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Structural and immunocytochemical characterization of the adhesive tendril of Virginia creeper (*Parthenocissus quinquefolia* [L.] Planch.)

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Summary. The tendrils of Virginia creeper (*Parthenocissus quinquefolia*) do not coil around their supports. Rather, they adhere to supporting objects by flattening against the support surface and secreting an adhesive compound which firmly glues the tendril to the support. In this study, microscopic and immunocytochemical techniques were utilized to determine the nature of this adhesive. Following touch stimulation, epidermal cells of the tendril elongate toward the support substrate, becoming papillate in morphology. Following contact with the support surface, an adhesive is produced at the base of the papillate cells. The adhesive appears as a highly heterogeneous, raftlike structure and consists of pectinaceous, rhamnogalacturonan (RG) I-reactive components surrounding a callosic core. In addition, more mobile components, composed of arabinogalactans and mucilaginous pectins, intercalate both the support and the tendril, penetrating the tendril to the proximal ends of the papillate cells. Following adherence to the support, the anticlinal walls of the papillate cells are devoid of RG I side-chain reactivity, indicating that extensive debranching of RG I molecules has taken place. Furthermore, a large amount of RG I backbone reactivity was observed in the contact area. These results may indicate that the debranched RG I molecules diffuse into and permeate the contact region, forming an integral part of the adhesive compound. These results indicate that Virginia creeper adheres to objects by a composite adhesive structure consisting of debranched RG I, callose, and other, less-well characterized mucilaginous pectins and that this structure subsequently becomes lignified and very weather-resistant upon the ultimate senescence of the tendril.

Keywords: *Parthenocissus quinquefolia*; Adhesive; Tendril; Vine; Rhamnogalacturonan I; Immunocytochemistry.

Abbreviations: AGP arabinogalactan protein; RG I rhamnogalacturonan I fraction.

Introduction

Vines have evolved several different mechanisms for utilizing stationary objects in order to compete for sunlight without investing energy in a large woody trunk (Darwin 1875, Jaffe and Galston 1969). The three most prominent climbing mechanisms are twining stems, coiling tendrils, and adhesive organs (tendrils or adventitious roots). A plant may also use more than one of these mechanisms to scale an object. For example, redvine (*Brunnichia ovata*) produces coiling tendrils that, once securely wrapped around an object, produce adhesive compounds that cement the tendril in place (Meloche and Vaughn 2008). Twining stems and coiling tendrils are limited in the diameter of objects they can ascend (Scher et al. 2001; Vaughn pers. obs.), whereas vines which use the adhesive mode of attachment are able to climb large-diameter and flat objects which pure tendril climbers cannot ascend. Despite the large number of species using adhesives alone or in conjunction with coiling, there have been few studies on these tendrils or this very effective adhesive (Endress and Thomson 1976, 1977), one that must support many kilograms of vine as it ascends an object.

Several members of the genus *Parthenocissus* (most notably Boston ivy and Virginia creeper) possess small, forked tendrils that develop flattened adhesive discs at their tips upon contact with a surface suitable for attachment. Earlier studies have suggested that there is a substantial amount of polyphenols present in the cells of the young (nonadhered) tendril (Endress and Thomson 1976) and that the adhesive substance in fully matured tendrils is most likely composed of an acidic mucopolysaccharide (Endress and Thomson 1977). The reagents used in those studies (e.g., ruthenium red, thorium) are general ones that recognize classes of compounds, not individual components, however. Since that time, significant advances in our ability to identify the components of plant cell walls

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via microscopic techniques have occurred, so now virtually every major and many minor components can be localized with either affinity probes or various antibodies (Vaughn et al. 1996, Sabba et al. 1999, Meloche et al. 2007). In this report, we use modern immunocytochemical methods to characterize this adhesive, as well as the changes in the cell wall composition that occur just prior to, and just after, contact with a surface is established. Our data indicate that the adhesive may be produced from the selective modification and remobilization of wall components of the papillate cells, primarily a debranched rhamnogalacturonan (RG) I, and a limited synthesis of new components as to form a complex and effective adhesive.

Material and methods

Plant material

Tendrils of Virginia creeper (*Parthenocissus quinquefolia*) were harvested from plants growing in their native environment. In preparation for this experiment, sheets of nylon-backed nitrocellulose membrane were fastened to the supporting structure just above elongating Virginia creeper stems. The Virginia creeper then produced adhesive pads that adhered to the nitrocellulose rather than the support wall, allowing their nondestructive removal for microscopy. Whole tendrils with adherent nitrocellulose were severed from the plant and immediately placed into fixative. Both mature and immature tendrils with adhesive pads were analyzed.

Light microscopy

Protocols for microscopy and immunogold and immunogold-silver labeling were as described in Meloche et al. (2007). Briefly, tendrils (with adherent nitrocellulose) were fixed in 3% glutaraldehyde overnight, dehydrated in ethanol at 4 °C (25 and 50% for 2 h each, 75% overnight, and absolute overnight). Following dehydration, specimens were infiltrated with London Resin White resin at 25, 50, 75, and 100% for 24 h each, at −20 °C. Specimens were placed on a rocker at room temperature for 24 h and then polymerized at 60 °C. Sections were stained with 1% toluidine blue and imaged on a Zeiss Axioskop with an Olympus Q-color 3 digital camera.

Immunogold-silver light microscopy

Semithin sections (350 nm) were blocked with 1% (w/v) bovine serum albumin (BSA) in phosphate-buffed saline (PBS) for 30 min at room temperature. Primary antibody was applied to sections (at various dilutions in PBS-BSA) and incubated in a moist chamber for 3 h. Sections were rinsed and incubated with secondary antibody (15 nm diameter gold; E-Y Labs, San Mateo, Calif., U.S.A.) diluted 1 : 20 in PBS-BSA for 1 h at room temperature. Sections were silver-enhanced (IntenSE, Amersham Scientific) for 20–30 min at room temperature.

Transmission electron microscopy

Material for standard transmission electron microscopy analyses was as described in Meloche et al. (2007). Briefly, tissues were fixed in 6% (v/v) glutaraldehyde with the addition of 1% (w/v) caffeine to precipitate phenolics, postfixed in 2% (w/v) osmium tetroxide and stained en bloc with 2% (w/v) uranyl acetate. After an acetone dehydration, samples were embedded in a 1 : 1 mixture of Spurr and Epon resins, using propylene oxide as the transition solvent. Samples with pale gold-silver reflectance were mounted on uncoated copper grids and examined with a Zeiss EM10CR electron microscope at 60 kV.

