

# Plant trichomes as microbial habitats and infection sites

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**Abstract** Trichomes, also simply referred to as hairs, are fine outgrowths of epidermal cells in many organisms including plants and bacteria. Plant trichomes have long been known for their multiple beneficial roles, ranging from protection against insect herbivores and ultraviolet light to the reduction of transpiration. However, there is increasing evidence that the presence of trichomes may have detrimental consequences for plants. For example, plant pathogenic bacteria can enter hosts through the open bases or broken stalks of damaged trichomes. Similarly, trichomes are considered a preferred site for fungal infection, and in this regard, the colonization and penetration of trichomes by fungi and oomycetes have been visualized using light, fluorescence, and scanning electron microscopy in a variety of plants from grasses to shrubs and trees. In addition to parasitic interactions, trichomes also form a host site for endophytic relationships with fungi, thereby serving as an unusual fungal niche. The replication and presence of plant viruses in trichomes have also been confirmed after inoculation. In contrast, the well-known beneficial *Azolla–Anabaena* symbiosis is facilitated through epidermal trichomes of the seedless vascular plant *Azolla*.

These observations indicate that plant trichomes are involved in multiple interactions in terms of providing microbial habitats and infection sites as well as functioning as protective structures. Trichome-related microbial parasitism and endophytism can, in many ways, be considered comparable to those associated with root hairs.

**Keywords** Epidermis · Infection · Niche · Trichome

## Introduction

Microbes are ubiquitous on Earth and can survive in even the most extreme of environments. Their habitats range from hydrothermal vents in the deep ocean to rocks, deserts, and the poles. In addition, they constitute the microbiota within multicellular organisms. Identifying novel microbial habitats or ecological niches reveals the tolerance and adaptation of microbes to different environments. Apart from saprophytic microbes, pathogenic microbes continue to find ways to exploit novel host resources, be it those of humans, domestic animals, or plants (Engering et al. 2013). Plant pathogenic microbes enter host plants through natural openings such as stomata, lenticels, and hydathodes on plant surfaces. Moreover, some plant pathogenic fungi can penetrate and infect plants directly using appressoria, penetration pegs, and infection hyphae. Identifying unknown infection sites and infection structures can reveal novel strategies whereby pathogens establish compatible interactions with the host plant (Kim et al. 1999; Petkar and Ji 2017).

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The surfaces of plant organs are characterized by a variety of protuberant cellular structures including papillae, trichomes, and other various emergences and projections (Werker 2000). These structures are distinguished from others based on their ontogeny and size. To microbes, plant surfaces studded with these structures might resemble to a jungle, where epicuticular waxes form a rough terrain, veins are grooves, and trichomes are trees (Vacher et al. 2016). Given that these protuberant structures do not have natural openings in their intact state, they are not traditionally regarded as habitats or infection sites for plant pathogenic microbes. Although studies have mainly focused on the beneficial roles played by plant trichomes in nature, there have been an increasing number of studies that have demonstrated trichomes to be microbial habitats and infection sites in a diversity of plants (Imboden et al. 2018; Łażniewska et al. 2012). In this review, I aim to highlight microscopic aspects of the trichome–microbe associations, both saprophytic and parasitic, involved in microbial habitation and infection in a variety of plants from grasses to shrubs and trees, thereby providing novel insights into the roles of trichomes as an often overlooked biological component in plant–microbe interactions.

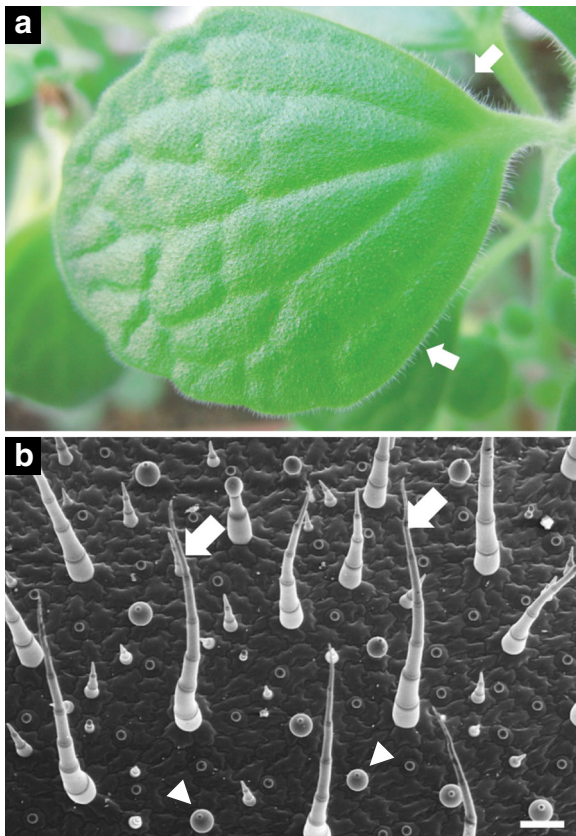
### Plant trichomes: structure and function

Trichomes (from the Greek meaning “hair”), often referred to simply as hairs, are commonly described as fine outgrowths of epidermal cells in organisms. Although trichomes are traditionally considered to be structures of plant origin and have been extensively studied in plants, they are also innate structures of prokaryotes and eukaryotic microbes. For example, bacterial trichomes are flexible structures comprising numerous contiguous cells (with diameters ranging from 3 to 6  $\mu\text{m}$ ) containing sulfur inclusions and enveloped by a common sheath of variable width ranging from 30 to 90  $\mu\text{m}$ , as shown in mats of long filaments (Nishino et al. 1998; Danovaro et al. 2017).

