Protoplasma
DOI 10.1007/s00709-008-0006-7

ORIGINAL ARTICLE

Unusual trichome structure and composition in mericarps of catchweed bedstraw (*Galium aparine*)

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Received: 13 February 2008 / Accepted: 23 May 2008

Abstract Catchweed bedstraw is famous for its ability to adhere to other objects due to the presence of numerous trichomes surrounding the stem and mericarps and on the surfaces of the leaves. These trichomes serve as an efficient vector for the movement of the propagules via animals. In this study, we examined the structure and composition of the mericarp trichomes by microscopic and immunocytochemical techniques to determine the distribution of polysaccharides. Trichomes present around the mericarps are distinguished by a pronounced hooked tip, resembling in many ways those on Velcro™. In semi-thin sections, the hooked area of the trichome contains little or no lumen but rather appears to be solidly composed of cell wall material. This solid hook appears to be divided into a plug-like zone of material and a highly thickened primary wall. These trichomes are also compositionally unique. They contain very little xyloglucan, even though other tissues in the plant reacted strongly with antibodies that recognize these polysaccharides. The distribution of pectin epitopes on these hooked trichomes was extremely distinctive, with each of the antibodies recognizing domains along the surface of the primary wall and/or in the plug area. Despite the heavily thickened nature of the walls of these trichomes, xylans were not present. Thus, the unique plugged, thickened, and hooked tip of these trichomes appears to be the result of a specific combination and distribution of various pectic polysaccharide molecules. This unusual wall composition may facilitate the formation of highly curved structures that might be difficult to form with the more rigid xyloglucans and xylans.

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Keywords Bedstraw · Cell wall composition · Immunocytochemistry · Trichomes

Abbreviations
RGI rhamnogalacturonan I

Introduction

Trichomes are one of the most variable of plant cell types. They range from unicellular, unadorned appendages to multicellular structures that are highly branched and ornamented (Fahn 1974; Evert 2006). Their variety in form is also reflected in their variety of functions: protection from insect herbivory and UV light, production and delivery of secondary compounds, collection of salt and other abiotics, protection of the leaf from excessive transpiration, and even increasing freezing tolerance (Levin 1973; Johnson 1975). Although our knowledge of trichomes has been greatly expanded by the study of two classic systems, the cotton ovule trichome (i.e. cotton fiber; Vaughn and Turley 1999) and the branched trichomes found on *Arabidopsis* leaves and stems (Hulskamp 2004), relatively little is known of the multitude of other trichome types.

One of the most unusual trichomes is found in catchweed bedstraw (*Galium aparine* L.). Catchweed bedstraw is a ubiquitous weed found throughout Eurasia and North America (Moore 1974; Malik and Vanden Born 1988), and it is an especially problematic weed in grasses and rapeseed (Defelice 2002). One of its most unique characteristics is its ability to cling to animal fur or human clothing for seed dispersal. In fact, the first hook-and-loop fastener system, Velcro™, was inspired by the hooked trichomes of the Burdock plant, whose trichomes look very similar to those

of catchweed bedstraw. Some of its common names, such as "stick-a-back", come from the childhood prank of surreptitiously sticking portions of the plant on another person's back (Defelice 2002). Herein, we use the less glamorous, but Weed Science Society of America-approved, common name for this weed, catchweed bedstraw.

These hooked trichomes are especially abundant around its flowers and subsequent seeds, allowing them to be transported for great distances on the fur of animals (Gorb and Gorb 2002; Gorb et al. 2003). Spreading of propagules is not the only advantage of this sticky habit, however. Catchweed bedstraw also utilizes this unique ability to stick to other plants and objects as an ecological advantage over other competing plants (Malik and Vanden Born 1988; Taylor 1999; Defelice 2002). Despite being a rather lax plant and with no ability to twine (nor possessing tendrils), bedstraw is able to scramble through, and even ascend over other plants. Thus, the bedstraw accomplishes a vine-like habit without being a true vine.

