Protoplasma 118, 206–218 (1983)

PROTOPLASMA

Experimental Studies of the Attachment of the Parasitic Angiosperm *Agalinis purpurea* to a Host[1]

Wm. V. Baird* and J. L. Riopel

Biology Department, Gilmer Hall, University of Virginia, Charlottesville, Virginia

Received January 17, 1983
Accepted March 24, 1983

Summary

Agalinis purpurea (L.) Raf. (*Scrophulariaceae*) is a facultative root-parasite. Haustoria, the organs specialized for host attachment and penetration, develop rapidly after exposure of the parasite's roots to host root exudate or specific haustoria-inducing molecules. Haustoria are competent to attach as early as 6–12 hours post-induction and reach a maximal level of attachment by 36 hours. Developing haustoria which have not contacted a host by 60–72 hours of development fail to attach when contact is established. However, this time course of attachment competency is extended for haustoria which have previously been attached to a host. Attachment is a non-discriminatory event and induced haustoria will adhere to a variety of biological and non-biological substrates. Adhesion is mediated through specialized haustorial hairs whose surface is covered with a papillate network. The papillae represent the cement which attaches the haustorium to its host. The proportion of papillate hairs on haustoria parallels the attachment competency.

Keywords: *Agalinis purpurea* (*Scrophulariaceae*); Attachment; Haustorium; Parasitic angiosperm.

1. Introduction

Angiosperm root parasites are a heterogeneous group of species distributed among eight families of flowering plants (Kuijt 1969). Many, like *Agalinis purpurea*, are facultative parasites with a normal photosynthetic potential and are capable of completing their life cycle without a host, although in nature they are invariably found attached to the roots of neighbouring plants. Typically these plants exert little physiological effect on their hosts (Tsivion 1979). Others, like *Striga* spp. and *Orobanche* spp., are obligate parasites. For these, host contact is essential for completion of their life cycle; contact must be established very early after germination and the effect of parasitism on the host plant can be severe (Eplee 1981, Musselman 1980).

The structure common to all root parasites is the haustorium, the unique organ effecting parasitism. Haustoria usually appear as knob-like structures distributed laterally along the root axis of the parasite. On casual observation they resemble short, bulbous lateral roots and are easily mistaken for nitrogen-fixing root nodules. However, they are different from both these organs in developmental origin, cellular organization and function (Musselman and Dickison 1975, Kuijt 1977).

It is instructive to consider haustorial development in four stages: initiation, pre-contact, attachment and penetration. Under optimal conditions the process is continuous and culminates in the establishment of a vascular tissue bridge between parasite and host. It has been shown that the onset of haustorium initiation requires specific physiological and environmental signals (Atsatt *et al.* 1978, Riopel and Musselman 1979). Typically few or no haustoria develop in the absence of host roots (Riopel 1979; Weber 1981). Recent evidence has shown that low molecular weight flavonoids can function as the signal molecules responsible for haustoria induction in *Agalinis* (Lynn *et al.* 1981). These compounds and their analogs reveal a high degree of molecular specificity on the part of the

[1] Portion of a dissertation submitted by the first author in partial fulfillment of the Ph.D. in Biology at the University of Virginia.

* Correspondence and Reprints: Department of Molecular and Population Genetics, Bio.-Sci. Building, University of Georgia, Athens, GA 30602, U.S.A.

parasite (STEFFENS *et al.* 1982) which is in marked contrast to its broad host range.

Once induced, development will proceed to attachment with no further external or host influence. Following attachment, the host is penetrated by the endophyte (DOBBINS and KUIJT 1973b, TOTH and KUIJT 1977). However, in the absence of host contact, development ceases at the pre-contact stage and the differentiation of cells characteristic of the penetration stage is not observed (RIOPEL and MUSSELMAN 1979). Thus, attachment is evidently a pre-requisite for the continued maturation of the haustorium. A better understanding of the process would therefore clarify the role of attachment in the successful establishment of the parasite.

This paper focuses on haustorial attachment in *Agalinis purpurea* grown under experimental conditions in sterile culture. Structural, temporal and biochemical parameters of attachment are described.

2. Methods

2.1. Plant Material

Plants of *Agalinis purpurea* (L.) Raf. (*Scrophulariaceae*) were grown axenically, in 35 × 10 mm plastic Petri dishes, from vernalized seeds as previously described (RIOPEL and MUSSELMAN 1979). The nutrient medium contained modified MS salts (MURASHIGE and SKOOG 1962), 0.5% sucrose and was solidified with 0.9% agar (pH 6.8 before autoclaving). Experiments were conducted on 3-5-week-old plants. Host plants were germinated on moist filter paper and placed in contact with *Agalinis* after three weeks.

2.2. Induction of Haustoria

Haustoria were induced on *Agalinis* roots in one of three ways: (1) placement of roots of an axenically grown host plant (*i.e., Lespedeza sericea*) in liquid medium with *Agalinis*, (2) placement of *Agalinis* plants in a 0.1% solution of gum tragacanth (GT) which is known to contain at least two haustorial inducing molecules (LYNN *et al.* 1981, STEFFENS *et al.* 1982), (3) placement of a 0.5 cm diameter filter paper disk, previously treated with an alcohol extract of GT and air dried, into the medium near roots of *Agalinis*. *Agalinis* plants responded similarly to all three induction methods.

2.3. Release of Attached Haustoria

The release of attached haustoria was monitored during their exposure to solvents reported to solubilize specific classes of wall macromolecules. Haustoria which had been attached for 4-5 days were treated in 5 ml of the solutions for varying lengths of time up to 24 hours, after which the percent of released haustoria was determined. (a) *Waxes* were dissolved with ethanol, methanol, chloroform or acetone (JENSEN 1962); (b) *cutin/suberin* were extracted by refluxing in tetrahydrofuran with an excess of $LiAlH_4$ or in 14% BF_3 in methanol (ESPELIE *et al.* 1980); (c) *lignins* were dissolved with hydrofluoric acid or hydrogen peroxide (JOHANSEN 1940); (d) general, *non-cellulosic carbohydrates* were extracted with 10% nitric and chromic acid (JOHANSEN 1940), 1:1 H_2O_2 and glacial acetic acid (KROH and KNUIMAN 1982), or 0.5% oxalate followed by successive treatments of 4% and 17.5% NaOH (JENSEN 1962); (3) *pectins* were extracted in boiling H_2O, 0.1 M $NaCO_3$ (JARVIS *et al.* 1981), 2% HCl followed by 2% NH_4OH (FACEY 1950), 1% disodium EDTA (KIRBY and ROBERTS 1971), 3% sodium hexametaphosphate (GRUSAK *et al.* 1980), or refluxed in 0.1 M ammonium oxalate in oxalic acid, acetate or citrate buffer (NISHITANI and MASUDA 1982; JARVIS *et al.* 1981); (f) *hemicelluloses* were extracted in 3% H_2SO_4 (RAY and BAKER 1965) or in 1.0 N NaOH followed by 6.0 N NaOH (GRUSAK *et al.* 1980, ASAMIZU and NISHI 1980, REID and WILKIE 1969); (g) *callose* was extracted in saturated solutions of calcium or stannous chloride (REYNOLDS and ROBERTS 1976), DMSO or 1% NaClO (VITHANAGE *et al.* 1980), and (h) *proteins* were solubilized by heating in a buffer containing 2% SDS and 5% 2-mercaptoethanol (LAEMMLI 1970). Also, commercially available glycolytic enzymes were monitored for their ability to release attached haustoria. These enzymes were used as 2% solutions in 0.1 M K-phosphate buffer (pH 5.5) at 30 °C. The proteinases, pronase and trypsin, were used at pH 7.2.