Plant trichomes are unicellular or multicellular appendages that originate exclusively from epidermal cells and develop outwards on the surface of various plant organs (Werker 2000). This definition suggests a close morphological relationship between trichomes and root hairs, implying that aerial trichomes and “subterranean trichomes” (or root hairs) cover nearly all the surfaces of most plants (Wagner 1991). Moreover, trichome and

root hair development in *Arabidopsis* is known to involve common genes and gene products (Wagner et al. 2004). In this context, the microbial associations that have been characterized for root hairs, including parasitism, endophytism, and symbiosis, might also be anticipated to be features of trichomes. Trichomes have been observed on all vegetative and reproductive organs in angiosperms (Ma et al. 2016); they can, for example, be observed on leaf surfaces with the naked eye and are responsible for the pubescence of certain leaf types (Fig. 1). The beneficial functions ascribed to trichomes range from protecting the plant against insect herbivores and ultraviolet light to reducing transpiration and increasing tolerance to freezing (Hülkamp 2004; Kim 2018). Functionally, they can be classified into two types: (i) glandular trichomes for extracellular secretion of mucilage and volatile compounds, and (ii) non-glandular trichomes for physical defense against certain stress factors. Glandular trichomes possess a basal cell, a short or long stalk, and a globose secretory head (Fig. 1b), whereas non-glandular trichomes are composed of a basal cell and a long (up to 300  $\mu\text{m}$ ) multicellular stalk (Kim 2013). Analytical electron microscopy has detected calcium phosphate in the non-glandular trichomes of Loasaceae plants as a structural component with antiherbivore function, comparable to the bones and teeth of animals (Ensikat et al. 2016). Plant protection chemicals are deposited on the trichomes as well as on the epidermis (Fortunati and Balestra 2018).

In addition to leaves, trichomes are present on the surface of stems (Fig. 2a). Glandular trichomes contain osmiophilic compounds in the head (Fig. 2b). The stalk of these trichomes comprises parenchymal cells beneath the head. Non-glandular trichomes appear as long thorns on the surface, which are distinct from ribbons (Fig. 2c). Microtome-sectioned trichomes reveal the highly vacuolated cells comprising the stalk (Fig. 2d). Some cells may contain dense cytoplasm in light micrographs. Although most trichomes have a head and a stalk filled with parenchymal cells, others have an empty space or lumen within (Tucker et al. 1984). These trichomes are referred to as cavitated or inflated trichomes, the lumen of which can easily be mistaken for an intercellular space (Marinho et al. 2016). There are three types of domains that plant trichomes present to microbes: (i) the trichome surface from the base to the tip, (ii) the parenchymal cells within trichomes, and (iii) the trichome lumen. Each of these domain offers different types of nutrients and environments to the associated microbes.



**Fig. 1** The adaxial leaf surface of *Plectranthus tomentosus*. **a** Photograph. Trichomes (arrows) are prevalent on the surface. **b** Scanning electron micrograph. Note glandular trichomes (arrowheads) and non-glandular trichomes (arrows). Bar = 50  $\mu\text{m}$ . A and B: from Kim 2013, with permission from the publisher

## Trichome–bacterium associations

### Colonization around intact trichomes

It is widely accepted that plant pathogenic bacteria cannot cause infection in intact host plants and can only enter host plants through wounds or natural openings (Liu et al. 2016). However, since the 1960s, there have been reports that plant pathogenic bacteria can use plant trichomes in two ways, as habitats and infection sites (Ivanoff 1961; Layne 1967; Leben and Daft 1964; Schneider and Grogan 1977). Microbial colonization patterns have been related to topographical surface features caused by veins, stomata, trichomes, epidermal cell junctions, or depressions in the cuticle, which are the sites where bacteria are most commonly observed (Andrews and Harris 2000; Beattie and Lindow 1995). For example, many of the apparently intact but non-