Surprisingly, there has been relatively little immunocytochemical investigation of the composition of the cell walls of trichomes outside of the well-studied cotton fibers (Vaughn and Turley 1999) and *Arabidopsis* root hairs (Freshour et al. 1996), and even these studies have not taken advantage of the great number of new antibodies available for probing plant cell walls (e.g. those available through the Complex Carbohydrate Research Center and Plant Probes). However, it is clear that even simple trichomes may have wall constituents that are different than neighboring atrichoblasts. For example, Freshour et al. (1996) determined that a pectin epitope recognized by the CCRC-M7 antibody was present in atrichoblasts but not trichomes (root hairs) in *Arabidopsis*, and Vaughn and Turley (1999) described the presence of a pectin sheath on cotton fibers that is absent from atrichoblasts. In this study, we performed an anatomical and immunocytochemical investigation of the trichomes of bedstraw to determine the composition and distribution of polysaccharides in these unusual trichomes. Our data indicate that trichome morphology and composition are unique as far as we are aware. The presence and distribution of numerous pectin epitopes, coupled with the lack of xylans and xyloglucans, might account for its unique structure.

Materials and methods

Biological materials

Plants of catchweed bedstraw were collected in Stoneville, Leland and Greenville, MS, USA in natural stands of the species. Young seedlings, mature plants and plants in the reproductive phase were collected for microscopy. In addition, seed was harvested and plants grown in a portable growth chamber under 400 μM/m^2/s photosynthetically active radiation in a soil mix (Meloche et al. 2007). Some samples were examined directly in an unfixed condition with an Olympus stereo microscope, and images were collected with an Olympus Q-Color3 digital camera.

Light microscopy and immunocytochemistry

Small tissue pieces were fixed in 3% (*v*/*v*) glutaraldehyde in 0.05 M PIPES buffer (pH 7.4) for 2 h at room temperature and then washed twice (15 min each) in cold PIPES buffer before dehydration in an ethanol series. After reaching 100% ethanol, the samples were transferred to −20°C. LR White resin was added in 25% increments, with a 25% increase each day, until 100% resin was reached. After the samples had remained in 100% resin at −20°C for 24 h, they were warmed to room temperature, and then agitated on a rocking shaker for 24 h. Samples were then transferred to flat-bottomed BEEM capsules and polymerization took place at 55°C for 2 h.

Samples were cut out of the blocks and re-oriented by mounting on acrylic stubs, so that the desired orientation of the hooked trichomes was achieved. In other instances, the trichomes from plastic-infiltrated (but unpolymerized) specimens were severed, and the isolated trichomes were flat embedded as described by Bowling and Vaughn (2008b). Sections were obtained with a Delaware Histoknife on a Reichert Ultracut Ultramicrotome set at 0.35 μm and dried onto chrome-alum-coated slides on a slide-warming tray. Sections for light microscopic examination were stained for 10–30 s with 1% (*w*/*v*) toluidine blue in 1% (*w*/*v*) sodium borate on the warming tray, washed with distilled water, mounted with Permount, and observed with a Zeiss photomicroscope. Images were collected using an Olympus Q-Color 3 digital camera.

For immunocytochemistry, small circles (1/2–1 in. in diameter) were drawn with a wax pen on the cleaned and chrome-alum-coated slides, and sections were collected in that circle and allowed to dry down on a slide-warming table. Slides were placed in a humid chamber and 1% (*w*/*v*) bovine serum albumin (BSA) in phosphate-buffered saline, pH 7.2 (PBS–BSA) was added to the wax pencil circle, and the sections were incubated for 30 min. The PBS–BSA was decanted and primary antibody (Table 1) from neat to 1:20 dilution in PBS–BSA (depending upon the antibody) was added to each circle (a volume of ~100 μl), with controls consisting of a non-reactive serum or PBS–BSA alone. Slides were incubated in the primary antibody for 3–4 h. After the incubation, slides were washed three to four times with PBS–BSA. Secondary antibody (goat anti-rat IgG or goat anti-mouse IgG) coupled to 15 nm colloidal gold (EY Labs, San Mateo, CA, USA) was diluted 1:20 in PBS–BSA

Table 1 Antibodies used in these studies, their dilutions, and their specificities

