Protoplasma (2002) 219: 227–237

PROTOPLASMA

Attachment of the parasitic weed dodder to the host

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Received January 29, 2001
Accepted November 28, 2001

Summary. The parasitic weed dodder (*Cuscuta pentagona* L.) invades a number of potential host species, but the mechanisms responsible for ensuring tight adhesion to the wide variety of host surfaces have yet to be identified. In this study, a battery of microscopy protocols is used to examine the host–parasite interface in an effort to deduce these mechanisms. As the dodder shoot approaches the host tissue, epidermal cells in the parasite shoot elongate and differentiate into secretory type trichomes. The trichome cell walls are malleable, allowing them to elongate towards the host and bend their walls to conform to the shape of the host cell surface. The presence of osmiophilic particles (probable cell-wall-loosening complexes) at far greater numbers than found in other species presages the expansion and malleable nature of the epidermal cells. In addition to the changes in cell shape, the dodder trichome cells secrete an electron-opaque cementing substance that covers the host–parasite interface. When probed with antibodies that recognize cell wall components, the cement reacted only with antibodies that recognize chiefly de-esterified pectins but not other common wall constituents. These data indicate that dodder utilizes both a cementing layer of pectin and a radically modified epidermal cell wall to secure the parasite to the perspective host.

Keywords: *Cuscuta pentagona*; Osmiophilic particle; Parasitic weed; Pectin; Cell wall; Immunocytochemistry.

Introduction

Dodders (*Cuscuta* spp.) are the most successful and widespread parasitic weeds, occurring virtually everywhere where crops are cultivated and on noncrop areas as well (Kujit 1969, Malik and Singh 1979, Dawson et al. 1994). Unlike most parasitic weeds that are selective on only several species of plants, dodders can invade plants as diverse as the green algae *Chara* spp., ferns, gymnosperms, and a wide variety of angiosperms (Gaertner 1950, Dawson et al. 1994). The ability of these parasitic weeds to invade such a large range of species (and with an incredible variety of surface morphologies) indicates that they must have highly adaptable mechanisms for host attachment. Unfortunately, the nature of the mechanism(s) is unknown.

Previous researchers have divided the parasitic organs of dodder into an "upper haustorium" (Lee and Lee 1989) or "adhesive disk" (Dawson et al. 1994), that includes the dodder shoot tissue external to the host, and the "endophyte", those portions of the dodder actually penetrating the host. With notable exceptions (Heide-Jorgensen 1987, Lee and Lee 1989), most of the studies of dodder have described processes in the endophyte rather than the upper haustorium (Kujit and Toth 1976). In addition, some of the studies on the upper haustorium appear contradictory. For example, Weinert and Barckhaus (1975) described an elaboration of cuticular material that occurred at the dodder–host interface, that they believe reduced the loss of moisture at the contact point and possibly functioned in cementing the dodder to the host. In contrast, Heide-Jorgenson (1987) reported that the "cement" that was present between the dodder and the host, although containing some particles of cuticle from the ruptured epidermis, was not actually the cuticle but some substance that originated from the dodder epidermal cells as an excretion. Moreover, the substance described in dodder is much more electron opaque than most cuticles and is different in morphology as well. In several other parasitic weeds, the production of cement involves some sort of polysaccharide excre-

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tion (Joel and Losner-Goshen 1994, Losner-Goshen et al. 1998, Neumann et al. 1999). These species are primarily parasites of roots rather than shoots, however; and shoot parasites generally twine around the host, whereas the root parasites simply adhere to the host surface. Thus, there might be different mechanisms involved in dodder parasitism, which strictly involves shoot to aerial-organ parasitism. Moreover, the widely different shapes of the surfaces being attacked by dodders will require different sorts of modification of the parasite epidermis so as to make the tightest possible contact (Lee and Lee 1989). The mechanism by which the parasite accomplishes this is also unknown.

In this report, light and electron microscopy, immunocytochemistry and affinity-gold techniques are used to determine the changes in the dodder required for successful attachment to the host. Moreover, three different types of surfaces (shoots, petioles, and leaves) that are invaded are examined to determine if the same kinds of processes are used on surfaces of different nature.

Material and methods

Plant material

Seeds of *Cuscuta pentagona* L. were treated with sulfuric acid as described previously (Sherman et al. 1999) to enhance germination. After washing in sodium bicarbonate and distilled water, the seeds were placed on top of soil mixtures in which grew impatiens (*Impatiens sultanii*) seedlings approximately 9 cm tall. Samples were taken at various developmental stages from initial curling of the dodder stem around the host tissue through final stages of haustoria formation, ranging from 2 to 5 weeks after inoculation with seed. Plants were maintained under a constant illumination of ca. 800 μmol/m^2 s (mixed fluorescent and incandescent bulbs) at 22 °C in a Conviron growth chamber. These growth conditions allowed for sustained growth of the impatiens plants and the rapid growth of dodders on this tissue.

Microscopy

For transmission electron microscopy, samples were fixed in 6% glutaraldehyde in 0.05 M piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES), pH 7.4, for 2 h at room temperature. The samples were then washed in two exchanges of 0.10 M cacodylate buffer, pH 7.2, for 15 min each and postfixed for 2 h in 2% (w/v) osmium tetroxide in cacodylate buffer. After a brief distilled-water rinse, the samples were en bloc stained with 2% (w/v) uranyl acetate overnight at 4 °C. The samples were then rinsed in water, dehydrated in an acetone series, transferred to propylene oxide, and embedded in a 1 : 1 (v/v) mixture of epon and Spurr resin. Infiltration of the resin occurred slowly over the course of three days, the last day on a rotating shaker to facilitate penetration. The dodder and host tissues were embedded in flat embedding molds (BEEM Inc.) and the specimens cut out and remounted on acrylic stubs to obtain a number of tissue orientations. Sections with a thickness of 0.35–0.55 μm were obtained with a Histoknife (Delaware Diamond Knives Inc., Bear, Del., U.S.A.), stained with 1% Toluidine Blue in 1% sodium borate, and examined with a Zeiss microscope to determine tissue orientations. Ultrathin sections (pale gold-silver reflectance) were obtained with a diamond knife (Delaware Diamond Knives Inc.) and were poststained with uranyl acetate and Reynolds lead citrate before observation in a Zeiss EM 10 CR electron microscope. Quantification of osmiophilic particles or cell-wall-loosening complexes was made by counting the particles on 20 micrographs printed to a final magnification of ×27,000 of elongating trichome cells. The number of particles per trichome cell profile was averaged and the high and low extremes were recorded. Similar protocols to measure osmiophilic-particle densities were used by Samajova et al. (1998).