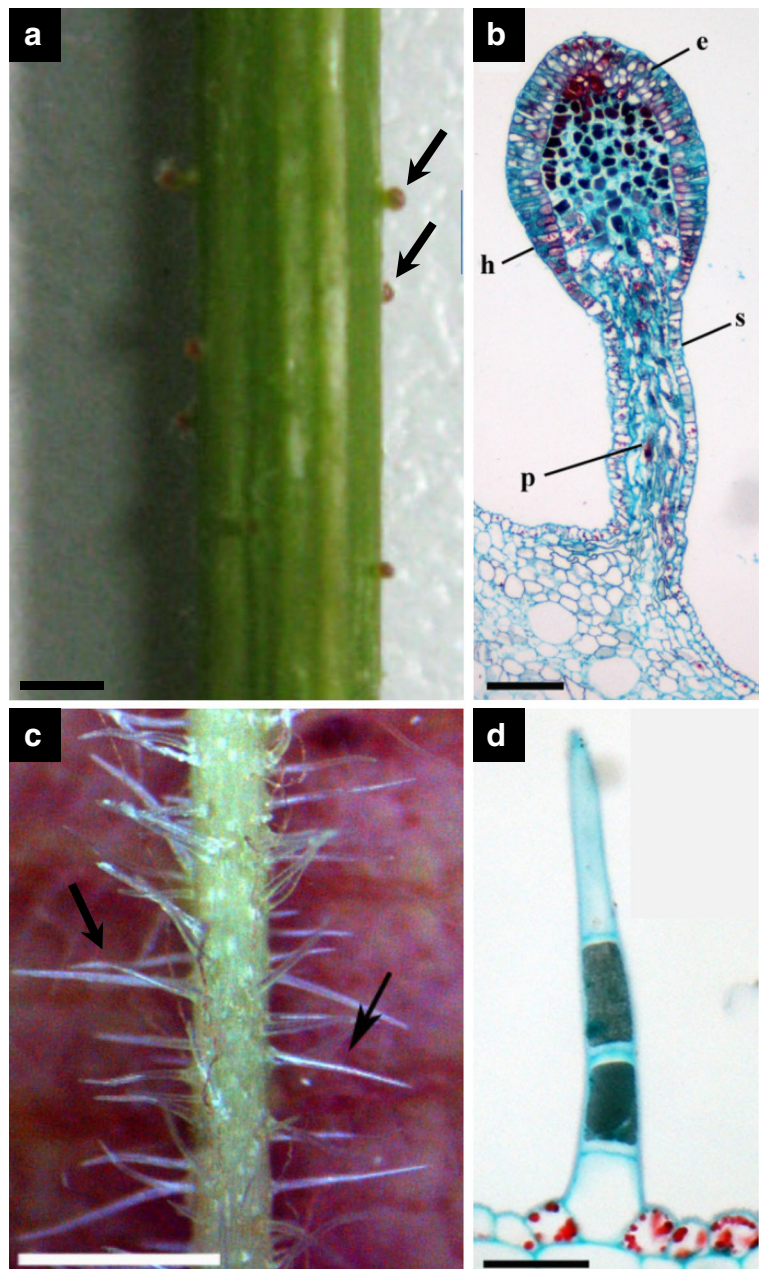
living trichomes of tomato leaves have been known to be colonized by *Pseudomonas syringae* pv. *tomato* (Pietrarelli et al. 2006; Schneider and Grogan 1977). Scanning electron microscopy has also revealed the occurrence of *P. syringae* pv. *syringae* cells at the base of trichomes (Mansvelt and Hattingh 1989), which may be related to the abundance of nutrients exuded from cracks in the cuticular layer or from the large number of ectodesmata located around the base of trichomes (Beattie and Lindow 1995). Aqueous pores ranging from 0.45 to 1.18 nm in radius are typically located in cuticular ledges at the base of trichomes (Schönherr 2006).

### Entry through damaged trichomes

Plant pathogenic bacteria can also gain access to plant interiors via the damaged parts of trichomes, such as opened bases or broken stalks, as infection sites. Most plant trichomes are fragile and readily collapse under slight pressure (Huang 1986), and with organ development, trichomes are gradually lost (Ma et al. 2016). *P. syringae* pv. *syringae* is known to enter pear leaves through the open base of trichomes and survive on trichomes (Mansvelt and Hattingh 1987), and in this regard, trichome bases can frequently be destroyed as a consequence of bacterial multiplication (Huang 1986). In the presence of free water, *P. syringae* pv. *tomato* residing on or near trichome bases have been shown to readily invade tomato fruit through these openings and multiply inside the broken trichome bases (Getz et al. 1983). Similarly, following destruction of the head cells of the peltate glandular trichomes of oregano (*Origanum vulgare* subsp. *hirtum*), intracellular colonization of *Pseudomonas putida* was observed within the damaged head cells (Fig. 3a and b) (Karamanoli et al. 2012). In such cases, the invading bacterial cells are assumed to tolerate or benefit from the metabolites exuded from glandular trichomes.

Evidence indicates that plant pathogenic bacteria colonize intact trichome bases using nutrients obtained from neighboring host tissues. Following the destruction of trichomes, the bacteria multiply within the remnants of these trichomes and further invade host tissues. Given that the blister symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* are typically observed near trichomes, it is assumed that this bacterium may enter leaves through broken trichomes and thereafter invade neighboring host tissues (Chalupowicz et al.

**Fig. 2** External and internal structures of trichomes of *Vitis* species. **a** and **b** Glandular trichomes (arrows) on the stem of *Vitis shenxiensis*. e = epidermal cells; h = head; p = parenchymal cells; s = stalk. **c** and **d** Non-glandular trichomes (arrows) on the abaxial leaf vein of *Vitis hancockii*. Bars = (a) 1 mm, (b) 100  $\mu$ m, (c) 0.5 mm, (d) 50  $\mu$ m. a–d: from Ma et al., 2011, with permission from the publisher

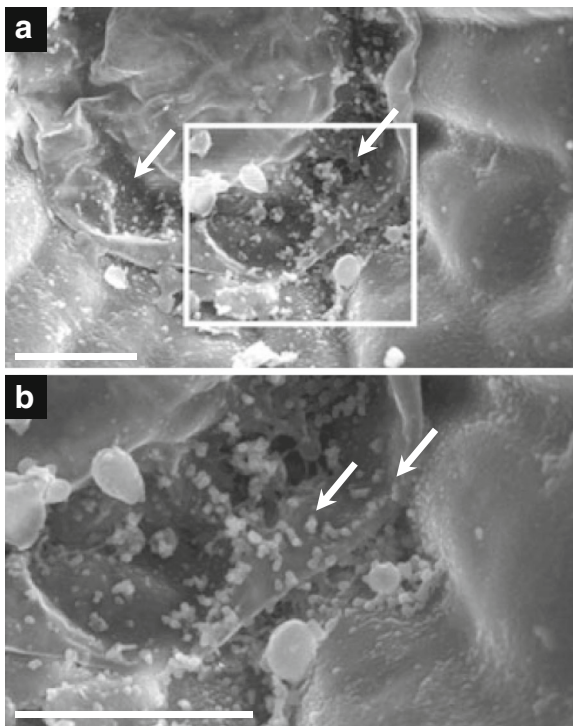


2017). Trichome bases also provide the fire blight pathogen *Erwinia amylovora* access to xylem vessels, which allows the further distribution of this pathogen in apple leaves (Bogs et al. 1998). The kiwi canker pathogen *P. syringae* pv. *actinidiae* is also found near broken trichomes and enters leaf tissues through such apertures (Renzi et al. 2012). Given the acknowledged passive nature of host infection by plant pathogenic bacteria, it is probable that the symptoms observed on seemingly

unwounded host organs are attributable to bacterial entrance through the damaged sections of trichomes such as opened bases or broken stalks.