Immunogold transmission electron microscopy

Sections with pale gold reflectance (ca. 100 nm) were collected from block faces of the same material used for light microscopy and mounted on uncoated 300-mesh gold grids. The grids were floated, specimen side down, as described by Meloche et al. (2007). A minimum of four grids were examined for each antibody treatment.

Results

Structure of the Virginia creeper tendril

Virginia creeper tendrils undergo a remarkable transformation from the free-growing tendril to one in contact with the nitrocellulose-coated substrate. Prior to contact with a substrate, the forked tendrils are elongated, with a small pad at their tip (Fig. 1A). These younger tendrils are isobilateral in cross section (Fig. 1C). A layer of epidermal cells surrounds a relatively undifferentiated central cortex region with a small vascular bundle present in the center of the tendril. Following contact with a substrate, these small pads flatten against the substrate and become strongly adhered (Fig. 1B). In fact, early attempts to collect tendrils adhered to a painted wall resulted in the removal of a patch of paint (not shown). Subsequently, we used nitrocellulose backed with nylon to facilitate the removal of an intact tendril–substrate surface. The epidermal cells in direct contact with the substrate divide and elongate exceptionally to become papillate cells (Fig. 1D for an early stage, Fig. 1E for maturity). The papillate cells change morphology to mimic the shape of the object to which it adheres; in this example, the nitrocellulose-coated wall. Along the interface of the nitrocellulose and the adhesive pad, areas of extracellular deposits strongly stained with toluidine blue may be identified that are the adhesive (Fig. 1E, F). Within the zone of adhesive, pockets of material that stain a distinctive shade of turquoise blue after toluidine blue staining (indicative of phenolics) occur as large, distinct deposits (Fig. 1F). In the adhered tendril, gelatinous fibers occur as a cluster in the center of the organ, internal to the vascular strands (Fig. 1E).

Electron microscopic observations indicate a further complexity of the zone including the adhesive and papillate cells. Papillate cells contain an unusual raft of wall material at the base of the cells, proximal to the nitrocellulose (Fig. 2A). The depth of the raft varies by cell and section but can be as deep as 3 μm as determined from longitudinal sections through this zone. In the sites of

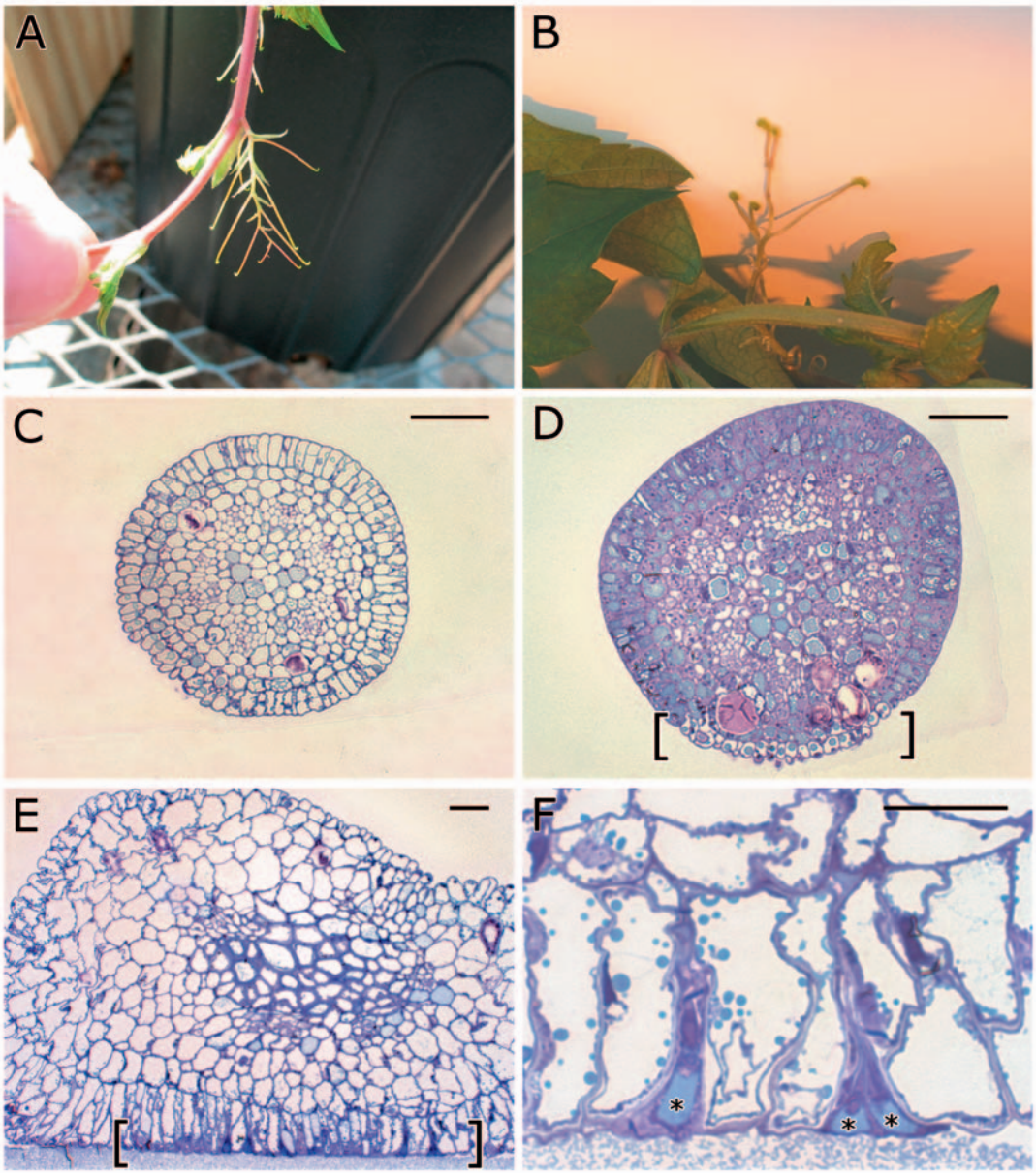


Fig. 1. Light micrographs of young (**A**, **C**, and **D**) and mature (**B**, **E**, and **F**) Virginia creeper tendrils. **A** Virginia creeper tendrils before contact with a substrate showing forked morphology of branchlet. **B** Tendrils fully adhered to a nitrocellulose-covered substrate. Note flattened tips of tendrils. **C** Young tendrils are isobilateral in cross section. **D** After touch stimulation, a small group of differentiated cells and an overall shape change may be noticed (brackets). **E** After the tendril has made contact, a zone of papillate cells (brackets) develops along the edge of the tendril in contact with the support. **F** Within and between the papillate cells, an extensively stained and heterogeneous zone of adhesive is noted (asterisks). Bars: 50 μm