Antibodies and dilutions	Recognition
Pectins	
JIM5 (neat)	Highly de-esterified homogalacturonans
JIM7 (neat)	Highly esterified homogalacturonans
LM5 (1:8)	1→4 galactan side chain of RGI
LM6 (1:8)	1→5 arabinan side chain of RGI
CCRC-M2 (1:8)	RGI, unknown epitope
CCRC-M10 (1:8)	RGI
CCRC-M22 (1:8)	De-arabinosylated RGI
CCRC-M38 (1:8)	Pectic mucilage, de-branched RGI
Xyloglucans	
CCRC-M1 (1:8)	Fucosylated xyloglucans
LM15 (1:8)	Xyloglucans, not fucosylation-dependent
Xylans	
LM11 (1:20)	Low substituted xylans
Arabinogalactan proteins	
CCRC-M7 (1:8)	AGP/RGI epitope
Extensin	
LM1 (1:8)	Extensin

and the slides were incubated for 1 h. After that incubation, the slides were washed twice in double-distilled water from a squirt bottle and silver-enhanced (IntenSE, Amersham or GE Biochemicals) for 15–30 min. The sections were washed with distilled water, dried with compressed air, and mounted with Permount before observation with a Zeiss photomicroscope.

Scanning electron microscopy

For scanning electron microscopy, small pieces were fixed in 6% (*v*/*v*) glutaraldehyde in 0.05 M PIPES buffer (pH 7.4) for 24 h at 4°C. Samples were washed in PIPES buffer and dehydrated in ethanol as described for light microscopy samples (above), except that the samples remained at 4°C until processed further. They were then critical-point dried in a Balzers critical-point drying apparatus (CPD030) and sputter coated with gold–palladium in a Hummer X coater to a thickness of 15 nm. Samples were observed with a JEOL 840 scanning electron microscope operating at 15 kV. Digital images were collected with a Kevex digital acquisition program.

Results

Gross morphology and scanning electron microscopy of surfaces

Catchweed bedstraw plants are trailing/scrambling plants with a four-sided stem interrupted by nodes with whorls of leaves and stipules (Malik and Vanden Born 1988; Defelice 2002). The leaves, stem and carpels surrounding the flowers/seeds (mericarps) are all covered in trichomes, with those on the stems angled down and those on the leaves angled mainly up relative to the long axis of the stem (Fig. 1a & b). Stem trichomes appear most frequently on the four corners of the stem, along the ridge of thickened tissue (Fig. 1b). Leaf trichomes are concentrated towards the leaf periphery, although there are some less frequent (and smaller) trichomes over the lamina (Fig. 1b). The trichomes on the leaves and mericarps are distinctly hooked, whereas those on the stems are not. Trichomes on bedstraw stems are generally broader and shorter (~100 vs. ~200 μm) than the hooked trichomes present on leaves and mericarps (compare Fig. 1a with b). Thus, virtually all the structures on this plant have some sort of trichome that could be used to cling to another object from some direction. Even in the material that we fixed, dehydrated and run through the critical point dryer, evidence of hairs and lint remained attached to these abundant trichomes (e.g. Fig. 1b), demonstrating just how effective a snare these trichomes are. The trichomes on the mericarp are extremely dense, essentially forming a solid cover over this surface. Moreover, the hooked portions of the trichomes are oriented in all directions so as to facilitate potential contacts with would-be agents for spreading of the seed.

As previously demonstrated (Gorb et al. 2003), the trichomes surrounding the flowering structures have a large hollow base, but at the hooked trichome tip, cell wall material fills in the lumen, making the tip/hook area completely cell wall (Fig. 1c & d). In contrast, the trichomes present along the ridges of stems have no hook at the top and the lumen goes right to the tip, as is typical of trichomes of other plants (not shown). Atrichoblasts at the base of the trichome are often swollen or enlarged in comparison with adjacent epidermal cells. Stem and mericarp trichomes, especially, were often broken from field-collected material, although at least portions of the highly thickened wall of the hook region remained intact.