### Trichome–fungus associations

Since the 1970s, there have been reports of various associations between fungi and trichomes (Kim et al.



**Fig. 3** Scanning electron micrographs of the base of a broken trichome of *Origanum vulgare subsp. hirtum*. **a** Head cells (arrows) of the broken peltate glandular trichome. **b** Higher magnification of the rectangular area in (a). Note the colonization of *Pseudomonas putida* cells (arrows) in the head cells of the broken trichome. Bars = 20  $\mu\text{m}$ . **a** and **b**: from Karamanoli et al. 2012, with permission from the publisher

1974; Lindsey and Pugh 1976). When trichomes are heavily colonized by hyphae, fungi have been observed to grow on and in trichomes on the leaf surface. Since trichomes are not generally recognized as microbial habitats or infection sites, the fungal associations with trichomes have not been intensively studied to date. However, as evidence has accumulated showing that trichomes are fungal habitats and infection sites, the notion that trichomes are simple interspersed pavement cells has gradually been dispelled. Trichomes appear to facilitate fungal adhesion to an organ surface, promote their growth, and provide a suitable microenvironmental humidity (Calo et al. 2006; Łażniewska et al. 2012).

#### An overlooked plant source of novel fungal species

In addition to thorns, trichomes are also regarded as an unusual fungal niche harboring many unknown fungal taxa (Cantrell et al. 2011). In this regard, novel genera of

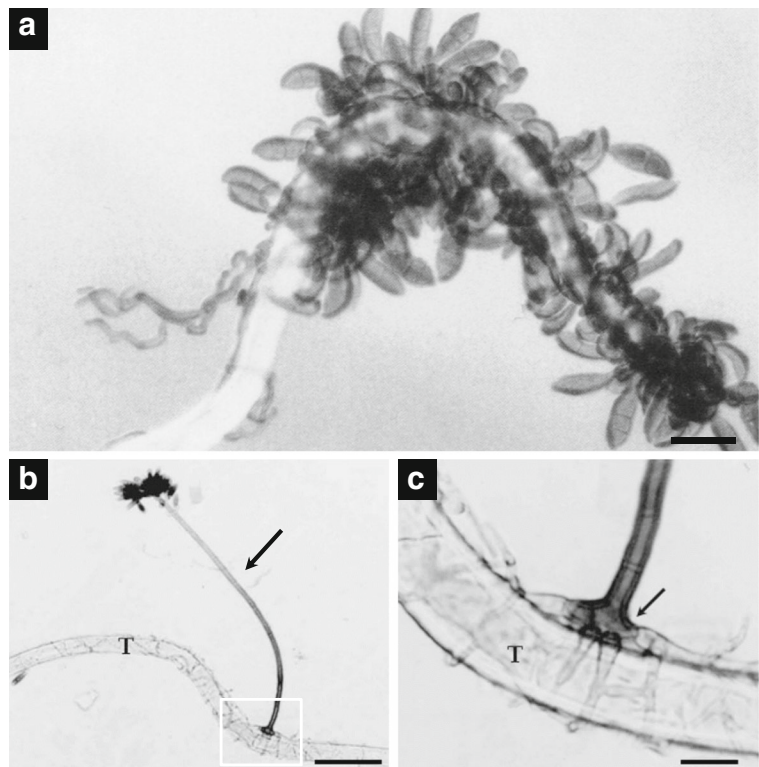
hyphomycete fungi have been isolated from the trichomes of native tree species in the Cerrado, the vast tropical savanna region of Brazil (Dornelo-Silva and Dianese 2004). For example, *Trichomatomyces byrsonimae*, the type species of the new genus *Trichomatomyces*, was first observed on the foliar trichomes of the woody plant *Qualea* (Fig. 4a) (Dornelo-Silva and Dianese 2004). The mycelium of this species grows superficially on the trichomes, and the conidia are elliptic and fusiform. The new genus was designated based on its association with foliar trichomes. Other trichome-inhabiting hyphomycetes were consecutively reported from the same Brazilian group. *Vesiculohyphomyces cerradensis* was found on the foliar trichomes of *Caryocar brasiliense* (Fig. 4b) (Pereira-Carvalho et al. 2009). Similar to *T. byrsonimae*, the mycelium of this fungus grows superficially on the trichomes, and it bears long conidiophores, at the base of which are rhizoidal foot cells (Fig. 4c). These examples thus emphasize the importance of trichomes as an overlooked plant source of novel fungal species in biodiversity surveys.

#### Tea anthracnose

Tea (*Camellia sinensis*) anthracnose is a fungal disease caused by *Discula theae-sinensis* (syn. *Colletotrichum theae-sinensis*, *Gloeosporium theae-sinensis*) (Yamada and Sonoda 2014). The fungus infects the host shrub only through the foliar trichomes, and forms irregular oval spots surrounding trichomes on the leaf surface (Hamaya 1982). The author inferred that tea varieties lacking foliar trichomes would be less susceptible to this disease than trichome-bearing varieties.