contact between nitrocellulose and raft, the raft material appears to accommodate the shape of the nitrocellulose (Fig. 2B). The raft is very heterogeneous, both from cell to cell and internally, although often divided into zones with similar appearance. In general, the raft is composed of as many as 5 distinct regions, from the most distal: (1) a finely granular wall with small pits of a less opaque material; (2) a much less electron-opaque area intercalated by dense areas (Fig. 2C); (3) a platelike area with thin ($>$0.1 μm) bands of relatively low opacity in a more dense but homogeneous background (Fig. 2C); (4) a very electron-opaque area containing aggregates of material that appear to be phenolics; and (5) the pitted primary wall of the papillate cell. A cuticular layer was generally found on cells prior to contact, although there was often no clear cuticle after the papillate cells had made full contact with the nitrocellulose. In addition to the raft material, extracellular deposits of only slightly greater density

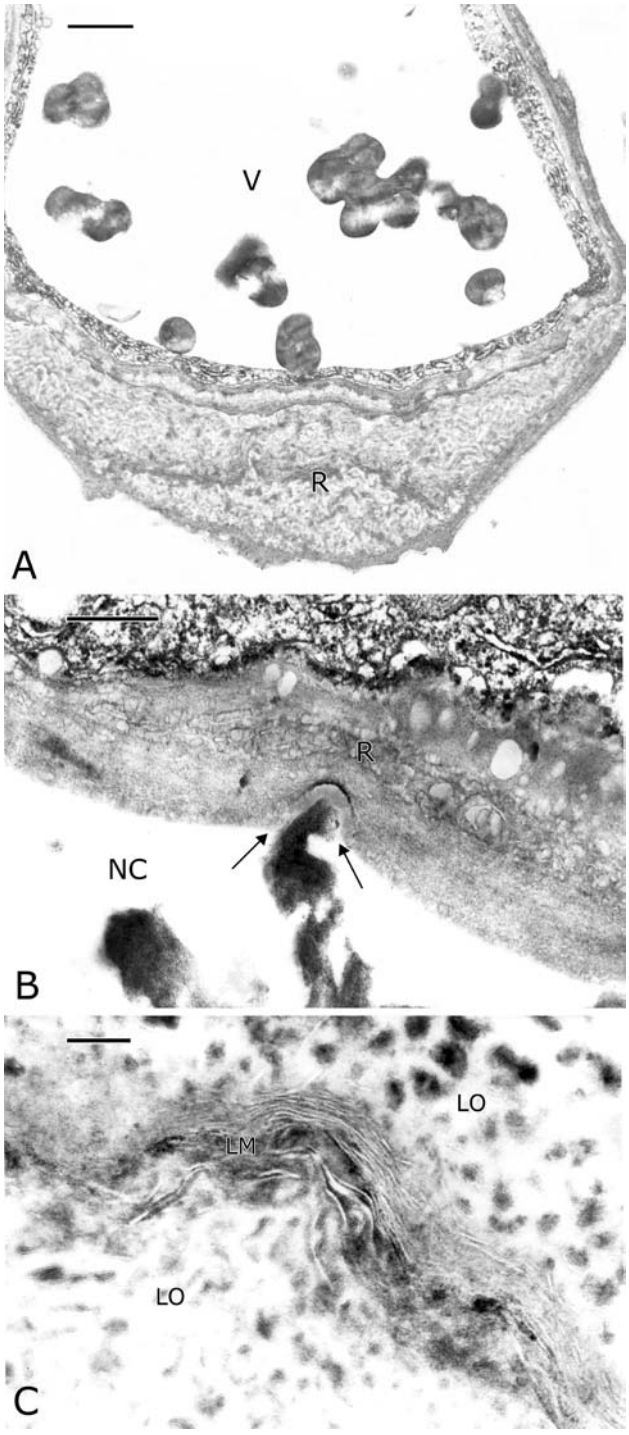


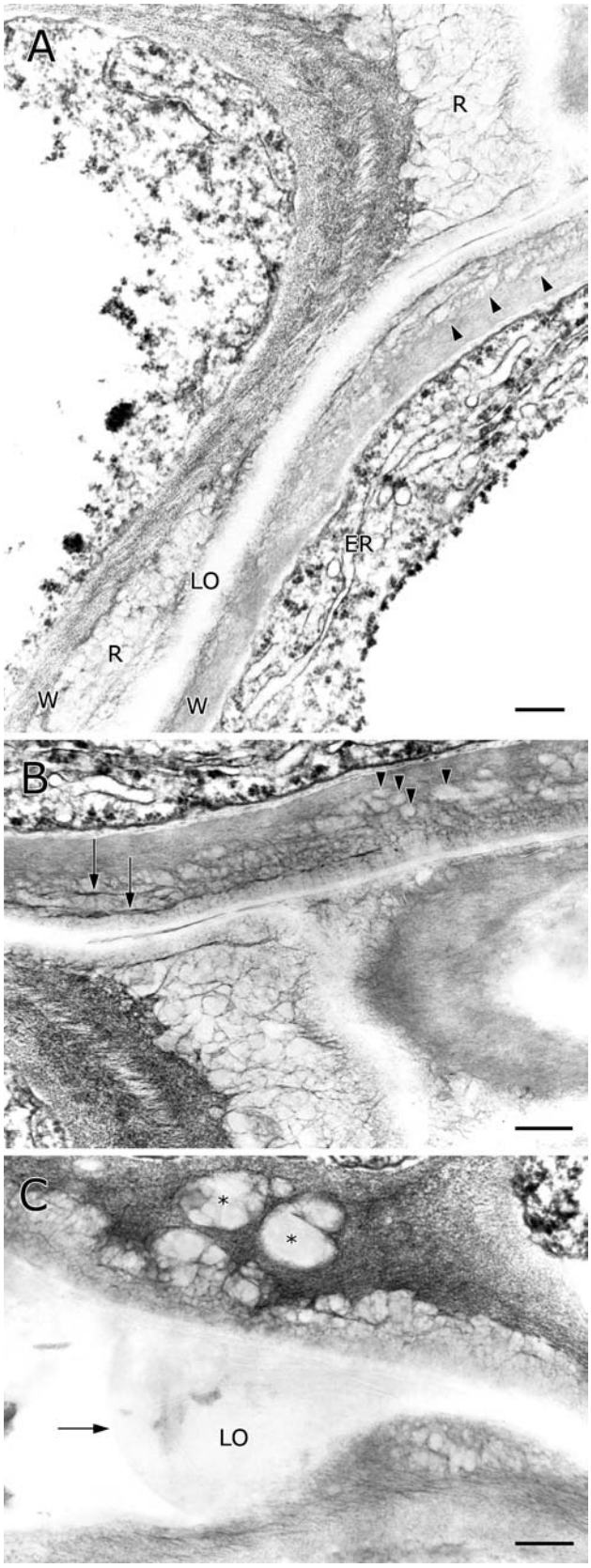
Fig. 2 A–C. Electron micrographs of papillate cells. **A** A low-magnification micrograph of a papillate cell just before contact with the nitrocellulose. Note the raft (*R*) of highly heterogeneous wall and adhesive material at the base. An extensive layer of endoplasmic reticulum can be seen between the vacuole and the plasma membrane. *V* Vacuole. **B** Accommodation of the raft material (*R*) to the nitrocellulose (*NC*). The raft material appears to be soft enough so that it is indented by the nitrocellulose (arrows). **C** Details of the lamellate material (*LM*) that runs through low-opacity zone (*LO*) of the raft. Long filaments of electron-translucent material run through a more electron-opaque background. In the low-opacity layer, globular deposits of highly electron-opaque material are observed. Bar: A, 1 μm; B, 0.5 μm; C, 0.2 μm

than the plastic were noted that not only draped the bases of the papillate cells (Fig. 3A, C) but also spilled into spaces between the papillate cells and into the nitrocellulose as well. This material has a different shape and opacity than the cuticle and is almost impossible to discern in sections that are processed for immunocytochemistry (see below), possibly because it has an opacity similar to that of the plastic. Furthermore, for specimens processed for ultrastructure, this material is not rendered substantially more electron opaque by osmium, uranyl acetate, or lead citrate. Occasionally, this material shows lamellate structures within the larger homogeneous deposits.

Aside from the rafts at the base of the papillate cells, the walls and intercellular spaces of these cells are also unusual. For example, the anticlinal walls are distinctly pitted, often with clear areas, or threadlike areas, where wall material has obviously been distended and/or material lost (Fig. 3A–C). In some cases, the voided areas of wall are of substantial size (>2 μm in length), are circular in outline, and are bordered with very electron-opaque fibrillar wall material. Especially towards the outer tangential wall end of the anticlinal wall, the fibrils appear to be oriented in a regular manner. It is possible that these apparent voided areas might be areas where new material