Immunocytochemistry

We used a battery of antibodies, which recognize a large percentage of the wall components (Table 1), to determine the wall characteristics of mericarp trichomes of bedstraw. CCRC-series antibodies were purchased from Carbosource, Complex Carbohydrate Research Center, University of Georgia. The JIM-series and the LM-series antibodies were purchased from Plant Probes, UK. A section serial to the antibody-labeled sections was stained with toluidine blue (marked "TOL" in Figs. 2 and 3) to show all of the trichome components. These figures include a non-median section plane (Fig. 2, TOL) that emphasizes the sides of the

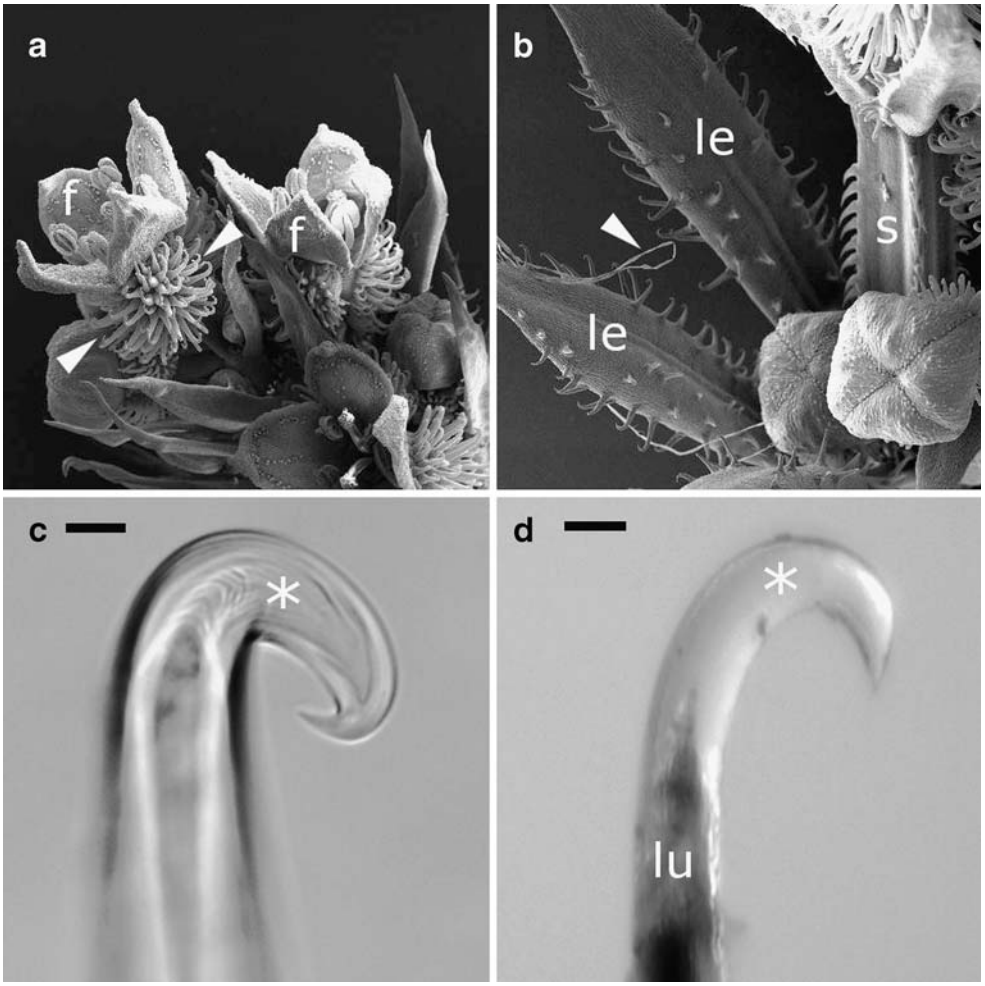

Fig. 1 Characteristics of the catchweed bedstraw plant, with **a** & **b** taken with a scanning electron microscope and **c** & **d** taken through a stereo microscope. **a** Low magnification view of the flowers (*f*) and subtending clusters of hooked tendrils that surround the flower and fruit (*arrowheads*). **b** Comparison of orientations and relative sizes of the various trichome types in catchweed bedstraw. Those on the stem (*s*) are oriented downwards, whereas those on the leaves (*le*) are oriented upwards. A strand of lint that was captured by the trichomes (*arrowhead*) and survived the fixation, dehydration and critical point drying, is connected to the sample. **c** & **d** Details of the trichome from the mericarp surrounding the flowers. The terminus and hooked area of the trichome is a solid wall (*), although the stalk of the trichome has a lumen (*lu*). *Scale bars* = 20 μm

hooked tip and a near-median section plane (Fig. 3, TOL), where the hook area is cut in almost perfect longitudinal section.