Fluorescence microscopy has revealed the presence of infection hyphae of *D. theae-sinensis* in the cell walls and lumens of foliar trichomes 12 h after conidial inoculation (Fig. 5a) (Yamada and Sonoda 2014). The lumen or cavity was distinctly observed between the two cell walls of the trichomes. Septate hyphae invaded the neighboring tissues through pore-like structures at the base of the trichomes 7 days after inoculation (Fig. 5b). Bright-field light microscopy showed a cross section of a spot extending from the infected trichome to a leaf vein (Fig. 5c). The corresponding fluorescence micrograph revealed the occurrence of callose deposition as a bluish fluorescence (Fig. 5d). Such a physiological response may be associated with the triggering of host defense mechanism against the hyphal proliferation from the infected trichomes, resulting in the small round spots on the leaf

**Fig. 4** Light micrographs of fungi on trichomes. **a** Colony of *Trichomatomyces byrsonimae* on the trichome of *Qualea* species. Bar = 20  $\mu\text{m}$ . **b** Hyphae of *Vesiculohyphomyces cerradensis* on the trichome (T) of *Caryocar brasiliense*. Note the long conidiophore (arrow). Bar = 75  $\mu\text{m}$ . **c** Higher magnification of the rectangular area in (b). Note a foot cell (arrow) of the conidiophore. Bar = 10  $\mu\text{m}$ . **a**: from Domelo-Silva and Dianese 2004; **b** and **c**: from Pereira-Carvalho et al. 2009, with permission from the publishers



surface. This pathosystem represents a good example of fungal growth in cavitated or inflated trichomes.

#### Maize seedling blight

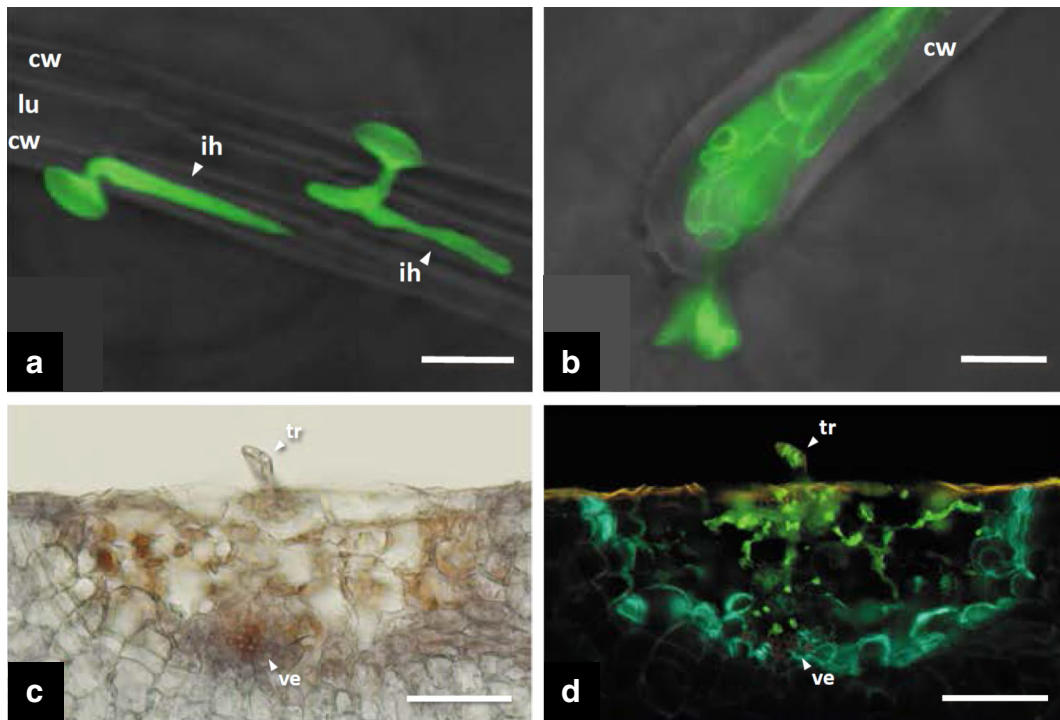
Maize seedling blight is caused by *Fusarium graminearum* and other *Fusarium* species (Hampton et al. 1997). There are three types of trichomes on the maize leaves, namely, two-celled, one-celled prickle, and long macro-trichomes, all of which have been observed to be wrapped and infected with the hyphae of *F. graminearum*, *F. proliferatum*, and *F. verticillioides* (Nguyen et al. 2016). Scanning electron microscopy has revealed the hyphae of *F. graminearum* growing on the base, middle, and apex of macro-trichomes (Fig. 6a) (Nguyen et al. 2016), whereas differential interference contrast microscopy has shown fungal hyphae growing on the base (Fig. 6b) and middle sections of a macro-trichome (Fig. 6c). For a better observation of the hyphae associated with trichomes, the chlorophyll in leaf tissues was cleared using a saturated chloral hydrate solution.

#### Rice spikelet blast

The rice blast fungus (*Magnaporthe oryzae*) is assumed to infect rice spikelets through the cuticle or trichomes. Inoculated conidia of the blast fungus germinate and form appressoria on the base of the large trichomes on rice spikelets (Koga 1995). Scanning electron microscopy of the freeze-fractured spikelets revealed hyphal development at the base of large trichomes 2 days after inoculation. Conidiophores were found to erupt from large trichomes 4 days after inoculation. However, it was not confirmed whether the hyphae penetrated the trichome cell walls or originated from the secondary invasion to the trichomes from penetration of the spikelet cuticle.

#### Barley head blight

*Fusarium graminearum* is the causal fungus of barley head blight. Two types of trichomes are found on barley leaves: (i) prickle-like trichomes (those with a large pointed protuberance), and (ii) domed trichomes (those with a smaller, rounded protuberance) (Imboden et al.