of low opacity has been inserted, rather than truly areas with no wall material. The papillate cells have virtually no middle lamellae nor plasmodesmata connecting them to adjacent papillate cells, although strands of wall material (probably residual middle lamellae) are sometimes observed between the cells. Within the cell, the cytoplasm is rich with ribosomes, Golgi bodies, and smooth endoplasmic reticula. Plastids contain prominent protein bodies. Small vacuoles contain globules of very electron opaque material (Fig. 2A). All material in the cell seems to be concentrated toward the outer tangential wall adjacent to the raft, generally with the central vacuole displaced away from the raft material.

Immunocytochemical determination of the cell wall

A battery of antibodies was used to probe sections of Virginia creeper tendrils at the tissue level (Table 1). Although many of the antibodies were observed to bind to some part of the cells of the contact face, only a few of these antibodies appeared to bind more heavily to the papillate cells and the raft material than to the other cells of the tendril. Four of these are shown in Fig. 4A–H. These antibodies fell into two classes: those raised to *Arabidopsis thaliana* seed coat mucilage (CCRC-M34 and CCRC-M36) and those raised to RG I (CCRC-M2 and



CCRC-M22). The pattern of binding appears different for each of these antibodies, with some recognizing relatively few domains, while others reacted with virtually all areas. Additionally, three antibodies bound to the walls of all of the cells in the tendril except the walls of the cells of the contact face. These antibodies recognize side chains of RG I such as 1,4-β-D-galactose oligomers (LM5, Fig. 4I), 1,5-α-L-arabinose oligomers (LM6, Fig. 4J), and methyl-esterified homogalacturonan (JIM7, not shown). Antibodies that recognize arabinogalactan proteins (AGPs), such as CCRC-M7, JIM8, and JIM13, were also enriched in the adhesive zone. However, all antibodies recognized other cell components, some in the wall and some in small vacuoles which were present in the papillate cells and in cells away from the touched surface. In contrast, antibodies to xyloglucan (CCRC-M1) labeled all cells relatively uniformly but did not label the adhesive interface.

Transmission electron microscope immunolocalizations were used to confirm the nature of some of the labeling patterns obtained at the light microscopic level with the immunogold-silver technique and also to probe sections with antibodies where only limited amounts of serum or ascites fluid was available (Figs. 5 and 6). As predicted by the heterogeneous appearance of the raft, this wall material is also highly heterogeneous in composition, although there does seem to be some order in terms of which polysaccharides occupy each layer of the raft. Callose (as detected by monoclonal anti-callose) occupies the less opaque zone intercalated by darker particles and seems to be concentrated on the less opaque material (Fig. 6A). Several of the pectinaceous mucilage antibodies label the zones surrounding the callose layer, with ones such as CCRC-M36 present only in the outermost layer (Fig. 6B). Antibodies to RG I (CCRC-M2, CCRC-M22) label these same areas but also material in all layers of the raft with the exception of the callosic layer (Fig. 6C). Antibodies