The reaction of trichome sections to pectin antibodies was extremely diverse, both in terms of intensity of reaction and the distribution of the epitopes within the trichome (Fig. 2). The antibodies JIM5 and JIM7 represent the two ranges of homogalacturonan esterification. JIM5 (which recognizes highly de-esterified homogalacturonans) was weakly reactive along the stalk of the trichome, but was enriched in material on the outer surface of the hooked tip. In contrast, JIM7, which recognizes more highly esterified homogalacturonans, labeled a zone of the wall adjacent to the plasma membrane and a smaller domain at the periphery of the trichome tip (Fig. 2).

The pattern was similarly complex for antibodies that recognize rhamnogalacturonan I (RGI) or side-groups of RGI. LM5 (which recognizes the 1→4 galactan side chain of RGI) was reactive only in the outer surface of the tip, whereas LM6 (which recognizes the 1→5 arabinan side chain of RGI) was reactive along the inner surface of the trichome wall and in the lumenal material at the tip (Fig. 2). These two antibodies produce a very similar pattern to that obtained with JIM7 but distinct from those of other RGI antibodies. CCRC-M22, an antibody to de-arabinosylated RGI, was strongly reactive with the plug material in the lumen of the tip of the trichome, and weakly reactive with the trichome stalk (except for material at the inner edge and on the outer edge of the hook). CCRC-M38, which recognizes an epitope present in pectic mucilages (possibly de-branched RGI; Bowling and Vaughn 2008a), strongly reacted with all areas of the trichome wall. In contrast, CCRC-M2, -M7 and -M10 antibodies reacted strongly with material at the plasma membrane/wall interface with weak (CCRC-M10) or no (CCRC-M2 and CCRC-M7) reaction with the wall proper. The reaction of CCRC-M7 may represent the ability of this antibody to label arabinogalactan protein (AGP) epitopes as well as RGI (CCRC web site; Freshour et al. 1996; Ligrone et al. 2002). Thus, these trichomes have very specific and diverse pectin types distributed uniquely throughout their wall.

Antibodies to other wall components also displayed unusual distributions and/or patterns (Fig. 3). Although thickened cells of the stem xylem and collenchyma