2.4. Scanning Electron Microscopy

Roots with haustoria were placed in Karnovsky's fixative (KARNOVSKY 1965) in 0.1 M cacodylate buffer, pH 7.2, containing 0.1% sodium azide (MINASSIAN and HUANG 1979). Fixation was for 2 hours at room temperature or overnight at 4 °C. Some specimens were post-fixed either in cacodylate buffered 2% osmium tetroxide or following the osmium-thiocarbohydrazide-osmium method of KELLEY *et al.* (1975). These specimens were then dehydrated through an ethanol series, critical point dried in a Tousimis Samdri PVT-3 (Rockville, Md., U.S.A) and coated with 100 Å of gold-palladium alloy using a Technics Hummer (Alexandria, Va., U.S.A). Specimens were examined with the aid of an ETEC Autoscan operated at 20 kV accelerating voltage.

3. Results and Observations

3.1. Attachment Substratum

Laboratory and field observations of soil grown plants reveal that haustorial attachment frequencies are variable (25-80%). In the soil most haustoria are usually attached to adjacent roots but others are either not attached or attached to a variety of inanimate objects. This has been reported for other root parasites as well (KUIJT 1969, WERTH and RIOPEL 1979). To evaluate the factors that might be required of the host surface to insure successful attachment of *Agalinis*, we induced haustoria in the presence of an assortment of natural and artifical attachment substrates.

Agalinis attached to roots of all plants tested, including both herbaceous and woody species (Table 1). We selected *Lespedeza sericea* (*Leguminosae*) as a control host for comparisons of attachment with artifical substrates representing a variety of surface textures and chemical compositions. Data for these experiments are summarized in Table 2 and show that haustoria will attach to almost all substrates presented. Few or no attachments (< 20%) were made to metallic or metal-

Table 1. *Host plants to which Agalinis attached*

Dicotyledons	Monocotyledons
Arenaria graminifolia	*Agrostis* sp.
Astragalus cicer	*Danthonia curva*
Bursera rubra	*Digitaria vestita*
Castilleja coccinea	*Festuca rubra*
Delphinium sp.	*Holcus lanatus*
Dianthus armeria	*Juncus maritimus*
Lespedeza sericea	*Panicum amarulum*
Oenothera biennis	*Phalaris* sp.
Penstemon digitalis	*Poa pratensis*
Petunia sp.	*Setaria macrostachya*
Potentilla buccoana	
Quercus sp.	Gymnosperms
Trifolium repens	*Pinus echinata*
Vernonia incana	*P. strobus*
Vicia villosa	*P. taeda*

Table 2. *Attachment to experimental substrates*

Substrates		Percent attached[1]
I. *Biological materials*		
A. Control host (*L. sericea*)	roots	70
	stems	60
	leaves	60
	seed coats	60
B. Applicator sticks		60
C. Pith of *Sambucus canadensis*		70
D. Cork		60
E. "Whatman" filter paper (25 → 2 μm)		50-70
II. *Non-biological materials*		
A. Molecular membrane filters (10.0 → 0.025 μm)		
"Nucleopore" (polycarbonate)		80-100
"Gelman" (cellulose triacetate)		80-100
"Milipore" (cellular nitrate and acetate)		70-90
B. Plastic Petri dishes (polystyrene) culture/non-culture		90/85
C. Metal-coated plastic Petri dishes (50–100 Å of carbon or platinum, or 200 Å of gold/palladium)		0-20
D. Glass Petri dishes		40-50

[1] Percentages were determined 48 hours after induction as a ratio of attached to induced haustoria and normalized to 100% for highest value obtained.

coated surface, apparently because of toxicity of these substrates which resulted in necrosis of the haustoria. Artificial substrates often yield higher attachment percentages (> 70%) than *Lespedeza* roots. This results in part from the uniformity of artificial surfaces and in part from our ability to obtain juxtaposition of large portions of the parasite root and the artifical surface. This is not possible with host roots, and as a result haustoria would frequently initiate where contact with a host root was impossible, contributing to a lower ratio of attached to induced haustoria. The percentages undoubtedly reflect this aspect of attachment and are presented as a justification for our use of plastic Petri dishes as the artificial attachment substrate in subsequent haustoria release and time course experiments.

3.2. Structural Basis of Attachment

Hairs are one of the most conspicuous features of the haustoria of many species of parasitic *Scrophulariaceae*. In *Agalinis*, hair formation is well advanced by 24 hours after induction (RIOPEL and MUSSELMAN 1979), and hair initiation continues throughout the pre-contact stage. Older hairs continue to elongate, and new ones arise in an acropetal sequence. The haustorial apex is typically glabrous and subtended by a ring of hairs (Fig. 1). These hairs are important to the attachment process. They are the first haustorial structures to come in contact with the host surface (Fig. 2), and their structure indicates that they may be functionally different from other hairs along the root axis. The most interesting difference is that while the surface of root hairs is smooth (Fig. 3) the surface of most haustorial hairs bears a papillate covering (Fig. 4). Papillae are present on haustorial hairs prior to physical contact with a host. Occasionally discrete papillae are found on the haustorial apex as well.

The relative abundance of papillate hairs on developing, attached and unattached haustoria is illustrated in Fig. 5. Papillae are found on the largest percentage of hairs by 24–36 hours post-induction. After this, the percentage of papillate hairs remains approximately constant for attached haustoria but decreases with age for unattached haustoria. Examining just the most apical hairs on the older haustoria shows that attached haustoria have a much larger percentage of papillate hairs than do unattached haustoria. Note also that the total number of hairs per haustorium continued to increase throughout the 72-hour experiment.