Fig. 3 A–C. Ultrastructural aspects of the side wall breakdown of papillate cells. **A** The side walls (*W*) between two papillate cells appear pitted and extracted. Much lighter, ovular areas (arrowheads) are found throughout the side walls of the papillate cells. Some areas of particularly extensive breakdown appear almost reticulate (*R*). The middle lamella region between the two cells now seems to be filled with a material that has a very low electron opacity (*LO*). **B** A higher magnification image of **A** showing details of the ovular-shaped areas (arrows) and a patch of reticulate wall. The wall containing the ovular areas appears somewhat lamellate. The clear areas which appear between successive lamellae (arrowheads) may be devoid of wall material. **C** Very large lesions in the side walls are noted (asterisks). A material of very low electron opacity (*LO*) also accumulates between the walls of the papillate cells and sometimes extends into the space between the papillate cells and the nitrocellulose support. Bars: 0.25 μm

Table 1. Antibody labeling of tendril tissue

Antibody	Antigen	Antibody binding to:			Other binding
		Contact face	Targeted vesicles	Other walls	
JIM5	de-esterified pectins	+[a]	−	(+)[b]	Binds to walls of touch-stimulated cells, but a very minor component of these walls
JIM7	highly esterified pectins	−[c]	−	+	Depleted from walls of contact face cells and from gelatinous fibers/vascular tissue
JIM8	lipophilic AGPs	+	+	+	Binds to inner wall/plasma membrane of all cells and to the contents of targeted vesicles
JIM12	extensin	−	−	−	No binding
JIM13	AGP	+	+	+	Strongly binds to contents of targeted vesicles, the inner wall/plasma membrane of all cells, and the middle lamellae of vascular and g-fibers
JIM19	unknown cell surface antigen	(+)	−	−	Very small amount of binding to contact face
JIM20	extensin	−	−	−	No binding
JIM132	Zinnea tracheary element walls	−	−	−	No binding
LM1	extensin	−	−	−	No binding
LM5	RG I galactan side chains	−[c]	−	+	Depleted from contact face cells
LM6	RG I arabinan side chains	−[c]	−	+	Depleted from contact face cells
LM7	partially ME-HG (non-blockwise)	(+)	(+)	−	Very light labeling of some vesicles; no labeling of walls
LM10	(unsubstituted) xylan	−	−	+	Heavy labeling of xylem and gelatinous fibers
LM11	xylan (arabinan-substituted)	−	−	+	Heavy labeling of xylem and gelatinous fibers
CCRC-M1	fucosylated xyloglucans	+	−	+	Heavy labeling of all walls
CCRC-M2	some RG I's	+[a]	−	(+)	Very heavy labeling of adhesive, plasma membrane, and PM-associated vesicles
CCRC-M7	AGPs	(+)	−	+	Mostly labels vasculature/gelatinous fibers and the walls of some epidermal cells
CCRC-M10	RG I	−	−	+	Labels inner wall–plasma membrane interface and some vesicles, but in vascular area only
CCRC-M22	RG I (backbone?)	(+)[a]	+	+	Heavily labels inner wall and plasma membrane and (usually) membranes of targeted vesicles; labels a (minor) component of the adhesive
CCRC-M31	Arabidopsis seed coat mucilage	(+)	−	−	Labels some (isolated) cells of the contact face
CCRC-M34	Arabidopsis seed coat mucilage	+[a]	+	+	Labels walls of the contact face, the middle lamellae of gelatinous fibers/vasculature, and some small targeted vesicles
CCRC-M36	Arabidopsis seed coat mucilage	+[a]	−	+	Inverse of LM5 and LM6; light labeling of other walls
CCRC-M38	Arabidopsis seed coat mucilage	+	−	+	Heavily labels all walls

[a] Antibody binds preferentially to material of the contact face
[b] (+), antibody binding weakly present
[c] Antibody binding dramatically reduced only in the contact face area

raised to AGPs label the outer layers of the raft, the opaque areas within the callosic layer, and areas between cells and continuing into the nitrocelulose (Fig. 5). Notably, none of these antibodies label the globular electron-opaque material that is thought to be phenolic on the basis of toluidine blue staining (Fig. 1C, D).

Aside from the labeling of the raft, several but not all of the antibodies label areas between the cells and even into the spaces between nitrocellulose strands, as is suggested in some of the immunogold-silver localizations at the light-microscopic level. The anti-AGPs label the distal ends of the papillate cells and include label between the papillate cell and progressing 50–100 μm deep into the nitrocellulose (Fig. 5). The gold particles appear to be in spaces, not on the particles themselves, indicating that this material is not merely adsorbed to the nitrocellulose, but rather fills the pores of the substrate. Label does not go deeper than 200 μm from the contact face between the adjacent papillate cells, however. Even the antibodies raised to pectinaceous mucilages do not all label in these spaces. Some, such as CCRC-M36, label areas confined to the outermost layer of the raft (Fig. 6B), whereas other epitopes appear to regularly label nitrocellulose and intracellular spaces (Fig. 5). Thus, the most mobile adhesive material is of a different composition than the less mobile material present solely in the rafts.