For scanning electron microscopy, larger tissue pieces of haustoria forming on leaf surfaces, petioles, and stems were fixed in 6% (v/v) glutaraldehyde in 0.05 M PIPES buffer, pH 7.4, for 2–4 h. The samples were subsequently washed in two changes of 0.10 M cacodylate, pH 7.2, and postfixed in 2% osmium tetroxide in cacodylate buffer at 4 °C overnight. This relatively long postfixation in osmium improved the conductivity of these larger specimens. The specimens were then washed in distilled water and dehydrated with ethanol. Samples were critical-point dried and mounted on aluminum stubs with silver paint. After coating with gold-palladium, the samples were observed with a JEOL 740 scanning electron microscope.

Antibodies and antiserum employed

Most of the antibodies employed have been used in several previous studies from our laboratory (Sabba et al. 1999; Vaughn and Turley 1999, 2001) and the reactions are described in detail in those reports and more briefly herein. To detect pectins, the monoclonal antibodies JIM5 and JIM7 were used to detect primarily de-esterified and primarily esterified pectins, respectively. Although there is some controversy as to the exact epitopes which are recognized by these monoclonals, the JIM5 monoclonal recognizes primarily a highly de-esterifed epitope in samples fixed in glutaraldehyde and osmium and embedded in epoxy resins (see discussion in Losner-Goshen et al. 1998). The pectin serum raised by Staehelin and colleagues (Moore and Staehelin 1988) appears to recognize a similar epitope to that of JIM5 in similarly prepared sections.

Immunocytochemistry

For immunocytochemical localizations, some samples were prepared as for standard transmission electron microscope analysis, whereas others were processed by a less rigorous fixation and embedding scheme (detailed in Sabba et al. 1999). Samples embedded by either protocol were handled in the same matter for immunocytochemistry.

Ultrathin sections (with pale gold reflectance, the thinner silver sections tend to fold in the many incubations in these protocols) were obtained with a diamond knife on a Reichert Ultracut ultramicrotome and mounted on uncoated 300-mesh gold grids. These samples were floated, specimen side down, on the following solutions: 1% (w/v) bovine serum albumin (BSA) in phosphate-buffered saline (PBS) (20 mM phosphate buffer [pH 7.4] with 0.85% [w/v] sodium choride), 30 min; primary antibody or antiserum, 3 h; BSA-PBS, 4 times 2.5 min each; secondary antibody or Protein A conjugated to 15 nm diameter gold, diluted 1 : 20 in PBS-BSA, 30 min; PBS, 4 times 2.5 min each. After washing in distilled water, the grids were poststained with uranyl acetate (3 min) and lead citrate (30 s) before observation. The tissues for immunogold localization were from several different fixations, and included eight different block

faces for each antibody localization. The cellulase-gold probe was prepared and utilized as described previously (Sabba et al. 1999).

Quantification of immunogold and cellulase-gold was performed as described previously (Sabba et al. 1999), with only mature haustorial formations used in these analyses.

Immunocytochemical labeling of controls

Several control experiments were run for each of the antibodies. In all cases, a secondary-antibody-only (goat anti-mouse, goat anti-rat, or Protein A each coupled to colloidal gold diluted 1 : 20 in PBS-BSA) control was run to eliminate the effects of nonspecific binding. In all cases, there was less than 0.3 gold particles per square micrometer of background labeling. For JIM7 and callose labeling, the monoclonals were mixed with either 1 mg of citrus pectin (from Sigma for JIM7) per ml or 0.1 mg of pachyman (from Sigma for callose) per ml for 1 h prior to incubation of the grids. Both of these pretreatments gave a >95% reduction in immunolabeling on the sections. Some grids were pretreated with 0.1 M sodium carbonate for 18 h at 4 °C prior to incubation to chemically de-esterify pectins (Moore and Staehelin 1988, Sabba et al. 1999). The sodium carbonate pretreatment resulted in the near complete elimination of labeling of JIM7 (which recognizes highly esterified pectins) but increased the labeling by the polygalacturonic acid (PGA) backbone antiserum and JIM5 monoclonal in areas not previously labeled. A 30 min treatment of the grids with 6% (w/v) sodium *m*-periodate eliminated all vicinal-OH groups on polysaccharides and greatly reduced the labeling of both the antixyloglucan and cellulase-gold labeling of the sections (>80% reduction in labeling) but did not affect the labeling of the callose monoclonal. Carboxymethylcellulose (0.1 mg/ml) was preincubated with the cellulase gold-probe 1 h prior to incubation of the grids and resulted in a >85% reduction of the cytochemical labeling of the cellulase-gold probe.