Close examination of the parasite-host interface indicates that the papillate hairs take an active role in attachment. The papillae are deformed into a thin, mesh-like network at the contact interface (Fig. 6), and this is exaggerated when one hair attaches to another (Fig. 7). Separation of the attached partners demonstrates that the adhesion of the papillate haustorial hairs to the host

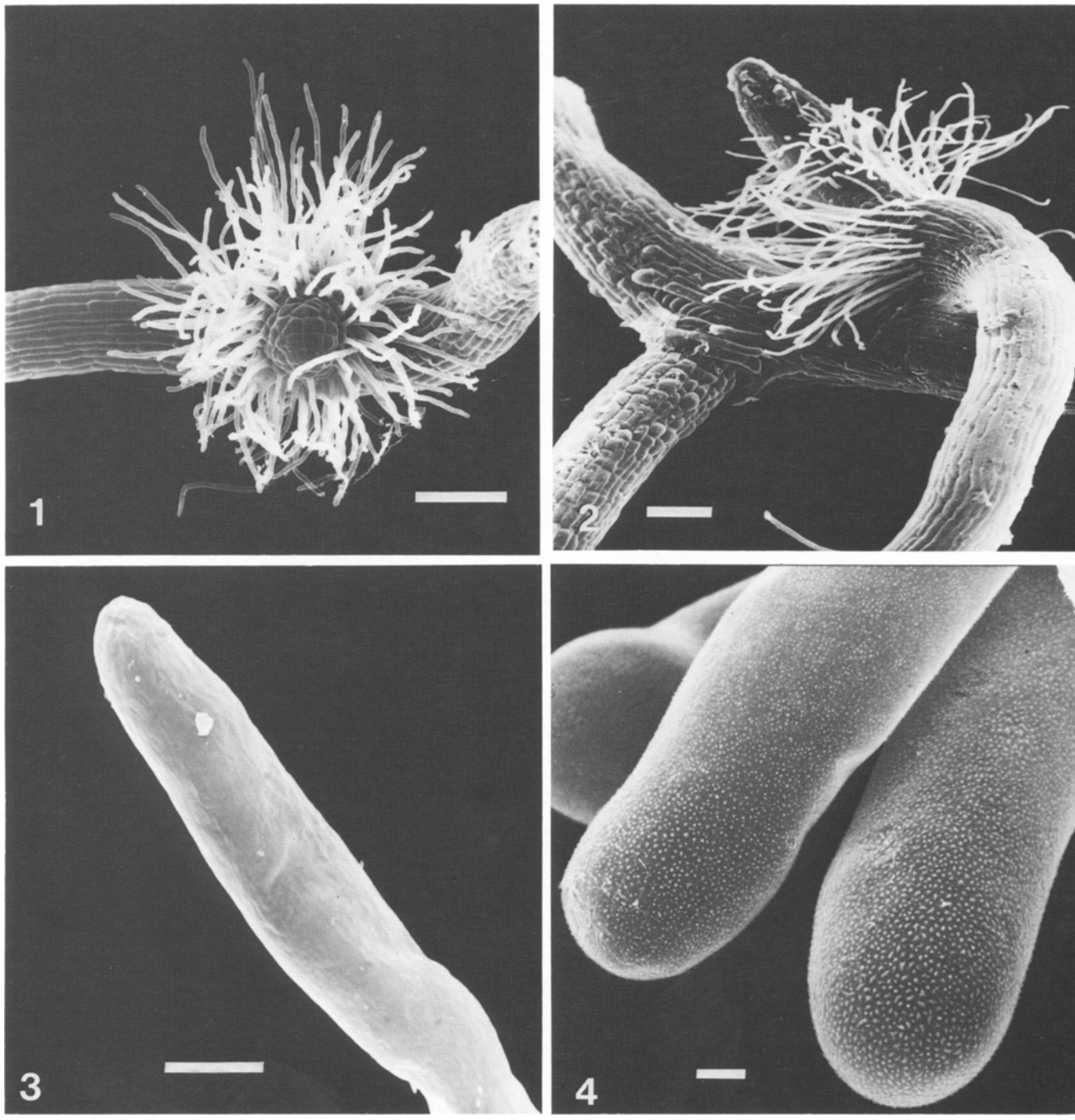

Fig. 1. 48-hour-old haustorium (× 150). Bar represents 100 μm

Fig. 2. 24 hour haustorium in contact with a host root (× 115). Bar represents 100 μm

Fig. 3. Root hair on 48 hour induced root (× 1,500). Bar represents 10 μm

Fig. 4. Haustorial hair on 48 hour unattached haustorium (× 3,600). Bar represents 1 μm

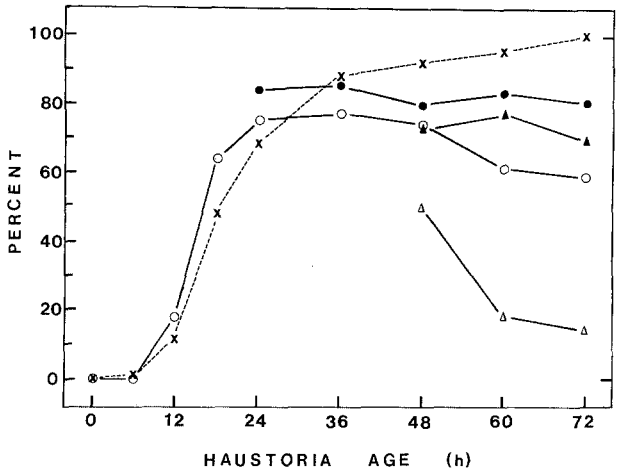

Fig. 5. Comparison of haustoria age (h = hours) and relative percent of haustorial hairs which have papillae. (×) Percent of total hairs per haustorium normalized to 72 hour haustoria. (●) Percent papillate hairs per attached haustorium. (▲) Percent of most-apical hairs which have papillae per attached haustorium. (○) Percent papillate hairs per unattached haustorium. (△) Percent of most-apical hairs which have papillae per unattached haustorium

has established a structural bond. As a result of this separation, a portion of the host surface that corresponds to the contact interface is retained by the hair, or occasionally the hairs may tear loose from the haustorium and remain attached to the host. Two examples of the former, each originally attached to different host cell types, are shown in Figs. 8 and 9. As in Fig. 6, the papillae appear to have coalesced in the contact region between the two appressed surfaces.

Another very interesting characteristic of the haustorial hairs can be observed in Fig. 10. Once the hairs have contacted the host root they continue to grow along it, following closely the contours of the root surface. Haustorial hairs have never been observed to breach the epidermal layer of the host or to penetrate individual epidermal cells, although they will grow into a crevice when one is encountered.