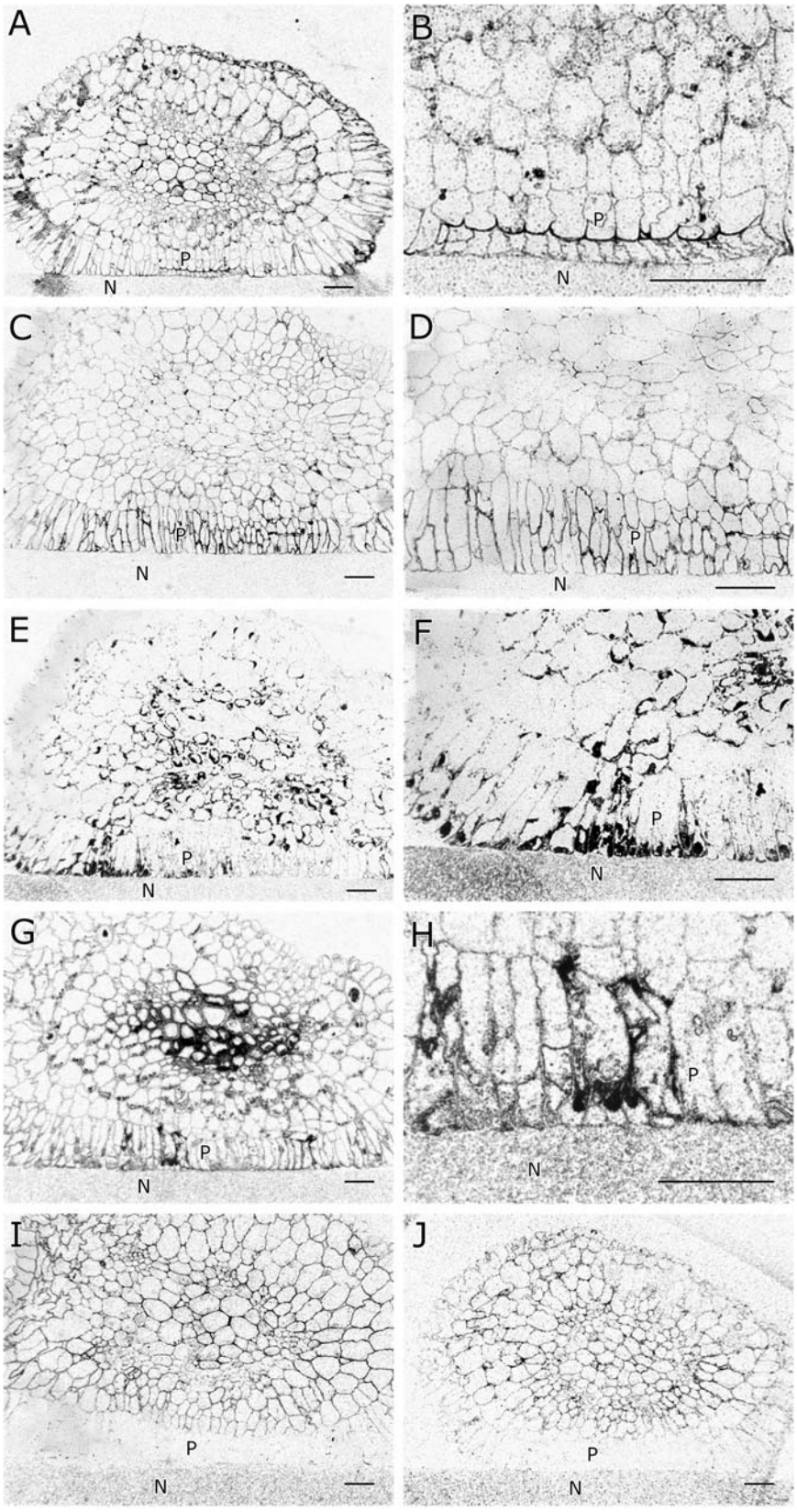


Fig. 4 A–J. Immunogold-silver localizations of wall epitopes in sections from the same block face as those shown in Fig. 1C and D. Although the antibody to RG I side chains (LM5) shows weak labeling in the papillate cells (*P*), antibodies to the RG I chain itself (CCRC-M2 and CCRC-M22), and antibodies to pectinaceous (RG I-type) mucilage (CCRC-M34, CCRC-M36, CCRC-M38) are highly enriched in the papillate cells and/or the adhesive layer. In addition, antibodies to AGPs are enriched in both the papillate cells and the adhesive. Bars: 50 μm

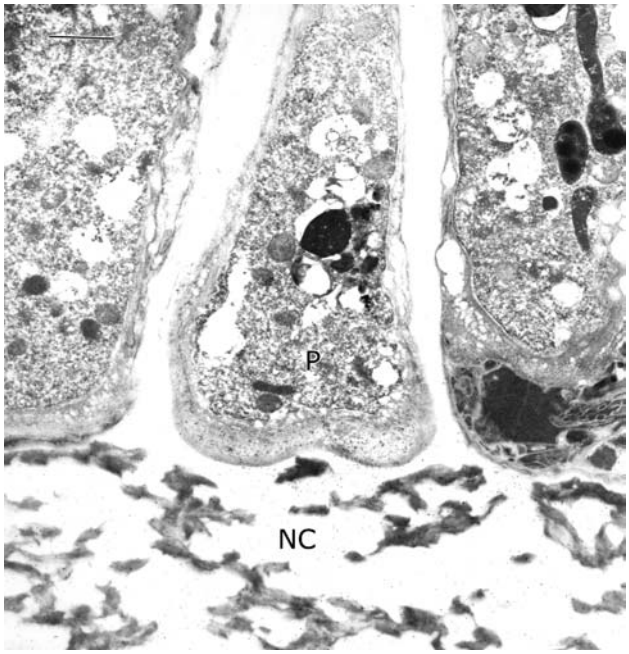


Fig. 5. Immunogold localizations of AGPs in the papillate cells of Virginia creeper. The papillate cells (*P*) are strongly labeled towards their base and including the raft at the bottom of the cells, but are impoverished at areas away from the contact zone. In addition, AGP reactivity is found between the cells and escaping for almost 100 μm into the nitrocellulose (*NC*). Bar: 1.0 μm

Discussion

Nature of the adhesive

Virginia creeper tendrils adhere to objects with a 3 μm thick raft of sticky polysaccharides that accumulate at the point of contact between the substrate and the papillate cells. In addition, some more mobile components intercalate intercellular spaces and even interstices in the substrate. This unique binatured system allows firm points of contact between the papillate cells and a large zone of cementing activity that extends into surfaces that would be too small for the papillate cells to penetrate. In the case studied in this report, even the tiny pores in the nitrocellulose membrane (ca. 100 μm) are filled in with several sorts of polysaccharide, as indicated by immunocytochemical localization of these epitopes. Moreover, the shape change that occurs in the papillate cells to mirror the surface of the substrate, and their ability to deform their walls along irregularities in the surface, facilitates their adhering to the substrate.