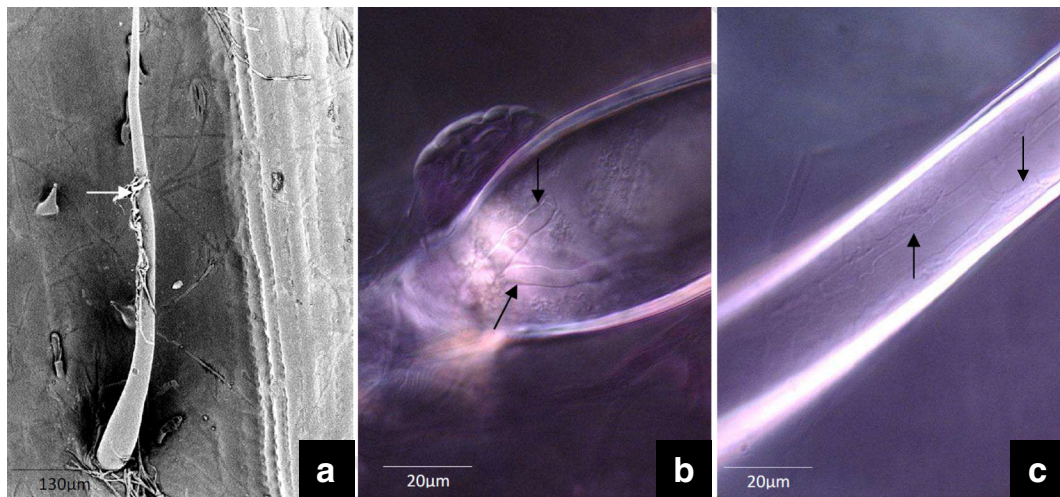


**Fig. 5** *Discula theae-sinensis* in tea leaves. **a** Fluorescence micrograph of infection hyphae (ih) in the lumen (lu) and cell wall (cw) of a trichome. Bar = 5  $\mu\text{m}$ . **b** Fluorescence micrograph of a hypha entering the mesophyll tissue from the trichome. Bar = 10  $\mu\text{m}$ . **c** and **d** Light and fluorescence micrographs of the cross-

section of a small round spot encircling an infected trichome. Note the hyphae emanating from the trichome (tr) to the surrounding vein (ve) and callose deposition (bluish white fluorescence). Bars = 100  $\mu\text{m}$ . **a–d**: from Yamada and Sonoda 2014, with permission from the publisher

2018). Inoculated conidia trapped by prickle-like trichomes have been observed to colonize the base of trichomes. The hyphae coil around the trichomes and

form hyphopodia and penetration hyphae that grow into the hypodermis. These observations suggest that prickle-like trichomes act as traps for conidia and are



**Fig. 6** Macro-trichomes of maize leaves inoculated with *Fusarium graminearum*. **a** Scanning electron micrograph of hyphae in the middle section (arrow) as well as at the base of the trichome. **b** Fluorescence micrograph of hyphae (arrows) invading the base of

the trichome. **c** Fluorescence micrograph of hyphae (arrows) invading the middle section of the trichome. **a–c**: from Nguyen et al. 2016, with permission from the publisher

the sites of fungal penetration. In addition, light microscopic observations have revealed hyphal growth on and in the trichomes of barley spikes and *Arabidopsis thaliana* leaves inoculated with *F. graminearum* (Skadsen and Hohn 2004).

#### Endophytism of *Trichoderma* in cacao stems

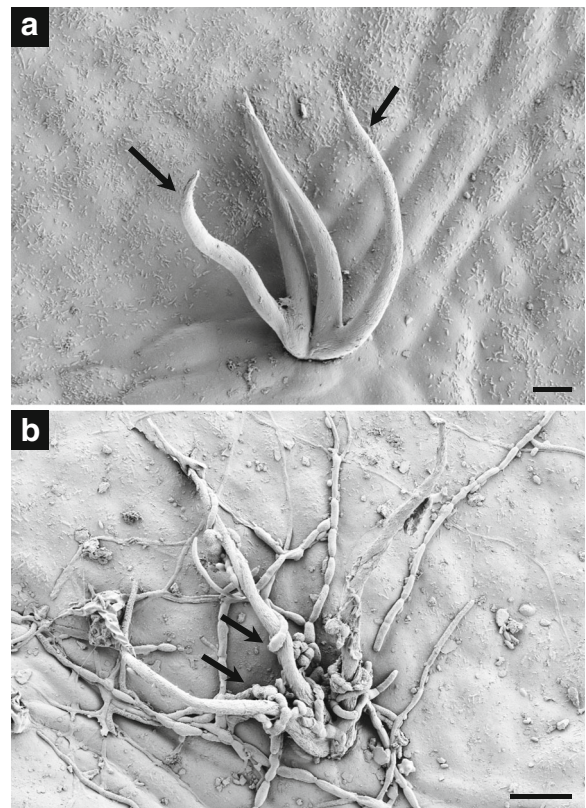
*Trichoderma* species are common soil saprophytic fungi and are widely used as biological control agents against a variety of plant pathogens. Four species of *Trichoderma*, including *T. harzianum*, show endophytic associations with cacao (*Theobroma cacao*) stems (Bailey et al. 2009). Light and scanning electron microscopy have revealed colonization of the stems of cacao seedlings inoculated with *Trichoderma* species, with the fungal hyphae forming appressoria-like swellings on glandular trichomes. *Trichoderma* mycelia have also been observed emerging from glandular trichomes after surface sterilization, and thus these fungi are assumed to enter glandular trichomes, survive surface sterilization, and establish an endophytic association with cacao stems.