3.3. Release of Attached Haustoria

Following the approach taken by investigators of plant cell wall chemistry, the basic chemical nature of the adhesive material was investigated using selective chemical solubilizations to release attached haustoria. Data from these experiments are summarized in Table 3. In general, haustoria were not released by treatments reported to solubilize waxes, cutins, suberins, lignins or proteins (0-3%), but were released when exposed to carbohydrate solvents. Chemical extractions of non-cellulosic carbohydrates released haustoria in large

Table 3. *Release of attached haustoria*[1]

Waxes:	ethanol	0[25]	chloroform	0[25]		
	methanol	0[25]	acetone	0[25]		
Cutin/suberin:	$LiAlH_4$	0[25]	BF_3(MeOH)	3[30]		
Lignin:	H_2O_2	2[30]	HF	0[25]		
Non-Cellulosic carbohydrates:	oxalate/NaOH	92[134]	nitric/chromatic acid	100[25]		
	H_2O/HOAc	95[40]				
Pectins:	H_2O	0[50]	EDTA	0[84]	pectinase[3]	11[114]
	Na_2CO_3	0[50]	$(NaPO_3)_6$	3[60]	Pectinol-AC[4]	8[50]
	HCl/NH_4OH	0[30]	oxalate	3[119]	Rohament-P[4]	5[90]
Hemicelluloses:	NaOH 0.5 N	76[110]	NaOH 1 N/6 N	94[110]	hemicellulase[3]	26[75]
	NaOH 6 N	80[30][2]	H_2SO_4	15[81]	Rhozyme Hp-150[4]	34[60]
Callose:	$SnCl_2$	0[25]	NaClO	84[25]		
	$CaCl_2$	0[20]	DMSO	26[15]		
Proteins:	SDS/BME	0[20]	trypsin[5]	0[25]	pronase[6]	0[27]

[1] The percent of relased haustoria follows each chemical or enzyme treatment and this is followed in brackets by the total number of haustoria in that treatment. This total includes only haustoria attached to plastic Petri dishes although haustoria attached to host roots demonstrated identical trends between and within categories.

[2] Percent of haustoria, not released by 1 N NaOH treatment, which were released by a subsequent 6 N NaOH treatment.

[3] Sigma; St. Louis, MO.

[4] Röhm Haas, Philadelphia, PA.

[5] Gibco; New York, N.Y.

[6] Calbiochem; San Diego, CA.

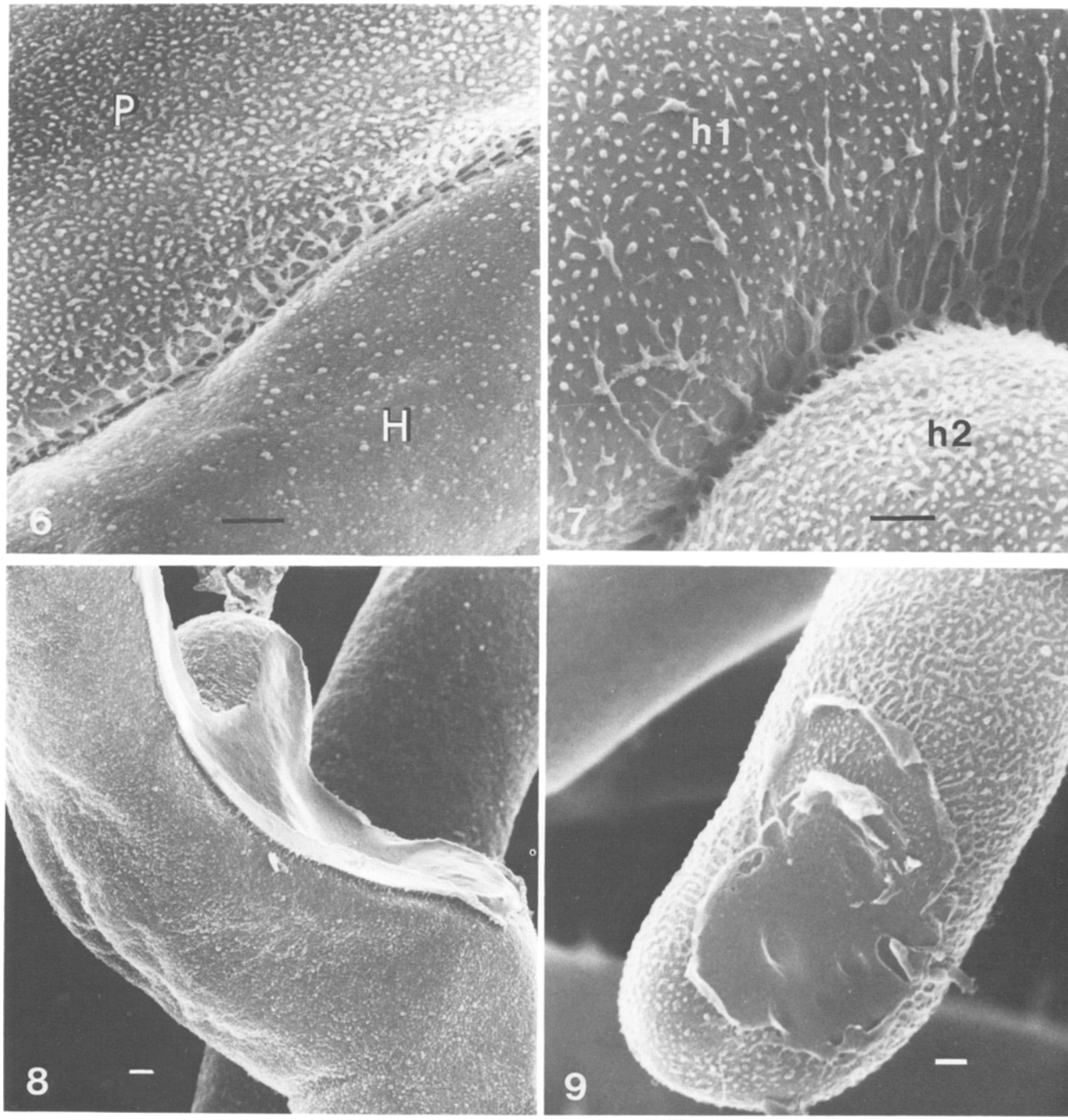

Fig. 6. Interface between haustorial hair of the parasite (*P*) and host root surface (*H*). Many papillae have coalesced and they bridge the gap at the contact region (× 10,000)

Fig. 7. Interface between two attached haustorial hairs (*h 1* and *h 2*) (× 10,500)