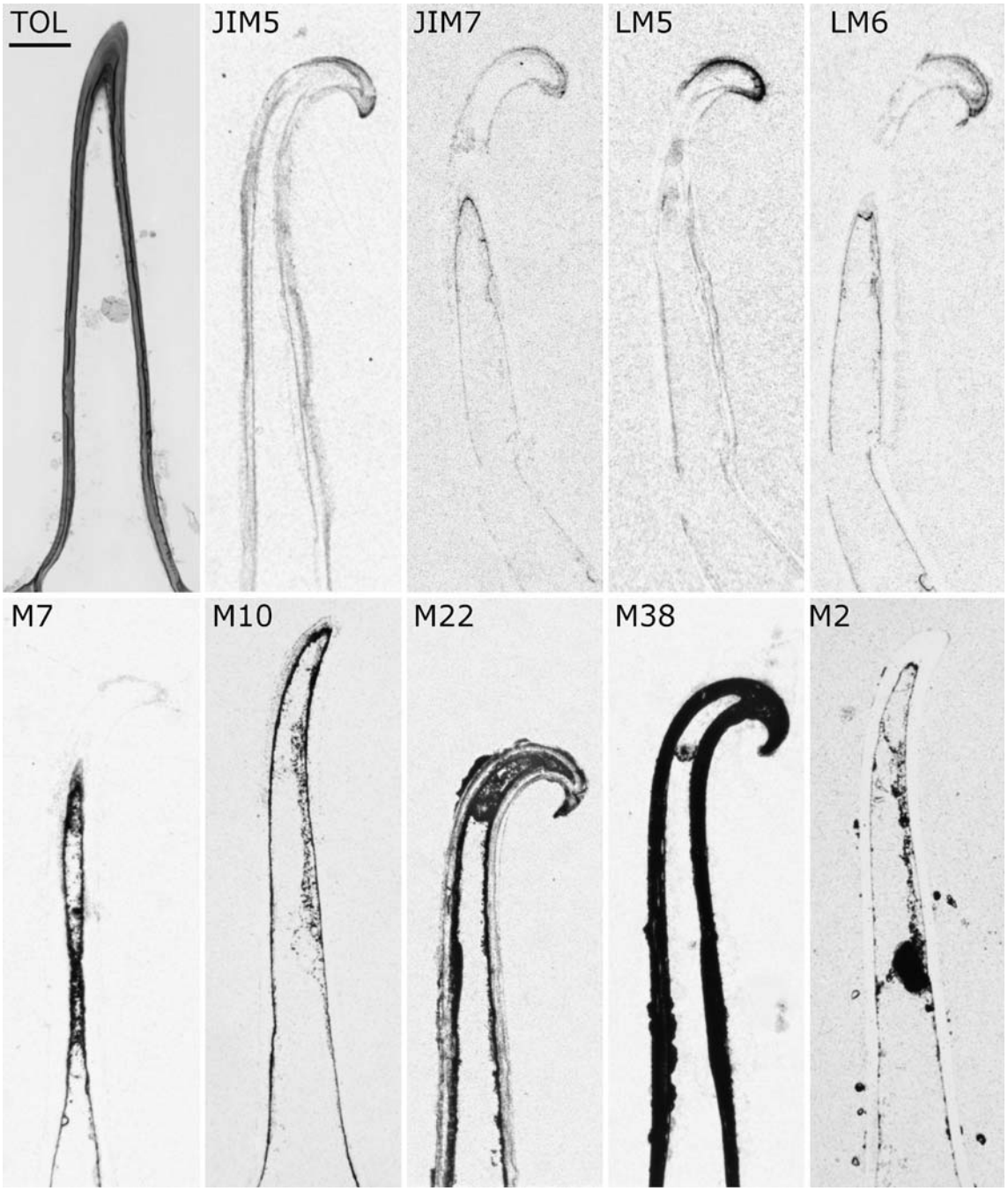

Fig. 2 Labeling of the hooked mericarp trichomes of catchweed bedstraw with antibodies that recognize various pectin epitopes and arabinogalactan proteins. A non-consecutive serial section labeled with the general stain toluidine blue (*TOL*) is shown for comparison. JIM5 reacts strongly with the walls and a band at the outside of the hook, whereas LM5, LM6 and JIM7 label only a small external region of the hook and weakly along the wall-plasma membrane interface. The RGI antibodies CCRC-M2, -M10, -M22, and -M38 each label a portion of the wall but each of the epitopes recognized is in a different domain within the trichome. The lumen of cytoplasm and vacuole does not extend into the tip, as vividly shown in the labeling by the CCRC-M7 arabinogalactan protein antibody. *Bar* = 50 μm

contained xylans (not shown), no reactivity to these epitopes was detected in the thickened trichome tip (Fig. 3, LM11). Likewise, antibodies to fucosylated xyloglucan (CCRC-M1) also failed to label the mericarp trichomes (not shown), although other cell types, including atrichoblasts, were labeled. Even the LM15 antibody that recognizes a broader group of xyloglucan epitopes (not specific for the fucosylated form) only bound strongly to wall material towards the tip region and not elsewhere in the trichome wall (Fig. 3). Extensins (detected with LM1 antibody) were also found in thickened cells and the tip region of the trichome, in similar locations to where JIM5,