#### Clematis leaf spot

*Phoma clematidina* is a fungal pathogen of leaf spot and stem rot in clematis (*Clematis* spp.). Two types of trichomes, short glandular trichomes and long non-glandular trichomes, are found on clematis leaves (Van de Graaf et al. 2002). Scanning electron microscopy has revealed conidial germ tubes penetrating the base of a non-glandular trichome on the adaxial leaf surface of clematis.

#### Oak leaf spot

Small brown spots with yellow halos are often observed on the adaxial leaf surface of infected sawtooth oak (*Quercus acutissima*). The fungal genera isolated from these spots include *Tubakia* and *Pestalotia* (data not shown). Field emission scanning electron microscopy has revealed radiate trichomes with two or four rays on asymptomatic leaves (Fig. 7a) (Jones 1986). Although bacteria are found on the leaf epidermis, the trichomes are rarely infested with fungal hyphae. In contrast, the trichomes on symptomatic leaves are commonly colonized by fungal hyphae (Fig. 7b). The hyphae grow on the leaf epidermis and coil around the rays of the radiate trichomes, with fungal colonization appearing to be



**Fig. 7** Scanning electron micrographs of multi-radiate trichomes on the adaxial surface of *Quercus acutissima* leaves. **a** Trichome on a healthy leaf. Note the four rays (arrows) radiating from the trichome. Bar = 10  $\mu$ m. **b** Trichome on a brown spot encircled with a yellow halo. Fungal hyphae (arrows) coil the trichome. Bar = 20  $\mu$ m. Courtesy of K. W. Kim

more intense near the trichome base than the tip regions. Transmission electron microscopy has also revealed the presence of fungal hyphae in the lumen of sawtooth oak leaf trichomes, with cell wall modifications of trichomes being observed in the vicinity of the fungal hyphae (Kim unpublished data). These observations indicate the pathogenic associations of the fungal hyphae with the oak species.

#### Trichome–oomycete associations

##### Bean blight

*Pythium aphanidermatum*, an oomycete plant pathogen, causes blight on the leaves and stems of bean. Zoospores of this fungus-like organism have been observed to



aggregate at the bases of trichomes and around trichome sockets on bean stems (Kim et al. 1974). Light microscopy has revealed the penetration of glandular trichomes by the infection hyphae of *P. aphanidermatum*. The authors also reported the emergence of an aerial hypha from a glandular trichome on an infected bean stem.

#### Oil palm bud rot

Trichomes have also been proposed as sites of pathogen infection on monocotyledonous plants. Bud rot of oil palm (*Elaeis guineensis*), caused by *Phytophthora palmivora*, is one of the most damaging diseases in South America. Zoospores of *P. palmivora* germinate and advance to the trichomes of oil palm leaflets (Sarria et al. 2016). Some of these zoospores form appressorium-like structures and penetration hyphae on the trichomes. Light and scanning electron microscopy have shown the hyphal emergence and sporulation of *P. palmivora* from the trichomes of oil palm leaflets.

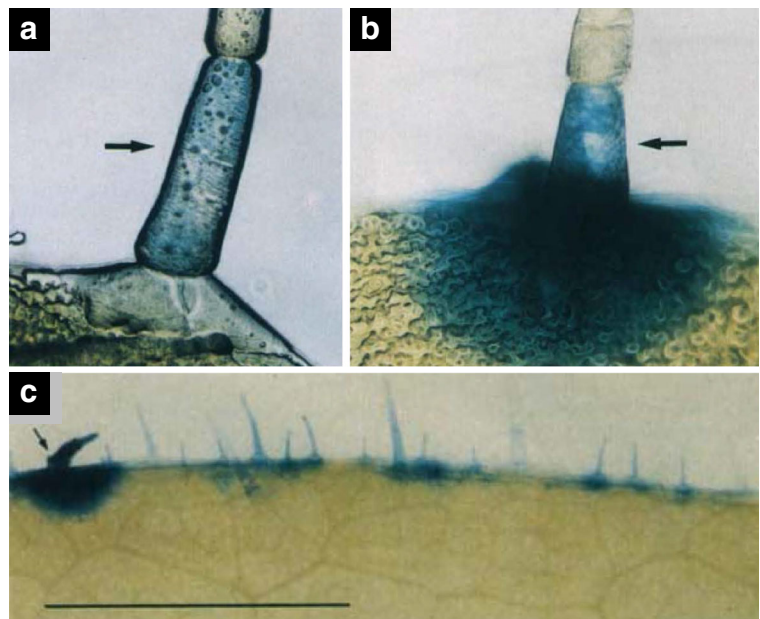
#### Trichome–virus associations

Although viruses are non-cellular microscopic entities, there have been reports of associations between plant viruses and trichomes, and the roles of

trichomes as viral entrance sites have long been proposed. Plants can be artificially inoculated with plant viruses by rubbing leaves with carborundum. When the trichomes of *Nicotiana* species are broken during mechanical inoculation,  $C^{14}$ -labeled *Tobacco mosaic virus* was deposited on the basal regions that trap and hold viruses in a site from which they can establish infection (Kontaxis and Schlegel 1962). To track virus movement within host tissues, micro-injection techniques into specific host cells have been employed. For example, the micro-injection of a *Potato virus X* (PVX) vector construct expressing the  $\beta$ -glucuronidase (GUS) gene into leaf trichome cells of *Nicotiana clevelandii* has been performed to reveal viral replication and cell-to-cell movement (Angell and Baulcombe 1995). The injected trichome cells showed GUS activity, thereby indicating PVX replication (Fig. 8a). Viral movement into the base of trichomes was also detected via the monitoring of GUS activity (Fig. 8b). The distribution of GUS activity was shown in both the injected trichome cells and in cells at the leaf margin (Fig. 8c).