Fig. 8. Branched haustorial hair previously attached to parenchyma cells of elder pith. A portion of cell wall remains attached (× 4,000)

Fig. 9. Haustorial hair previously attached to the leaf of *Lespedeza sericea*. A portion of leaf cuticle remains attached (× 5,000). Bars represent 1 μm

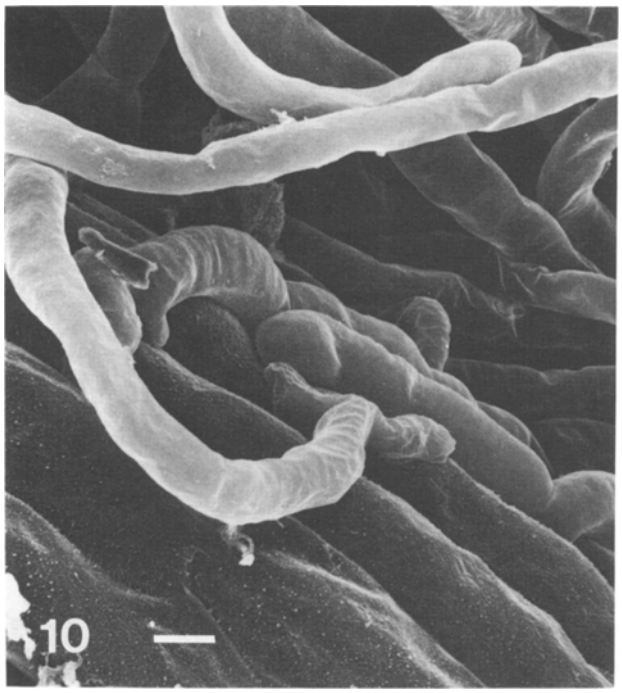

Fig. 10. Host-parasite interface showing haustorial hairs attached and growing along the host root surface (× 800). Bar represents 10 μm

numbers (> 90%). Although the more severe nitric/chromic acid treatment released all attached haustoria within 10-20 minutes of exposure, continued exposure completely dissolved the haustoria. This was the only treatment which altered the cellular integrity of the haustoria; in all other treatments released haustoria were intact.

Calcium chelating agents [EDTA, $(NaPO_3)_6$, Oxalate] and other solvents commonly employed to remove pectins (H_2O, HCl/NH_4OH) do not release haustoria to any appreciable extent (0-3%). Haustoria were released by dilute (76%) and concentrated (94%) alkali. Haustoria were not released by treatments with stannous or calcium chloride but were released by DMSO (26%) and dilute NaClO (84%). Also presented in Table 3 are data from similar experiments using commercially available enzymes. Although these results are consistent with those for the organic and inorganic solvents, they must be considered preliminary in view of the impure nature of these enzyme preparations.

Further verification of the papillae's importance in attachment is provided by examining haustorial hairs treated with these same solvents. Treatments that did not release haustoria also failed to remove papillae to any appreciable extent (Figs. 11 *a* and *b*). Similarly, the treatments previously demonstrated to release haustoria in significant quantities also removed most if not all of the papillae (Figs. 11 *c* and *d*). These data suggest that the papillae are composed of the same substance(s) as that which cements the parasite to the host.

3.4. Attachment Time Course

Our results from experiments using substitute attachment substrates have demonstrated the indiscriminate nature of haustorial attachment. This observation, combined with a previous report that a large number of mature, well developed haustoria failed to attach when presented with a host (RIOPEL and MUSSELMAN 1979), led us to investigate the time course of attachment. This allowed us to determine the relationship between haustorial maturation and attachment competency. In initial experiments, both live hosts and plastic Petri dishes were used as attachment substrates. Since trends in attachment to live hosts paralleled those for Petri dishes, and since increase in the proportion (ratio) of attached haustoria to induced haustoria can be obtained using Petri dishes; the data presented are from experiments using plastic Petri dishes as a substitute attachment substrate.

Table 4 summarizes experiments in which attachment efficiency was monitored at specific intervals over a four-day period. Some adhesion (2%) can occur as early as 6 hours after induction (exposure). At 12 hours, 16% of the haustoria have attached. However, these averages do not reflect the large variation in attachment percentage or the tenuous nature of these early attachments. Significantly higher levels of attachment ($t_7 = 3.61$, $p < 0.05$) are reached by 18 hours post-induction, at which time over 60% of the developing haustoria have

Table 4. *Attachment time-course*[1]

Haustoria age in hours	Percent attached $\bar{x} + SD$[2]
0	0
6	2.1 ± 2.6
12	15.6 ± 10.1
18	68.2 ± 4.2
24	83.2 ± 7.4
36	91.0 ± 4.3
48	94.6 ± 2.8
60	96.0 ± 4.0
72	95.0 ± 4.4
96	97.5 ± 3.0

[1] Developing haustoria constantly in contact with the attachment substrate.

[2] N = 8; average number of haustoria per time point = 75.