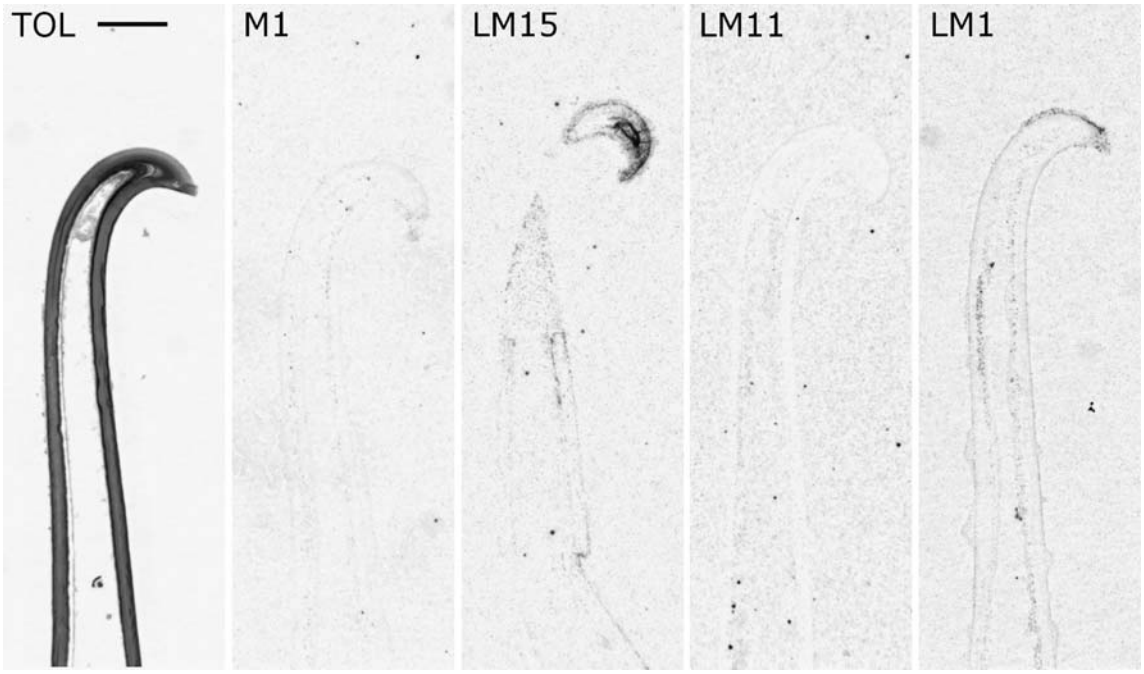

Fig. 3 Immunolabeling of xyloglucan (*M1* and *LM15*), xylans (*LM11*) and extensin (*LM1*). A serial section that has been stained with toluidine blue (marked "*TOL*") is shown for comparison. Unlike the pectin and arabinogalactan protein antibodies, none of these antibodies extensively label the trichomes, with the exception of a weak reaction of LM15 and LM1 towards the trichome tip. *Bar* = 50 μm

LM5 and LM6 epitopes are enriched. Thus, most of the traditional epitopes found in highly thickened cell wall types are not associated with the thickened cell walls of the bedstraw trichomes or are present only in restricted areas of the trichome.

Discussion

Abundance, shape and composition of trichomes promotes stickiness

Stems of catchweed bedstraw are well known for their ability to adhere to other structures. Most of the previous work on the trichomes of this plant has centered on those that surround the flowers and, subsequently, the seeds (Gorb and Gorb 2002; Gorb et al. 2003). These trichomes are hooked and have an obvious role in animal-based dispersal mechanisms. However, those on leaves are virtually identical in morphology and are no doubt important for the stickiness of bedstraw stems prior to the appearance of floral structures. Likewise, the trichomes that protrude from the stem are in a position where adherence would be greatest and they are oriented in a direction opposite to those on the leaves, thus catching objects in the opposite direction. Considering how strong the hooked trichomes are (Gorb and Gorb 2002), it is surprising that they have walls so enriched in pectin. However, these walls are also heavily thickened compared to other epidermal cells and the hook portion itself is solid.

In addition to the structure of these trichomes, their unusual composition might also facilitate stickiness. In the field-collected samples of bedstraw stem and mericarp trichomes, the tips of the hooks were occasionally observed to have broken (not shown), and it is this tip that is highly enriched in the very sticky de-esterified pectins and pectic mucilages. Thus, the ability to break on contact, exposing a sticky wall, could expedite the sticking of another organism or hair to the bedstraw. Although catchweed bedstraw has been compared to Velcro™ (Gorb et al. 2003), it appears that bedstraw is actually better in that it not only has the grappling effect of the hooks on the leaf and flower/fruit trichomes like Velcro™, but it also has a sticky substance as well as another trichome type oriented in a different direction to the hooked trichomes.