The data from the immunocytochemical studies gives us the strongest indication as to the molecular composition of the adhesive. Previous attempts using more general histochemical stains (Endress and Thomson 1977) gave

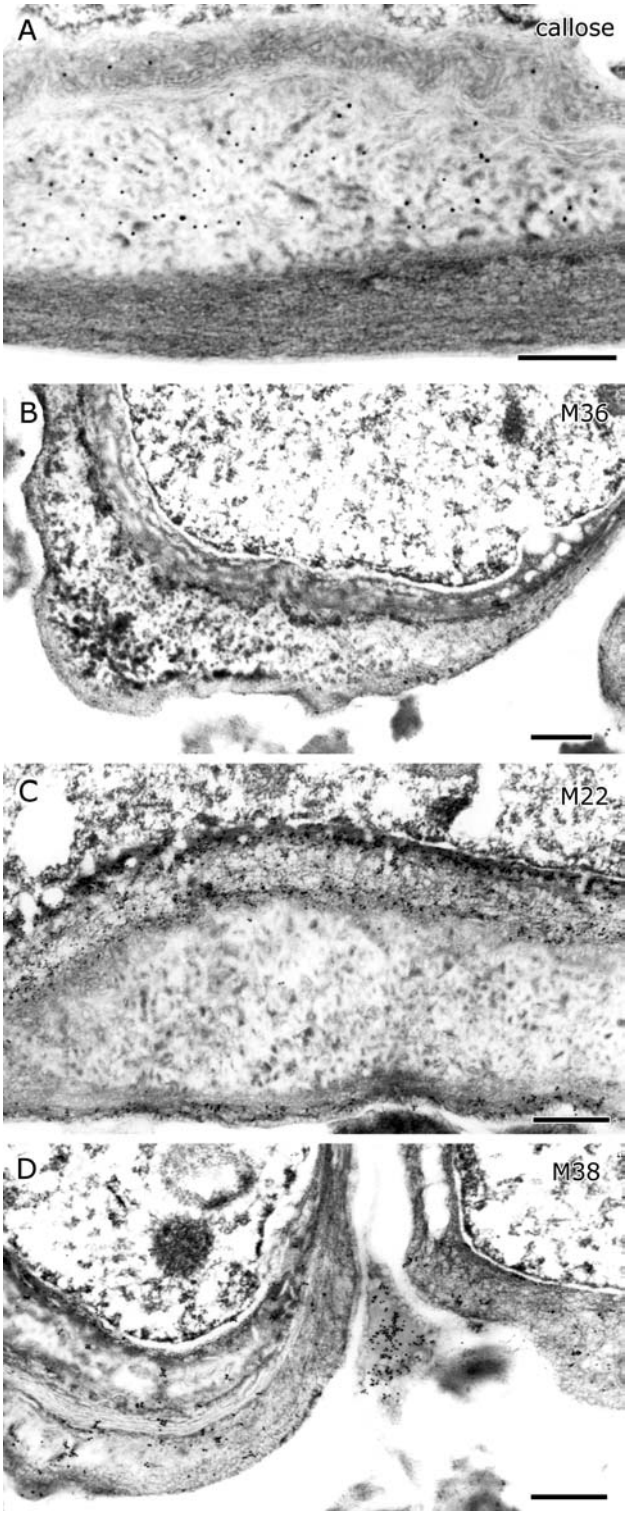


Fig. 6 A–D. Immunogold localization of various polysaccharide epitopes in the raft area of papillate cells. **A** Callose label is present in the zone of low electron opacity. **B** CCRC-M36 labels just the edge of the raft tissue, but no other layers within the structure (arrowheads). **C** CCRC-M22 labels all of the layers of the raft with the exception of the low-opacity (LO) zone. **D** CCRC-M38 labels areas similar to those labeled by CCRC-M22, even though this antibody was raised to mucilage rather than to RG I directly. Bars: 0.5 μm

only an indication of the sorts of molecules that might be involved in the adhesive. Rafts are highly labeled with antibodies that recognize RG I epitopes (CCRC-M2, CCRC-M22) and AGPs (JIM8, JIM13, AGP monoclonal), and also pectic mucilage antibodies (CCRC-M34, CCRC-M36, CCRC-M38), which have been shown to also recognize RG I on the basis of a screening of these antibodies by the staff of the Complex Carbohydrate Research Center (M. G. Hahn pers. commun., University of Georgia, Athens; www.ccrc.uga.edu/~mao/wallmab/Antibodies/antib.htm).

Interestingly, antibodies that recognize the 1→4 galactan (LM5), 1→5 arabinan (LM6), and highly esterified homogalacturonan (JIM7) side chains of RG I are conspicuously absent from the papillate cells and adhesive rafts (Fig. 4). With the loss of RG I side-chain epitopes, binding of antibodies which recognize the RG I backbone is lost from the papillate side walls and increased in the contact face and raft area. These data indicate that the pectic mucilages in the raft may be debranched RG I, formed by the loss of galactan, arabinan, and homogalacturonan side chains and the subsequent migration of these debranched RG I backbones from the side walls of the papillate cells into the contact area. This interpretation is also supported by the structural data, in which massive modifications to the primary wall are observed, suggestive of extensive extractions of certain components of the cell wall and middle lamellae (Figs. 2 and 3). Alternately, the rafts could be from de novo synthesized unbranched RG I, or even a combination of new synthesis and the modification and migration of existing RG I molecules. The presence of secretory systems composed of Golgi bodies and smooth endoplasmic reticulum indicates that at least some components are synthesized de novo. Because the pectinaceous mucilage antibodies all recognize RG I (M. G. Hahn, pers. commun.), it could be that the debranching of RG I alters its affinity for molecules of the cell wall, thus freeing it from its position in the wall of the differentiating papillate cells and allowing the molecules to migrate down into the adhesive pad and substrate, where they bind to components of the raft. One of the properties of mucilage is its ability to expand when hydrated, and of course this role would be one that facilitates the adhesive of the Virginia creeper in infiltrating all the nooks and crannies between cells and irregularities in the substrate. Recently, the polysaccharides composing the highly water-retentive cell walls of *Sphagnum* spp. have been found to be largely composed of an RG I-like polysaccharide (Kremer et al. 2004, Ballance et al. 2007). This further indicates the extensive water-holding capacity of this type of pectin.

Another major component of the raft is callose. Callose is usually centrally located within the raft (Fig. 6A) and it is consistently coated with pectin-containing layers (Fig. 6B–D). Callose swells when moistened, as indicated by its role in plugging sieve tubes and in spreading cell plates (Samuels et al. 1995). This spreading effect of callose is used to great effect in plugging damaged phloem elements and facilitating the spread of the cell plate membranes towards the parental side walls in dividing cells (Samuels et al. 1995, Vaughn et al. 1996, Parre and Geitmann 2005). This property of callose may be the most important in the raft formation, i.e., spreading the raft out over a greater area, while the whole time covered in a sticky pectic mucilage. An extracellular callose-containing structure, such as described here, has not been found in other systems, except perhaps in severely wounded tissues. Besides the spreading property of callose, this polysaccharide is also observed where insects or pathogens have invaded or when mechanical stresses have been applied (e.g., Vaughn 2003). It is possible that the production of callose in the contact region of the Virginia creeper pad is due to a similar mechanical-stress response of the papillate cells to the substrate.