Results

General morphological characterization

Haustorium formation in dodder begins as a swelling of stem areas proximal to the host tissue (Fig. 1A). A group of epidermal cells divide and then elongate dramatically, becoming unicellular secretory type trichomes (Figs. 1 B and 2 A). This congested mass of epidermal cells resembles a mass of fingerlike extensions from the dodder shoot (Fig. 2 B). As the epidermal cells are expanding, so-called "osmiophilic particles" (Robards 1969, Oleson 1980, Hoffman-Benning et al. 1994) or "cell-wall-loosening complexes" (Edelmann et al. 1995, Samajova et al. 1998) are produced from Golgi-derived vesicles (Fig. 2 B), which are subsequently deposited in the wall (Fig. 2 C). (For the purposes of this paper we will refer to these particles as

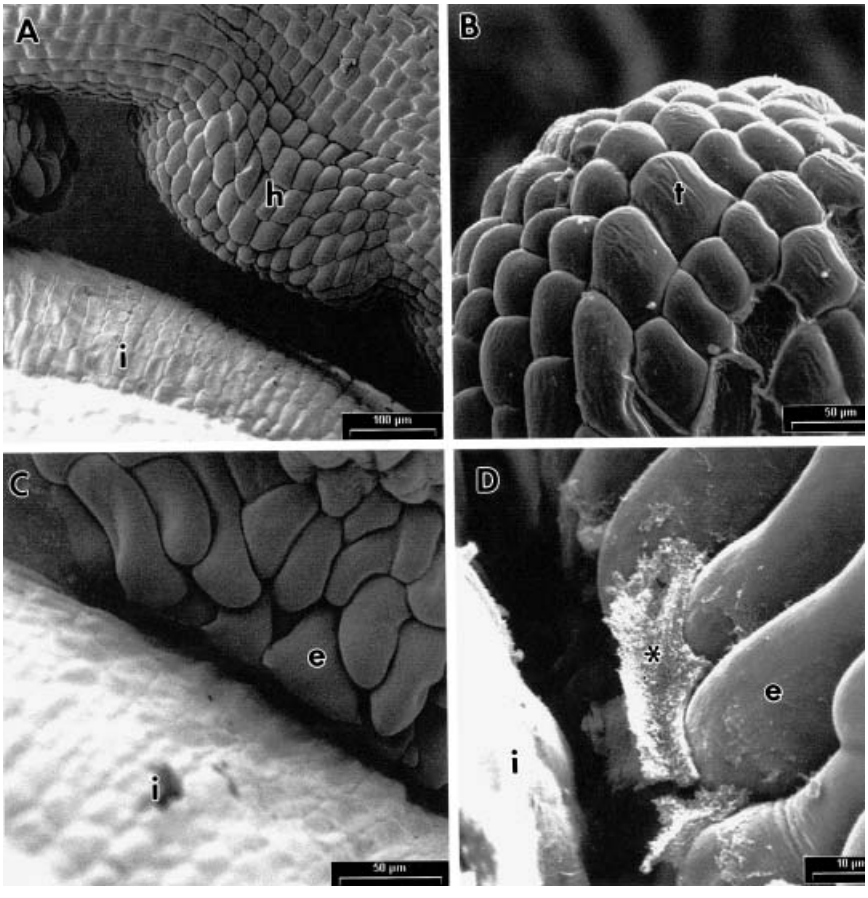

Fig. 1 A–D. Scanning electron micrographs of developing dodder haustorium and its attachment to the host. The specimens are oriented looking down the impatiens in the zone of dodder twining and attachment. **A** Developing haustorium (*h*) that is approaching the impatiens stem (*i*) consists of a mass of secretory trichomes, tightly bunched. **B** Haustorium (*h*) before contact with the host reveals a large cluster of fingerlike trichomes (*t*). **C** After contact with the host stem (*i*), epidermal cells (*e*) now conform to the surface morphology of the host. **D** A haustorium that was detached just slightly from the host shoot prior to processing for SEM reveals the presence of the extracellular cement (asterisk) that adheres the dodder to the host. *e* Epidermal cell; *i* impatiens stem