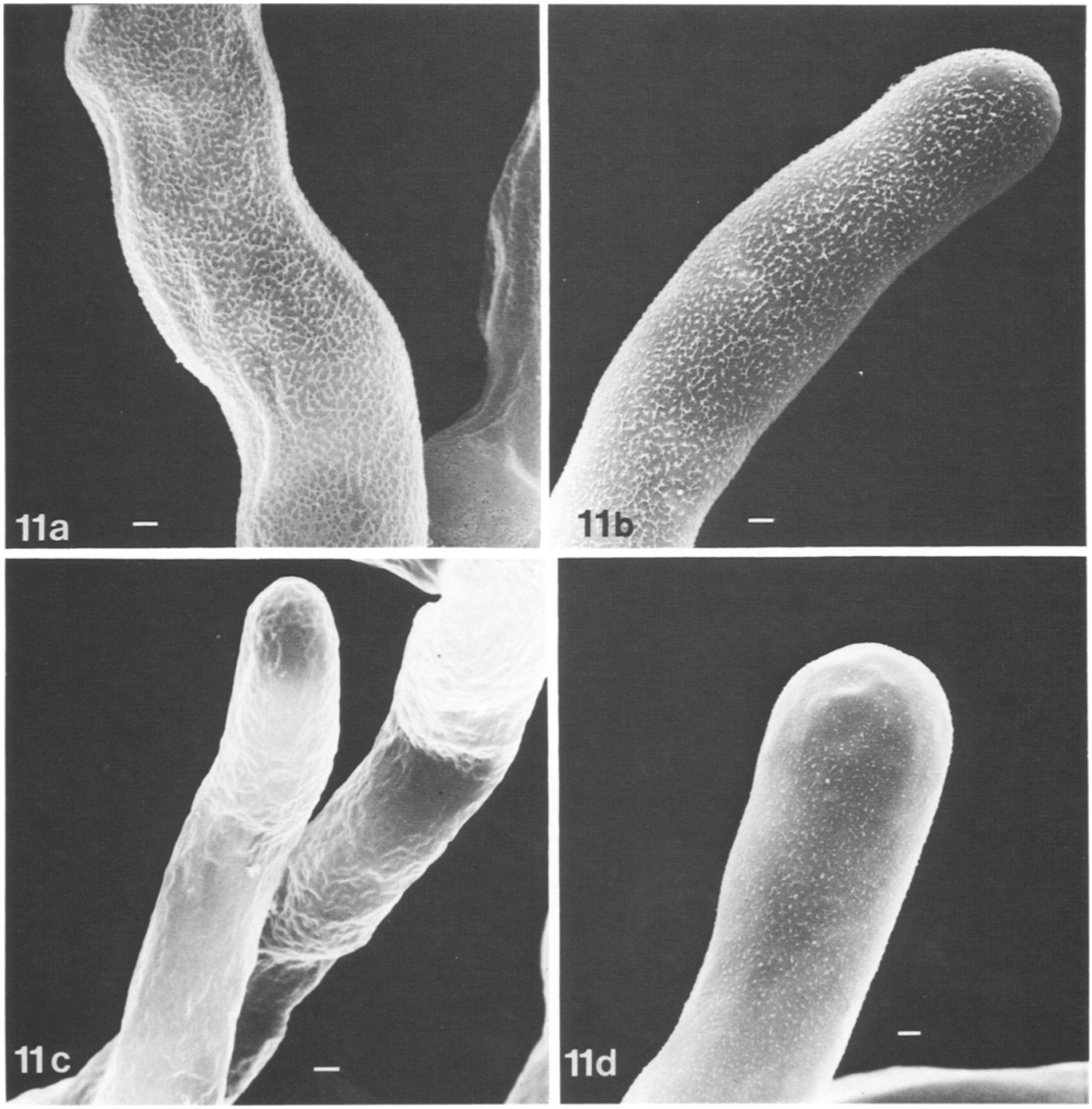

Fig. 11. 48 hour haustorial hairs exposed to various chemical extractions or enzyme treatments. *a* Pronase-treated hair (× 3,800). *b* Oxalate-treated hair (× 3,700). *c* Hemicellulase-treated hair (× 3,600). *d* 1 N NaOH-treated hair (× 3,600). (Bar in each photograph represents 1 μm)

attached. Maximal levels of attachment (> 90%) have been attained by 36 hours.

The length of time haustoria retain the capacity to attach to a host was then investigated. In these experiments haustoria were induced without a host, then at 12-hour intervals plants with developing haustoria were placed in contact with the substrate. Table 5 presents the results of these experiments. Haustoria of a particular age group were found to require a minimum of 18–24 hours, after host contact, to reach peak levels of attachment. The data for 24 (and 36) hours after transfer show that the percent attachment decreases as

Table 5. *Attachment of Agalinis after delayed contact*

Haustoria age[1] at time of contact with attachment substrate	Percent attached: $\bar{x}$ + SD[2] (Hours after contact) (12)	(24)	(36)
0	20 ± 13.4	85 ± 2.8	93 ± 2.0
12	45 ± 21	82 ± 7.0	86 ± 3.8
24	62 ± 12	76 ± 1.4	75 ± 2.1
36	43 ± 13	51 ± 1.4	53 ± 5.0
48	24 ± 1.4	27 ± 2	25 ± 0
60	3 ± 4.6	3 ± 4.2	5 ± 7.3
72	6 ± 4.4	0 ± 0	2 ± 3.8
84	3 ± 4.0	1 ± 2.1	0 ± 0
96	0 ± 0	0 ± 0	0 ± 0

[1] In hours.
[2] For each time point N = 3; average number of haustoria = 50.

Table 6. *Reattachment of haustoria after delayed contact*

Haustoria age[1] at time of dislodging	Percent reattached[2]: $\bar{x}$ + SD[3] (Hours after dislodging before attachment substrate was reintroduced) (0)	(12)	(24)
0	91.0 ± 10.3	82.0 ± 7.0	76.0 ± 1.4
12	90.1 ± 5.3	80.1 ± 9.3	69.0 ± 15.1
24	79.7 ± 8.3	74.1 ± 4.1	30.0 ± 0.0
36	72.5 ± 11.6	58.1 ± 7.3	30.0 ± 2.0
48	54.1 ± 7.0	29.6 ± 5.6	20.5 ± 3.5
60	32.0 ± 5.7	25.8 ± 4.3	14.0 ± 2.0
72	23.2 ± 4.5	19.6 ± 0.5	6.5 ± 1.5
96	14.3 ± 3.6	13.9 ± 0.5	11.7 ± 1.6

[1] In hours.
[2] Counted 24 hours after reintroduction of host.
[3] N = 4; average of 60 haustoria per time point.

the interval between induction and substrate contact is increased. There is a marked decrease in attachment frequency for haustoria older than 24 hours, such that by 60 hours or older, haustorial attachment percentages are not significantly different from zero ($p < 0.05$).

The capacity of previously attached haustoria, dislodged by gentle pressure to the roots, to reattach was also investigated. Attached haustoria of specific ages were dislodged and then returned to the substrate after 0, 12, or 24 hours. Twenty-four hours later the number of reattached haustoria was determined. Results of this experiment are summarized in Table 6. As in the previous experiment, a decrease in attachment percentage with increasing haustorial age is seen. In Fig. 12, the attachment competency for similarly aged, unattached or previously attached haustoria are compared during development. It is seen that attachment percentages are as high or higher, and that attachment competency (especially for haustoria 60 hours or older) is maintained longer, for previously attached haustoria than for unattached ones.

One explanation for this might be that previously attached haustoria are able to reattach because the cementing substances remains adhesive after the haustoria have been dislodged. However, the observation that older, dislodged haustoria reattach at consistently lower frequencies than younger haustoria seems to argue against this explanation. To investigate this possibility further, roots with attached haustoria were killed by placement in 5% solutions of glutaraldehyde or sodium azide for 6 hours, and then dislodged and immediately placed back in contact with the substrate.

