012, **85**, 021916.
134. K. Radotić, C. Roduit, J. Simonović, P. Hornitschek, C. Fankhauser, D. Mutavdžić, G. Steinbach, G. Dietler and S. Kasas, *Biophysical Journal*, 2012, **103**, 386-394.
135. M. Majda, P. Grones, I.-M. Sintorn, T. Vain, P. Milani, P. Krupinski, B. Zagórska-Marek, C. Viotti, H. Jönsson and E. J. Mellerowicz, *Developmental Cell*, 2017, **43**, 290-304. e294.
136. A. Peaucelle, S. A. Braybrook, L. Le Guillou, E. Bron, C. Kuhlemeier and H. Höfte, *Current Biology*, 2011, **21**, 1720-1726.
137. L. Beauzamy, J. Derr and A. Boudaoud, *Biophysical Journal*, 2015, **108**, 2448-2456.
138. H. Ma, L. A. Snook, S. G. Kaminskyj and T. E. Dahms, *Microbiology*, 2005, **151**, 3679-3688.
139. D. S. Damineli, M. T. Portes and J. A. Feijó, in *Pollen Tip Growth*, Springer, 2017, pp. 391-413.
140. A. Y. Cheung, S. Niroomand, Y. Zou and H.-M. Wu, *Proceedings of the National Academy of Sciences*, 2010, **107**, 16390-16395.
141. A. Candeo, F. G. Doccula, G. Valentini, A. Bassi and A. Costa, *Plant and Cell Physiology*, 2017, **58**, 1161-1172.