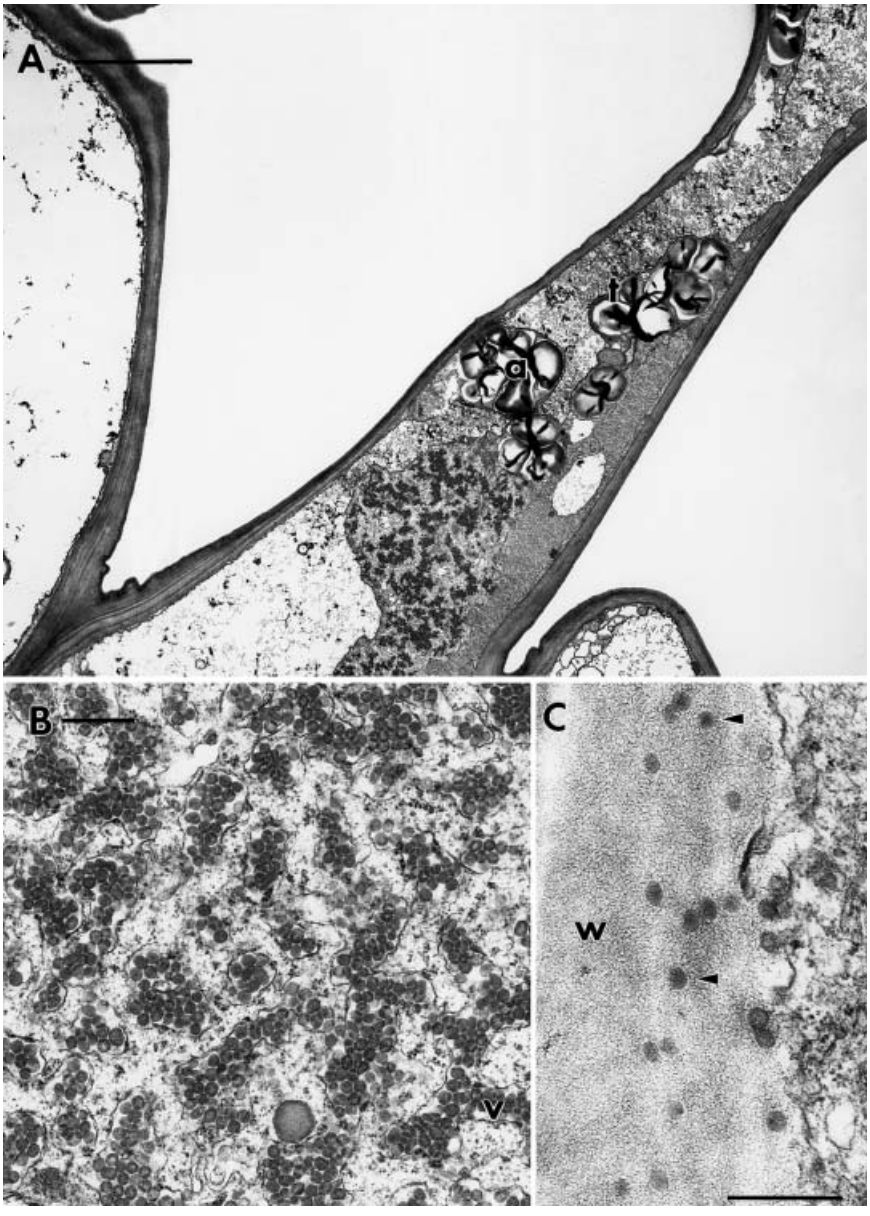

Fig. 2 A–C. Ultrastructure of the elongating trichome cells of the developing dodder haustorium. **A** Details of the elongating secretory trichome (*t*) prior to contact with the host epidermis. Amyloplasts (*a*) and large accumulations of vesicles characterize the cytoplasm. **B** Details of the cell-wall-loosening complex production and accumulation in vesicles in the trichome cytoplasm. Vesicles (*v*) containing groups of these particles are noted. **C** Wall (*w*) of the trichome cell revealing numerous cell-wall-loosening complexes. Two of the particles marked with arrowheads reveal less electron-opaque areas in the cell wall directly around the particle, perhaps indicating wall modification. Bars: A, 2.0 μm; B and C, 0.5 μm

cell-wall-loosening complexes as recent data from our laboratory indicates that, although the particles are electron opaque, they are in fact not osmiophilic. Samples fixed in only glutaraldehyde and poststained with uranyl acetate and lead citrate reveal these particles as strongly electron opaque even in the absence of osmium.) These cell-wall-loosening complexes are associated with the rapid elongation and the malleability of the epidermal cell wall (Edelmann et al. 1995). Quantification of cell-wall-loosening complexes in the elongating cells of rapidly elongating corn coleoptiles revealed an average of about 4 of these particles per cell profile (Hoffman-Benning et al. 1994) or 5–6 in elongating tissues in pea and bean (Edelman et al. 1995, Samajova et al. 1998). In the dodder trichome cells, as many as 474 (average 372) particles per trichome profile have been observed in the wall and as many as 742 per trichome profile if the particles present in Golgi-derived vesicles in the cytoplasm are included in the counts. If the correlation exists between elongation and the presence of these particles (Samajova et al. 1998, Cosgrove 1999), then the potential for elongation and wall loosening in these dodder trichomes is much greater than in these other species examined. In addition to the changes in cell-wall-loosening complexes, the dodder trichomes

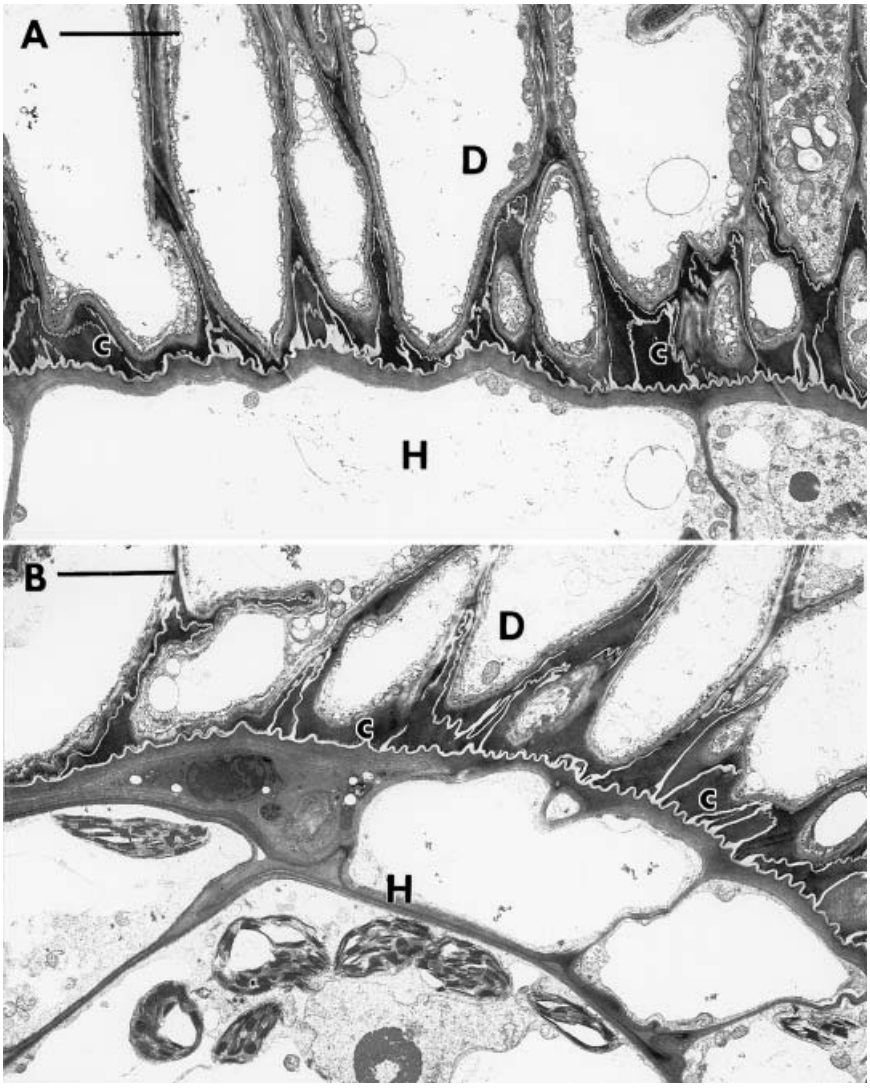

Fig. 3. Examples of dodder haustoria attachment to leaf lamina (**A**) or petioles (**B**). **A** In the attachment of the haustorium to the relatively flat leaf surface, the epidermal cells of the dodder (*D*) have formed a relatively flat interface to the host (*H*). The electron-opaque cement (*c*) forms a tight seal between the dodder and the host. **B** Unlike the relatively flat surface of the leaf lamina shown in **A**, attachment of the dodder (*D*) to the host petiole (*H*) involves a relatively rounded surface area. Regardless, a similar production of cement (*c*) fills in the spaces between the dodder and the host and the epidermal cells now conform to the more rounded surfaces as they do when the dodder has invaded a leaf lamina. Bars: 2.0 μm

begin to excrete an amorphous electron-opaque substance that accumulates in the space between the cuticle and the outer layers of the cell wall (not shown).