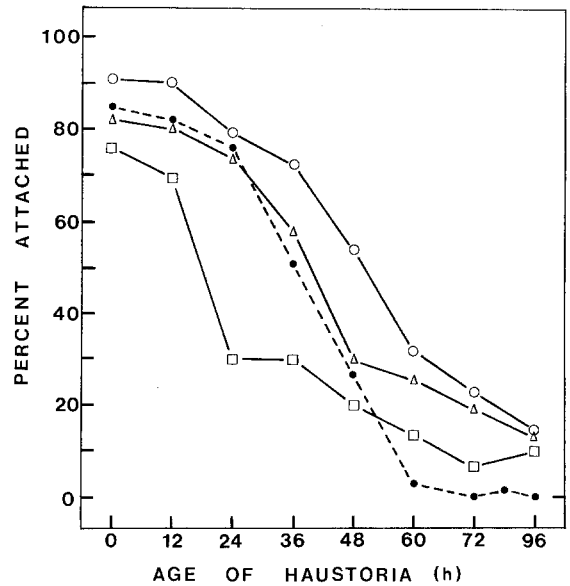

Fig. 12. Comparison of attachment percentages for unattached and dislodged haustoria after delayed contact with the attachment substrate. (●) Percent attachment of *unattached* haustoria versus age of haustoria when placed in contact with substrate. Percent attachment of *previously attached* haustoria versus age of haustoria at time of dislodging and then reintroduction of attachment substrate delayed for zero hours (○); delayed 12 hours (△); delayed 24 hours (□). Age of haustoria is in hours (h)

Although these haustoria have hairs with papillae, they were rarely if ever observed to reattach (Table 7). Reattachment at levels equal to those of intact haustoria (Table 6) or at levels of haustoria severed from the mother root (Table 7) would be expected if reattach-

Table 7. *Reattachment of treated haustoria*

Haustoria age[1] at time of treatment and dislodging	Percent reattached[2]: $\bar{x} + SD$[3]	
	Treatment[4]	
	Severed	Killed[5]
12	94.4 ± 3.2	0 ± 0
18	76.2 ± 13.0	0.9 ± 2.5
24	60.9 ± 20.7	3.8 ± 3.1
36	41.5 ± 9.6	1.3 ± 2.1
48	31.7 ± 16.7	0 ± 0

[1] In hours.
[2] Counted 24 hours after treatment.
[3] N = 4; average of 60 haustoria per time point per treatment.
[4] Haustoria constantly in contact with the attachment substrate prior to dislodging.
[5] Combined results for both glutaraldehyde and sodium azide.

ment was solely a function of the cement retaining its adhesive property.

4. Discussion

Cell attachment is well documented for both plants and animals. This information has come from studies on the interactions between cell types of a single species as well as from combinations (symbioses) involving two or more species (GRINNELL 1978, MARCHASE *et al.* 1978, TURNER 1978, DAZZO 1980, HARRISON and CHESTERTON 1980, REISERT 1981). Many of these studies examined molecular aspects of cell recognition phenomena. In most cases attachment is obligate for continued development and has a profound influence on the subsequent physiology and morphogenesis of the partners.

We have demonstrated that the hairs which grow from the apex of the developing haustorium of *Agalinis* play an important role in attachment. They are the first to contact a host, and show modifications (from typical root hairs) that participate in the formation of a structural bond with the host root surface. Only a few authors have mentioned these hairs, and typically as part of broad anatomical studies, (CHUANG and HEKKARD 1971, DOBBINS and KUIJT 1973 a, MUSSELMAN and DICKISON 1975, KUIJT 1977). WEBER and UHLARZ (1976) described hairs on the haustoria of *Pedicularis rostratospicata* (*Scrophulariaceae*) which appear to clasp the host root. This is similar to what we have found for *Agalinis* (Figs. 4 and 10). ATSATT and MUSSELMAN (1977) reported a mucilage-like material covering the root system of *Orthocarpus purpurascens* (*Scrophulariaceae*) grown in agar culture, and speculated on its origin and relationship to other anomalous secretions in parasitic flowering plants. Since attached haustoria were not examined these authors did not suggest any role for the hairs or the mucilage-like-material in attachment.

Many investigators have reported on the occurrence of a secreted mucilaginous layer associated with the root epidermis and especially the root cap (*e.g.*, ROUGIER 1981; and references therein). This root slime or "mucigel" (JENNY and GROSSENBACHER 1963) is composed chiefly of an acidic polysaccharide, most likely a pectic compound (HARRIS and NORTHCOTE 1970, GREAVES and DARBYSHIRE 1972, WRIGHT and NORTHCOTE 1974, PAULL *et al.* 1975), and has been implicated in binding of soil particles and microorganisms (JENNY and GROSSENBACHER 1963, DAZZO 1980, HINCH and CLARKE 1980). Our studies indicate that the haustorial hairs of *Agalinis purpurea* also secrete extracellular non-cellulosic polysaccharides which are important in attachment. Our findings, through not conclusive proof, suggest that the cementing substance is composed of alkali labile polysaccharides and can, therefore, be broadly classified as hemicellulose. This is significant in view of the work of LAPP and SKOROPAD (1978) in which these authors concluded that hemicellulose is responsible for the adhesion of appresoria of the fungus *Colletotrichum graminicola*. Our finding that hemicellulose, rather than pectin, may be the functionally significant component of the adhesive papillae also serves to emphasize the specialized synthetic nature of the haustorial hairs. It will therefore be important to make a more qualitative determination of the composition of the cementing material. However, this material is too diffuse and too slight in quantity to permit the application of current chromatographic and spectrographic methods.

The cementing substance is visualized as an extracellular, papillate coating over the surface of most haustorial hairs (Fig. 4) and occasionally the haustorial apex. The latter case is quite variable in its occurrence, and typically functioned much later in the attachment sequence prior to penetration. At the parasite-host interface the papillae become compressed into a reticulate pattern much as drops of an adhesive would respond when pressed between two surfaces (Figs. 6–9). The papillate morphology is not an artifact of specimen preparation for the SEM, because papillae were observed on non-coated as well as freeze-dried haustoria.

Functional specialization of the haustorial hairs is an

interesting finding and their potential role in host penetration seems clear. The observation that hairs of detached haustoria retain portions of the host surface (or tear away from the haustorium and remain attached to the host) indicates that these hairs are securely bonded to the host (Figs. 8 and 9). Also, the continued growth of attached hairs along the host root surface would serve to increase the effective surface area involved in adhesion (Figs. 2 and 10). As a result hair attachment secures the haustorium firmly to the host surface reinforcing the subsequent thrust of the penetrating endophyte (haustorial wedge). Also, soil surrounding the roots may participate as an additional stabilizing factor during host penetration.

During the pre-contact stage growth is characterized by an increase in cell size and number, and the haustorium is principally a parenchymatous organ with minimal internal differentiation. One exception to this is a group of densely protoplasmic cells which occupy the haustorial apex during this period (BAIRD and RIOPEL 1983). Development of hairs also continues. In our experiments haustoria attach in significant numbers by 12–18 hours post-induction and reach a maximal level of attachment efficiency by 36 hours. Haustoria 60 hours or older no longer attach when placed in contact with a host (Table 5).