The absence (or loss) of the galactan and arabinan side chains (as measured by the lack of antibody labeling of the papillate cell walls) is in stark contrast to walls in the remainder of the tissue. In other systems, the loss of RG I side chains has a profound effect upon the characteristics of the wall itself. For example, in ripening fruit, the loss of 1→4 galacan and 1→5 arabinan side chains on RG I has been shown to cause a softening of the tissue (Pena and Carpita 2004). If the loss of RG I side chains leads to fruit softening, then the loss of the side chains in the contact face of the Virginia creeper tendril might also be causing softening of this tissue. Softening of the tendrils would allow them to spread out against the contacted surface, thereby increasing the surface area for adhesion, and would also allow them to conform to any surface irregularities (e.g., Fig. 2B). Furthermore, the loss of the side chains might make the RG I molecules more mobile, allowing them to move toward and into the raft material or beyond, into the pores and/or cracks of the substrate itself. These data indicate that the debranching of RG I converts these molecules into a liquidlike "mobile phase" which allows them to diffuse out of the walls and into the adhesive raft and the substrate itself, culminating in an adhesive that has set and formed a solid structure. Moreover, homogalacturonans have been proposed to be a side chain of RG I, rather than an integral part of the backbone (Vincken et al. 2003). The selective loss of the JIM7-reac-

tive epitopes from the papillate cell walls indicates that either the homogalacturonans are cleaved from RG I as part of a general side-chain loss or a more extensive metabolism of the RG I backbone.

Comparisons with other adhesive tendrils

Relatively little is known of the anatomy of other adhesive structures in vines, the only other modern studies being those of Endress and Thomson (1976, 1977) on adhesive tendrils of Boston ivy and Groot et al. (2003) on the adhesive roots of *Ficus pumila*. Boston ivy, because it is related to Virginia creeper, is structurally very similar. Papillate cells on the touched surface appear to produce an adhesive. The adhesive material has some of the characteristics of those of Virginia creeper, although it appears to be less heterogeneous on the basis of the micrographs supplied in these reports (Endress and Thomson 1977). Furthermore, structures which may be akin to the rafts reported here are described in the text (as "thickenings of the wall") but are not illustrated. Endress and Thomson (1977) also reported that in Boston ivy, the material at the contact surface contains platelet-type structures. In Virginia creeper, we found the contact surface material to be much more amorphous. The anticlinal walls of the papillate cells of Boston ivy were also shown to be pitted, similar to the Virginia creeper, further suggesting similar or identical mechanisms in Boston ivy. Although no immunoctyochemical studies were undertaken, cytochemical stains that recognize mucopolysaccharides and/or pectin (ruthenium red, colloidal thorium) were strongly reactive with the adhesive, indicating a result similar to those for the Virginia creeper as well. In *Ficus pumila*, the adhesive is produced by a clustering of adventitious roots rather than a tendril. However, even there, the adhesive area reacts with light-microscopic stains that recognize pectic and acid polysaccharide moieties (Groot et al. 2003). Thus, it appears that a basic mechanism of adhering (regardless of the structure) involves the production of a pectic mucilage that sets and adheres the vine to a substrate. Even in the area that cements the dodder (*Cuscuta pentagona*) haustorium onto the surface of the host, a pectic substance is produced, although that one appears to be more akin to that found in the middle lamellae as it is enriched in de-esterified homogalacturonan (Vaughn 2002).

How Virginia creeper tendrils adhere

In conclusion, our data indicates that the papillate cells of Virginia creeper tendrils are uniquely suited for their ability to attach to objects. First, touch-stimulated epidermal cells expand greatly and assume the shape of the object that is contacted. After contact is made, a complex structure consisting of a callose pad surrounded by, and impregnated with, adhesive molecules related to debranched RG I forms at the base of these papillate cells. The function of the callosic core of this adhesive pad might be to facilitate spreading, similar to an expanding cell plate (Samuels et al. 1995) or could facilitate the sealing of the adhesive into the numerous small holes in the surface of the object support substrate. On the basis of immunocytochemistry, the adhesive molecules are found both in the sticky raft and in a more mobile phase between the papillate cells and into the substrate and possibly a third phase that coats the spaces between papillate cells. Many of these molecules appear to be RG I reactive and appear to be derived from the loss of side chains of RG I in the papillate cell walls and their remobilization to the raft and other adhesive areas on the tendril. AGPs may be produced de novo and serve as a highly mobile phase of the adhesive, penetrating areas of the substrate too small for the pad to penetrate.

Other compounds present in the tendril, and papillate cells in particular, might also influence the function of the tendril besides the polysaccharides described above. Endress and Thomson (1976) remarked on the presence of phenols in the related Boston ivy and speculated that these compounds might also be involved in the adhesive process. Phenolics are present in the vacuoles of all cells and are present in a zone just above the callosic layer in the raft as well. The widespread occurrence of phenolic compounds in the cells of the adhesive tip and tendril at large might indicate that these compounds are involved in the tanning of the structures, or in a polymerized form might add to the adhesive as well. For example, many insects are adhered to cell surfaces by breaking open trichomes that produce copious phenols, which polymerize upon exposure to air and mixes with polyphenol oxidase. The phenolic polymers trap and adhere the insect to the surface. However, most of the phenolic deposits observed in Virginia creeper are found within the adhesive (note the placement of the blue-green deposits in the light micrograph in Fig. 1F), not at its extremities, and the deposits are variable in their presence in any given cell, making them less likely to be the adhesive. There might be another role for these phenolic compounds, however. Only days after adhering to an object, the Virginia creeper tendrils go through a programmed cell death and senescence, leading to the breaking of vacuoles and the release of oxidative enzymes that would effectively tan the papillate cells and

the tendrils as a whole. This would ensure that the tendril would persist as a structure and one that is highly resistant to environmental conditions and is unpalatable to insects and other herbivores.

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