As the dodder makes contact with the host, the walls of the dodder epidermal cells accommodate the surface of the host plant and form a relatively tight connection between the host and parasite (Figs. 1 C, D and 3). The surface of the dodder attachment organ is modified by changes in the surface of the cell walls from the relatively pointed fingerlike extensions of the trichome cells to either flat or rounded surfaces characteristic of the host (Figs. 1 B, C, 3, and 4). To accomplish this change in surface morphology, the tips of the trichome cell walls actually appear to be bent backward into the cytoplasm (Fig. 4). Cell-wall-loosening complexes are especially prominent in the areas of the cell wall that have made contact with the host and the areas of infolding (Fig. 4), presumably allowing the dodder trichome cells to be malleable enough so as to form a tight seal with the host epidermis.

An electron-opaque "cement" forms a continuous coating between the dodder epidermal cells and the host (Figs. 1 D, 3, and 4). In addition to coating the surface, the cement also fills in all the crevices created by the infolding of the epidermal cells, thus creating a tight seal between the host and parasite. Pieces of apparently detached cuticle appear as electron-translucent areas in the electron-opaque cement between the host and parasite (Figs. 3 and 4). Some of these presumed cuticular pieces retain their morphology, whereas others appear without any recognizable shape. The cement coats all the cells of the host and

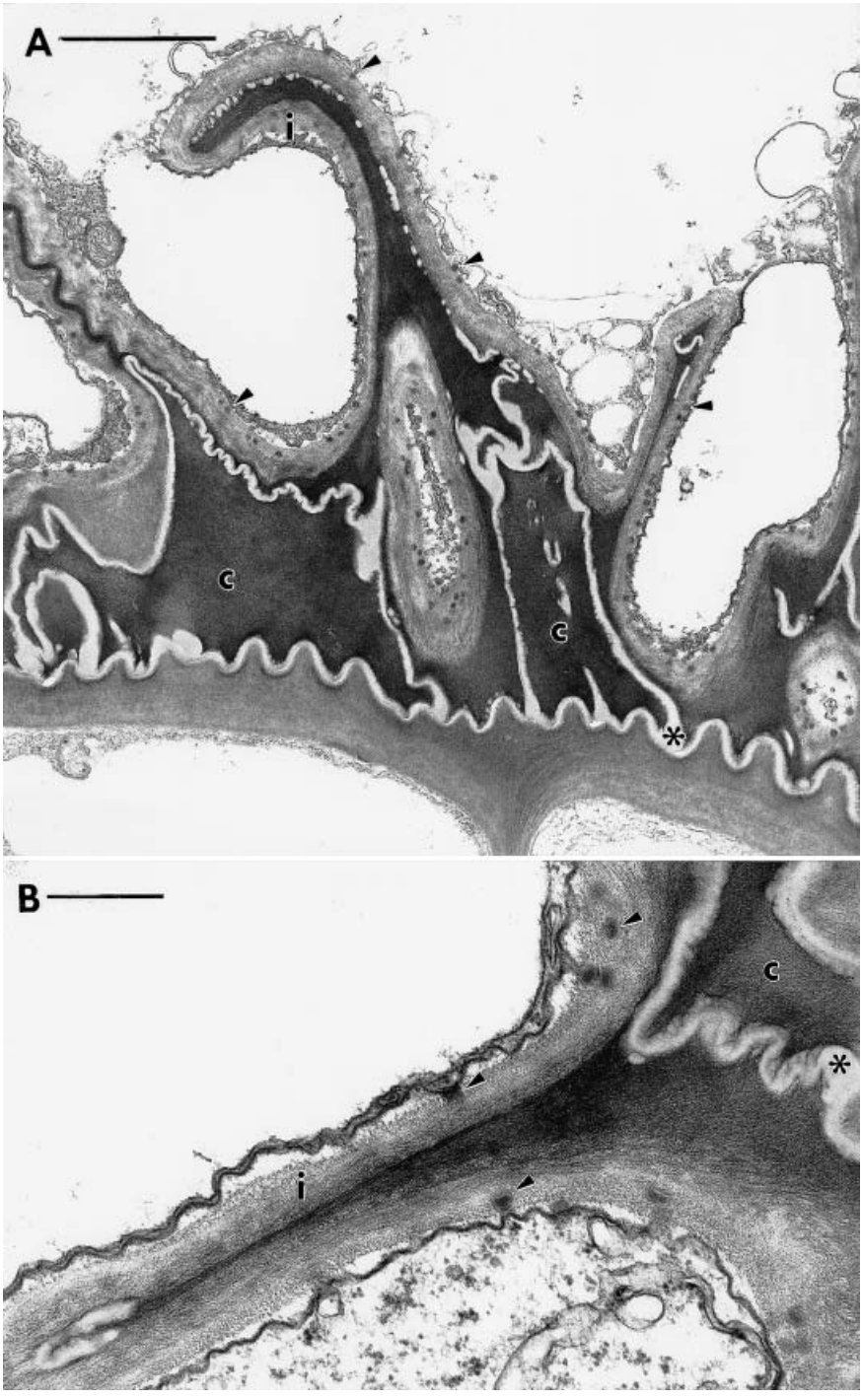

Fig. 4 A, B. Electron micrographs of dodder epidermal cells after contact with the host. **A** Note the accumulation of the electron-opaque cement (*c*) that adheres the dodder to the host. Invaginations (*i*) of the host wall show the malleable nature of these walls. Cell-wall-loosening complexes are found all along the plasmalemma–wall interface (some are marked with arrowheads). Although the area of contact contains cuticle remnants from both species, the cuticles (marked with an asterisk) are much less opaque than the surrounding cement. **B** Higher-magnification electron micrograph through one of the wall invaginations (*i*) of a dodder epidermal cell. The cell-wall-loosening complexes are found throughout the malleable cell wall layer. Cuticle (asterisk) is clearly discerned as less-electron-opaque areas surrounded by the much more electron-opaque cement (*c*). Bar: A, 2 µm; B, 0.5 µm

the epidermis to the edge of the haustorium and occasional slightly beyond this boundary (e.g., Fig. 1 D). Thus, the dodder utilizes both an extensively loosened cell wall and a cementing substance to ensure tight contact and adhesion to the host.