Regarding viral movement through plasmodesmata, transmission electron microscopy has revealed details of the trichome plasmodesmata of *N. clevelandii* leaves (Waigmann et al. 1997). The ultrastructural complexity of trichome plasmodesmata was found to increase from the tip to base cells and have neck

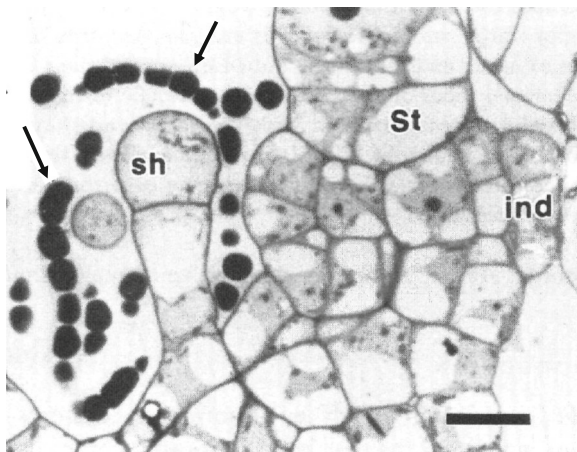
**Fig. 8** Histochemical analysis of the  $\beta$ -glucuronidase (GUS) activity in the trichomes of *Nicotiana clevelandii* leaves. **a** GUS activity in the injected trichome cell (arrow). **b** GUS activity at the base of the infected trichome (arrow). **c** GUS activity in the infected trichome (arrow) and in the leaf margin. Bar = 5 mm. **a–c**: from Angell and Baulcombe 1995, with permission from the publisher



regions with a similar dimension (~60 nm in diameter) to that of mesophyll plasmodesmata. Inclusion bodies, including the pinwheels and laminated aggregates of *Potato virus Y* (PVY), have been observed in the cytoplasm of potato leaf trichomes (Kogovšek et al. 2011), and the detection of viral RNA and particles was found to be consistent with the distribution of PVY in foliar trichomes.

### Trichome-cyanobacterium associations

*Azolla* is a genus of seedless aquatic ferns that form symbioses with the cyanobacterium *Anabaena*. These ferns have several types of trichomes, including epidermal and cavity trichomes, depending on organ location. Cells of the symbiont *Anabaena azollae* establish an intimate association with sporangia of the fern *Azolla mexicana* (Fig. 9). The branched epidermal trichomes of *A. mexicana*, referred to as sporangial pair hairs, are involved in the packaging of the *A. azollae* into sporocarps (Perkins and Peters 1993). In the leaf cavity of *Azolla caroliniana*, simple trichomes may be involved in the exchange of fixed carbon from the fern to *A. azollae* (Calvert et al. 1985). These observations suggest that trichomes represent the interface between the fern and its symbiotic cyanobacteria.



**Fig. 9** Light micrograph of a longitudinal section of a single sporangium of *Azolla mexicana*. Note the intimate association between *Anabaena azollae* (arrows) and the sporangium. Ind = indusium. sh = sporangial pair hair. St = stalk cell. Bar = 10  $\mu$ m. From Perkins and Peters 1993, with permission from the publisher

### Conclusions

Trichomes are known to play beneficial roles in plants, which are primarily associated with the protection of plants, as structural barriers to biotic or abiotic factors. However, as the outermost protruding cellular structures on the plant surface, trichomes can also function as a route for entrance or penetration, leading to microbial colonization on and in these structures. Since the 1960s, there have been reports of the detrimental roles of trichomes as potential sites of infection and microbial habitats, which, along with representing a source of undescribed species, are roles that are now generally acknowledged. Since intact trichomes have no surface apertures, they should not be regarded as natural openings on the plant epidermis. Nevertheless, these structures are readily damaged or broken, consequently providing openings via which bacteria, fungi, oomycetes, and viruses can gain access to host tissues.

Undoubtedly, further studies on trichome infection through detailed observations from a diverse range of plants will be necessary to gain a complete understanding of this phenomenon. Recent advances in specimen preparations including tissue clearing techniques opened unique opportunities for microscope-enabled research in plant pathology (Warner et al. 2014). Various clearing methods for multiscale plant tissues have been developed ranging from PEA-CLARITY to ClearSee (Yu et al. 2018). The notable increase in depth of imaging after tissue clearing may allow for three-dimensional analysis of multiple cell layers, revealing the spatial relationship of trichomes and microbes (Warner et al. 2014). Elucidation of the mechanisms and factors triggering trichome infection could unravel the overlooked nature of trichomes on the plant epidermis.

In addition to bacteria and fungi, archaea constitute a substantial component of the plant microbiome as well as the Earth's ecosystems (Moissl-Eichinger et al. 2018). Belonging to the third domain of life, archaea have the potential to interact with plants by: (i) plant growth promotion, (ii) nutrient supply, and (iii) protection against abiotic stress (Taffner et al. 2018). Future studies will employ workflows for amplicon-based next-generation sequencing methods for the analysis of trichome-archaeon interactions (Bang and Schmitz 2018). Trichomes per se do not affect bacterial diversity on *A. thaliana* leaves (Reisberg et al. 2012). As the trichomes provide anoxic or oxygen-depleted

ecological niches, they may represent a special habitat for methanogens and ammonium-oxidizing archaea (Moissl-Eichinger et al. 2018). A revised concept of trichomes as microbial habitats and infection sites will provide novel insights into the fundamental understanding of plant–microbe interactions in nature.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare no conflict of interest.

**Study of human participants and animals** This study does not contain any studies with human participants or animals.

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