Unusual aspects of cell wall composition in bedstraw

The labeling of various pectin antibodies on catchweed bedstraw trichomes is both qualitatively and quantitatively unique. Although one of the epitopes, recognized by CCRC-M38, is found throughout the entire trichome and is bound strongly by the antibody at all sites, each of the other pectin antibodies recognizes limited regions of either the trichome wall or hooked tip. The greatest number and diversity of these various epitopes are present in the hook

region itself, where a wall of both unusual thickness and shape is formed. Neither hooks nor solid lumens have been reported for other trichomes (Fahn 1974; Evert 2006). The totally solid nature of these trichomes may be the reason that xyloglucans are not required for wall strength. That is, the highly rigid nature of cell walls composed of xyloglucan and/or xylan and cellulose may be too rigid to undergo the rather radical bend required to form the hook. Rather, a highly diverse pectin assemblage is needed to construct such an unusual wall. Highly de-esterified pectins are present along the wall-cuticular wax boundary of the wall of developing cotton fibers (Vaughn and Turley 1999) and cotyledons of *Vicia faba* (Vaughn et al. 2007). In these cases, the highly de-esterified pectins form a thin layer that may be involved in interactions with the surface waxes. In the highly curved hook region of the bedstraw mericarp trichome, such an attachment between wall and wax would be critical and may explain the presence of the JIM5 epitopes on that surface of the trichome. Reactivity with other antibodies to homogalacturonans (JIM7), RGI (LM5, LM6 and M22), and extensins (LM1) is also enriched in this region. Whether these epitopes have functions related to the binding of wax or to the unusual curved architecture of this area is unknown.

We have seen a similar co-localization of extensin and highly de-esterified pectins in cotton fibers (Vaughn and Turley 1999) and in walls of cellulose-deficient, dichlobenil (DCB)-habituated BY-2 cells (Sabba et al. 1999). In the latter case, the association of extensins and de-esterified homogalacturonans can substitute for the normal cellulose-xyloglucan cell wall. Although we thought at the time that the DCB-habituated cell wall was a unique occurrence, it is possible that homogalacturonans and extensins produce a relatively strong wall in these trichomes as well.

As we learn more about the nature of the epitopes recognized by each of these antibodies, it may become more obvious why a given pectin epitope is so critically placed in each region of these trichomes. Heterogeneity of pectin epitopes exists in other cases, such as in roots of *Arabidopsis*, with LM6 epitopes predominating in the meristem and LM5 in the zone of cell elongation (McCartney et al. 2003). Why the cells require this difference in pectin epitope distribution is also unknown. However, what all these studies point out is that slight variations in pectin composition influence wall properties in unique ways. As cellulose and xyloglucan are relatively much more invariant, it may be that it is the pectins that determine the majority of the properties of the primary wall. In the case of the hooked trichome, the wall must be bent in a very atypical fashion, be plugged to become solid, and yet also thicken to a structure approximately 30 μm thick. None of these are characteristic of the more typical parenchyma cells, nor are they characteristic of other thickened cells, such as xylem elements, which generally have large amounts of xylans (e.g. Vaughn 2006; Meloche et al. 2007).

Originally, the prevailing concept of the plant cell wall was of a relatively invariant cellulose-xyloglucan matrix swimming in a sea of pectins and a few other polysaccharides and proteins. With the aid of a bevy of new antibodies, we are beginning to see that there is a tremendous microheterogeneity of walls, in that certain tissues or cell types have a complement of polysaccharides that can differ significantly even from adjoining cells. In our earlier study of water-conducting cells in bryophytes (Ligrone et al. 2002), we found that even within a single type of cell, the polysaccharides a given species uses for the construction of a certain type of cell wall can vary widely. Our current concept is that the plant cell has the ability to synthesize a number of polysaccharides. Adjusting the quantities and distribution of these various polysaccharides allows the plant to confer upon the wall various attributes that allow it to make cells with unusual shapes and compositions, and further, allows the plant to make distinct tissues and organs.

Acknowledgements Andrew J. Bowling was supported in part by the USDA-ARS Research Associate program funding. We thank the many visitors to our poster at the Weed Science Society of America meeting, especially A. DiTommaso, for their many comments on this species and the nature of trichomes in this and related species. Dr. Jarrod Leland graciously allowed the use of his stereo microscope and camera for the examination of living specimens. J. Paul Knox generously provided LM15 antibodies for this study. Production of the CCRC monoclonal antibodies was supported by a NSF grant to Michael Hahn, Complex Carbohydrate Center, U Georgia. Mention of a trademark, proprietary product or brand does not constitute an endorsement of that product or brand by the USDA.

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