Immunocytochemical characterization

To determine the composition of the dodder secretory cement and cell walls, a battery of antibodies were used to probe sections of the dodder through the area of host–parasite contact. These observations covered several of the stages of cement formation and epider-

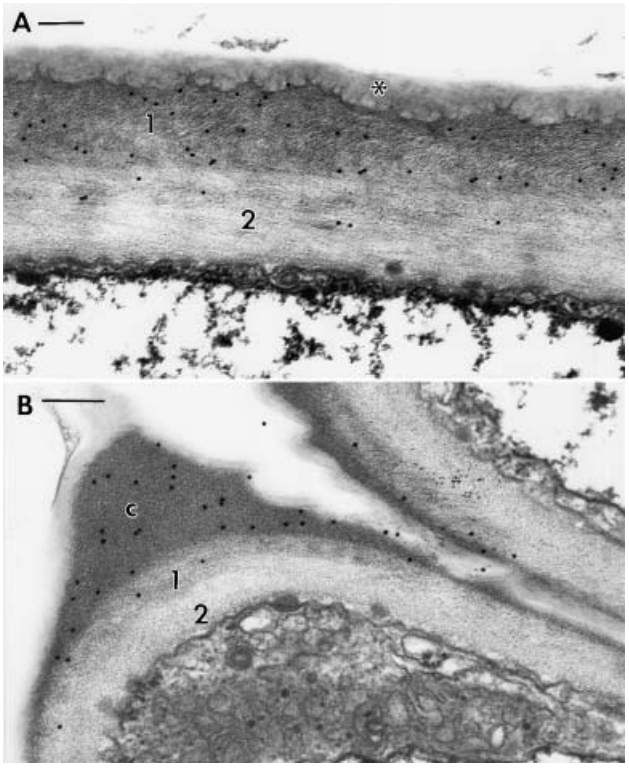

Fig. 5 A, B. Immunogold localization of pectins in epidermal cells of dodder prior to host contact. **A** Details of an elongating epidermal cell labeled with antibodies to the de-esterified PGA backbone. Note the strong labeling in the first layer (*1*) of the cell wall, with relatively little labeling in the second (*2*). The cuticle (asterisk) is unlabeled and appears as a continuous band across the epidermal cell wall. **B** Early stages of cement (*c*) formation in the epidermal cells prior to host contact. The cement is already strongly labeled with the anti-PGA backbone serum. Bars: 0.2 μm

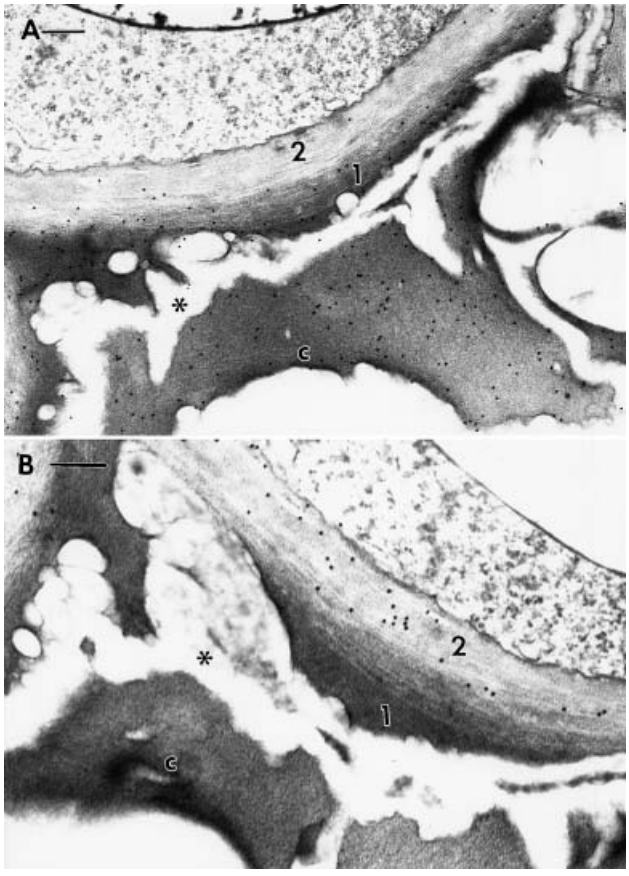

Fig. 6. Localization of PGA backbone (**A**) and xyloglucan (**B**) in the dodder epidermal cells after contact with the host is secured. **A** The cement (*c*) produced by the dodder and the outer layer (*1*) of the dodder wall are labeled strongly with anti-PGA backbone antiserum, whereas the inner layer (*2*) and the cuticle (asterisk) are virtually unlabeled. **B** With antibodies to xyloglucan the inner wall layer (*2*) is labeled heavily, whereas neither the outer layer of the wall (*1*) nor the cement or the cuticle (asterisk) are labeled. Bars: 0.2 μm

mal wall modifications, but quantitative analyses were limited to tissues that had formed a mature haustorium so that developmental differences would not confound the results.

The epidermal cells of the emerging upper haustorium before contact with the host are distinctly bilayered, with the outer portions of the wall labeled with anti-PGA backbone and JIM5 monoclonal antibodies (Fig. 5 A and Table 1). As the trichome cells elongate and approach the host, an electron-opaque substance is observed between the cuticle and wall and it also labels strongly with these same two antisera (Fig. 5 B). The cuticle is distinct at this stage as a much less electron-opaque mass at the extreme periphery of the dodder epidermal cells and is not labeled by any of the polysaccharide antibodies employed.

In the mature upper haustorium, the cement is strongly labeled with the monoclonal antibody JIM5 and the polyclonal PGA backbone serum, as is a layer of the cell wall in the trichome cells (Fig. 6 A and Table 1). The labeling is relatively uniform throughout the cement, including the portions that intercalate the spaces between adjacent epidermal cells. The label does not extend into electron-translucent areas that are probably the remains of the disturbed cuticular material of the host and parasite. The monoclonal antibody JIM7, which recognizes more highly esterified pectin groups, labels the cement layer and the outer layer of the epidermal wall only sparsely (Table 1) but strongly labels the cell wall in portions of the cell outside of the zone labeled with the de-esterified pectin antibodies. Sodium carbonate treatment of sections chemically de-esterifies the pectins. When these treated sections are probed with the JIM5 and the PGA backbone sera, only a small increase in labeling

Table 1. Quantification of immunogold labelling in dodder–impatiens interface

Compound recognized by antibody or antiserum used[a]	Nr. of gold particles per μm² of interface structure[b]				
	cement	invagination	epidermis	host wall	ML
PGA	47	17	23	2	31
De-esterified pectins	34	12	14	3	29
Esterified pectins	3	18	12	14	3
Xyloglucan	0	39	43	62	0
Cellulose	2	41	47	81	2
Callose	1	4	7	1	0
Extensin	3	2	2	2	8

[a] Antibodies are described in more detail in the papers of Vaughn and Turley (1999, 2001) and in the Material and methods section of this paper

[b] Cement, the excreted material occurring between the host and parasite; invagination, area of the dodder epidermal cell walls which have invaginated towards the cytoplasm; epidermis, the dodder epidermal cell wall which is not included in the invaginations but does include both layers of cell wall; host wall, the epidermis of the host bordering the dodder; ML, the middle lamellae of the dodder cells not from the epidermis

in the cement layer is noted, although labeling in the host and parasite walls was markedly increased by this treatment (not shown). These data indicate that the pectins present in the cement layer are the highly de-esterified types as they are labeled by antibodies that recognize de-esterified pectins and chemical de-esterification does not increase this labeling. When compared with labeling of these same antibodies in middle lamellae of the host or the dodder cells out of the contact zone, the densities were quite similar (Table 1). Thus, the production of the dodder cement was not too different from the production of the normal cell-to-cell adhesions found in middle lamellae in terms of pectin epitopes present and their labeling density. These data do not prove identity between the middle lamellae and the cement, however, as other substances such as glycoproteins, phenols, and other secondary plant products could be a component of the cement.

Other antibodies and probes were used to determine whether the pectins were present alone or as a complex with other common wall components. These probes included xyloglucan (Fig. 6B), callose, and extensin monoclonal antibodies and/or polyclonal sera and the cellulase-gold affinity probe. Although all of these labeled wall components, none labeled the cement layer significantly beyond background labeling (Table 1).

In the dodder epidermal cells, the zone of the cell wall containing de-esterified pectin was labeled by neither the xyloglucan antibodies (Fig. 6B) nor the cellulase-gold probe (Table 1). This biphasic wall structure and composition of these epidermal cells is shared by a number of other differentiated epidermal cells, in both secretory and nonsecretory forms (e.g., Vaughn and Turley 1999, 2001). Despite the tremendous contortions of the walls in the invaginations, the composition of the invaginated areas appears similar to areas of the wall not ingrown (Table 1). For example, one might expect an accumulation of callose in tissues that have been wounded or crushed, although no such increase is observed in the dodder trichome cells that have made contact with the host (Table 1). Thus, despite the very unusual morphologies that are generated in the dodder epidermal cells, these cells are much like other differentiated epidermal cells such as trichomes in terms of wall organization.

Discussion

Two mechanisms for attachment

The microscopic and cytochemical results described herein reveal that dodder uses two major mechanisms in the attachment of the upper haustorium to the host. The first involves the formation of epidermal cells with very malleable walls that allow these cells to conform to the surface of the host. These are no doubt due to the presence of large numbers of cell-wall-loosening complexes that change the wall in such a fashion that it now invaginates into its cytoplasm rather than breaking or cracking as the dodder makes contact with the host. That the outer layer of these trichome walls is enriched in de-esterified pectins may also aid in malleability of these walls compared to walls composed of the relatively more rigid xyloglucan-cellulose wall throughout. In addition to the changes in wall malleability, dodder excretes a layer of pectinaceous cement, similar to the middle lamellae in the presence of primarily de-esterified pectin residues. Both the mechanism of cell wall loosening and the production of a pectinaceous cell-to-cell adhesive are normal processes of the cell that the dodder has taken to an extreme in securing itself to the host.

Attachment mechanisms in other parasitic weeds

The first role of the forming haustorium is to establish a tight contact between the host and the parasite (Malik and Singh 1979, Kujit 1969). In all of the parasitic weeds investigated to date, an adhesive substance at the attachment site is noted; however, the amount of this adhesive substance or cement varies tremendously between the various parasitic species. In the parasitic weeds that invade roots, a relatively thick layer of mucilaginous substance is secreted (Kujit 1969, Heide-Jorgenson 1989). The reaction of these cements with stains for lipids and polysaccharides indicates the complex nature of these copious deposits (Joel and Losner-Goshen 1994). In the stem-twining parasites such as dodder, the production of the cements is more restricted to a thin band that coats both the host-parasite surface and the spaces between adjacent epidermal cells in the parasite. It is possible that the physical attachment of the host to a twining stem requires less of the cementing substance than in the more ephemeral sorts of attachment that occur in the parasites that invade through the root tissue, in which attachment is made without twining. However, the twining dodder stem would be exposed to more meteorological variables than would the root parasites, and as a result might require cement of a different nature to withstand these challenges. Thus, it is not unreasonable to think that species with different sorts of attachments may use different sorts of cement to perform this function of host attachment.

Why pectins?

Although pectins have been less a subject of investigation than the more abundant cellulose-xyloglucan fraction, they are potentially a much more variable and hence a more biochemically diverse group of compounds (Ridley et al. 2001). Variations in the side chains and degrees of esterification of the PGA backbone allow for an almost infinite variability in composition and possible functions. In the case of the cement, the pectins (and what other components might be present) have formed a relatively amorphous mass and contain chiefly de-esterified PGA residues, not unlike what is noted in the middle lamellae. This production of a cementing substance with a similar distribution and density of pectin epitopes to the middle lamellae indicates that the same cellular adhesive that allows cell-to-cell adhesion in one species is utilized to attach cells between species. The data accumulated only allow us to draw this analogy in terms of pectic and other polysaccharide wall components, however.

In previous studies by our group and others, de-esterified pectins were shown to be a major wall component of epidermal appendages such as hairs, trichomes, and fibers (e.g., Vaughn and Turley 1999). Although nontrichome epidermal cells of the dodder shoot have a simple wall without a pectin layer, the cells that will become trichomes differentiate a bilayered wall and then begin secreting the pectin that will form the cement. Early stages of this process reveal the appearance of esterified pectin in the Golgi vesicles, indicating that the precursors to the pectin cement are made as esterified molecules (reactive with JIM7 antibodies; not shown) and then enzymatically converted to de-esterified forms (Ridley et al. 2001). In the dodder trichome cells, pectins are also excreted so as to produce the cementing ensuring host adhesion.

Modification of epidermal cell wall structure

Besides the production of a cementing substance, an equally important step in successful dodder attachment is the modification of the epidermal cells, first to expand to ensure contact and then to conform to the shape of the host. The sorts of wall modifications described in the dodder wall are surely some of the more unique wall modifications known in higher plants.

A key element of host-to-parasite adhesion in the process is the appearance of the cell-wall-loosening complexes. These are associated with the elongation of deep-water rice and corn hypocotyls and a number of other species (e.g., Edelman et al. 1995, Hoffman-Benning et al. 1994, Oleson 1980, Kutchera et al. 1978, Samajova et al. 1998) in which rapid elongation growth is occurring. In the dodder epidermal cells, a much greater accumulation of particles (by factors of 50–100 the levels in a cell profile) both in the precursor form of Golgi-derived vesicles and wall-associated particles is observed than has been described in these other species. In fact, previous observations of cell-wall-loosening complexes in these other systems have never noted the particles associated with the Golgi, although indirect proof of this was obtained from inhibitor studies (Hoffman-Benning et al. 1994, Edelmann et al. 1995). In order for dodder to establish a

parasitic union before seed reserves are dissipated, the parasite must make unions to perspective hosts rapidly. The presence of large numbers of cell-wall-loosening complexes in the dodder trichome cells enables these cells both to elongate rapidly towards the host and to modify their surface to mirror that of the host.

In other experiments, we have shown that at least one of the components of the cell-wall-loosening complexes in dodder is the protein expansin (Vaughn et al. 2001). Expansin separates from the bulk of the particle as the Golgi vesicle fuses with the wall and is then distributed throughout the wall. The appearance of expansin not only allows the walls to expand but also makes them much more malleable in terms of cellulose-xyloglucan cross-linking. The presence of a layer of de-esterified pectins in these walls may add to this flexibility as well. This increased malleability of the wall probably accounts for their ability to modify their shape and so to mirror the surface of the perspective host. Despite the very strange conformations produced by these walls, there is no evidence of an accumulation of callose, although one might suspect such an increase in tissues that have been placed under such mechanically stressful conditions.

Despite the unusual wall conformations in the dodder epidermal cells, there has been relatively little discussion of these wall modifications in the literature. In some of the light micrographs in the paper of Lee and Lee (1989), epidermal cells with obvious wall invaginations are noted (e.g., Lee and Lee 1989: fig. 13), although there is no mention of them in the text of this paper. Heide-Jorgenson (1987) describes these invaginations in line drawing format and Dawson et al. (1994: fig. 21) provide the first electron micrographs of the wall invaginations and cement. Osmiophilic particles or cell-wall-loosening complexes in dodder tissues were reported from dodder hyphal cells by Tripodi and Pizzolongo (1967), although this report is their first mention in tissues other than hyphae.

In summary, dodder cells go through massive cellular changes in establishing a tight contact with the host. The epidermal cells near the prospective host become secretory trichomes that excrete a cement composed of chiefly de-esterified pectins, similar to the middle lamellae. This effective cell adhesive would maintain the close contact through the penetration of hyphae into the host tissue. An equally important component of the attachment mechanism occurs in the modification of the epidermal cell walls so as to conform to the surface of the host. The walls are first made malleable by the addition of cell-wall-loosening complexes so that, upon contact with the host, the walls conform to the new surface. The combination of radically modified epidermal cell walls and pectinaceous cement ensures a tight adhesion between host and parasite.

Acknowledgments

Rex Paul provided invaluable assistance in the preparation of samples for the SEM and digital data acquisitions. Lynn Libous-Bailey grew the plant material and provided excellent technical assistance with the photography. Antibodies and antisera were kindly provided by L. Andrew Staehelin, K. Roberts, M. Hahn, and J. Paul Knox. Helpful suggestions from Drs. R. Turley, R. Kloth, T.W. Barger, and two anonymous reviewers are acknowledged. This research is supported in part by a NRI grant to K.C.V.

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Verleger: Springer-Verlag KG, Sachsenplatz 4–6, A-1201 Wien. — Herausgeber: Dr. D. Menzel, Botanisches Institut und Botanischer Garten, Rheinische Friedrich-Wilhelms-Universität Bonn, Kirschallee 1, D-53115 Bonn, Bundesrepublik Deutschland. — Redaktion: Sachsenplatz 4–6, A-1201 Wien. — Satz und Umbruch: SNP Best-set Typesetter Ltd., Hong Kong. — Druck: Adolf Holzhausens Nachfolger, Holzhausenplatz 1, A-1140 Wien. — Verlagsort: Wien. — Herstellungsort: Wien. — Printed in Austria