In view of the continued growth of haustoria, the restriction of attachment competency to such a short period of time was not expected. We have also observed that dislodged haustoria can readily reattach with high frequency at ages when unattached haustoria cannot (Fig. 6). In effect, one influence of attachment is to prolong the period of attachment competency.

The precise cellular changes that characterize the pre- and post-contact period are not well defined. Our studies with *Agalinis* emphasize that host contact is an important developmental signal required to sustain haustorial differentiation and promotes within this organ cellular events, releated to host attachment and penetration, that would otherwise not occur. For example, in *Agalinis* host attachment results in the continued production of adhesion papillae on developing hairs. Comparing Tables 4 and 5 with Fig. 5 shows that changes in attachment competency correlate well with those for the production of papillate hairs. As attachment efficiency increases with haustorial age, the total number of haustorial hairs as well as the percent of these hairs which have papillae also increases. Similarly, the marked reduction in attachment efficiency for unattached haustoria 60 hours and older is accompanied by a decrease in the percent papillate hairs per haustorium. This is in contrast to attached haustoria where the percent of papillate hairs is maintained at a constant level over the 24- to 72-hour post-induction period.

We conclude that the development of the haustorium in *Agalinis* is influenced by at least two exogeneous signals. The first is a recognition event in which the parasite root receives a precise chemical stimulus from the host (RIOPEL and MUSSELMAN 1979, LYNN *et al.* 1981, STEFFENS *et al.* 1982). This sets in motion the initiation of the haustorium which reaches attachment competency in less than 24 hours. Without a second stimulus (tactile or chemical?) from the host, differentiation of the haustorium is arrested and ontogenetic changes characteristic of the penetration stage do not occur; although growth continues slowly for about a month. With contact and adhesion the haustorium shifts to the host penetration mode. Unlike the initiation signal, host attachment, though important to complete haustorial maturation, is not a discriminating event. The attachment surface is not required to convey, nor does the haustorium recognize, any specific chemical or physical information in order to effect attachment.

It should be emphasized that *Agalinis purpurea* has a broad host range (Table 1). The relevance of these findings for plants like *Striga* and *Orobanche*, which show distinct host preferences, is not known. It remains possible that in these more evolved host-recognition systems both the attachment and penetration stages will be discriminatory.

Acknowledgements

We thank Mrs. JUDITH KINNIER for invaluable technical assistance, Dr. LYTTON J. MUSSELMAN (LJM) for seed collections of *Agalinis purpurea*, and Mrs. CAROLYN BAIRD and Ms. DESEREE STUKES for typing of the manuscript. Supported by USDA Competitive Research Grant 5901-0410-9-0257, USDA Cooperative Research Agreement 58-7B30-0-196 (JLR) and a Biology Department Pratt Fellowship (WVB).

References

ASAMIZU, T., NISHI, A., 1980: Regenerated cell wall of carrot protoplasts isolated from suspension-cultured cells. Physiol. Plant. **48**, 207–212.

ATSATT, P., MUSSELMAN, L., 1977: Surface characteristics of roots and haustoria of *Orthocarpus purpurascens* (*Scrophulariaceae*). Beitr. Biol. Pflanzen **53**, 359–370.

– HEARN, T., NELSON, R., HEINEMAN, R., 1978: Chemical induction and repression of haustoria in *Orthocarpus purpurascens* (*Scrophulariaceae*). Ann. Bot. **42**, 1177–1184.

BAIRD, V., RIOPEL, J., 1983: Experimental studies of haustorium initiation and early development in *Agalinis purpurea*. (Submitted).

CHUANG, T., HECKARD, L., 1971: Observations on root-parasitism in *Cordylanthus* (*Scrophulariaceae*). Amer. J. Bot. **58**, 218–228.

DAZZO, F., 1980: Adsorption of microorganisms to the roots and other plant surfaces. In: Adsorption of Microorganisms to Surfaces (BRITTON, G., MARSHALL, K., eds.), pp. 253–316. New York: J. Wiley & Sons.

DOBBINS, D., KUIJT, J., 1973a: Studies on the haustorium of *Castilleja* (Scrophulariaceae). I. The upper haustorium. Can. J. Bot. **51**, 917–922.

– – 1973b: Studies on the haustorium of *Castilleja* (*Scrophulariaceae*). II. The endophyte. Can. J. Bot. **51**, 923–931.

EPLEE, R., 1981: *Striga*'s status as a plant parasite in the United States. Plant Dis. **65**, 951–954.

ESPELIE, K., DAVIS, R., KOLATTUKUDY, P., 1980: Composition, ultrastructure and function of the cutin- and suberin-containing layers in the leaf, fruit peel, juice-sac and inner seed coat of grapefruit (*Citrus paradisi* Macfed.). Planta **149**, 498–511.

FACEY, V., 1950: Abscission of leaves in *Fraxinus americana* L. New Phytol. **49**, 103–116.

GREAVES, M., DARBYSHIRE, J., 1972: The ultrastructure of the mucilaginous layer on plant roots. Soil Biol. Biochem. **4**, 443–449.

GRINNELL, G., 1978: Cellular adhesiveness and extracellular substrata. Int. Rev. Cytol. **53**, 65–144.

GRUSAK, M., THOMAS, R., MARSH, B., 1980: Wall regeneration by plasmolysed cells from tissue suspension cultures of *Sphaerocarpus dunnellii*. J. Cell Sci. **43**, 167–175.

HARRIS, P., NORTHCOTE, D., 1970: Patterns of polysaccharide biosynthesis in differentiating cells of mature root tips. Biochem. J. **120**, 479–491.

HARRISON, L., CHESTERTON, J., 1980: Factors mediating cell-cell recognition and adhesion. FEBS Letters **122**, 157–180.

HINCH, J., CLARKE, A., 1980: Adhesion of fungal zoospores to root surfaces is mediated by carbohydrate determinants of the root slime. Physiol. Pl. Path. **16**, 303–307.

JARVIS, M., HALL, M., THRELFALL, D., FRIEND, J., 1981: The polysaccharide structure of potato cell walls: chemical fractionation. Planta **152**, 93–100.

JENNY, H., GROSSENBACHER, K., 1963: Root-soil boundary zone as seen in the electron microscope. Proc. Soil Sci. Amer. **27**, 273–277.

JENSEN, W., 1962: Botanical Histochemistry. San Francisco: Freeman and Co.

JOHANSEN, D., 1940: Plant Microtechnique. New York: McGraw-Hill Co.

KARNOVSKY, M., 1965: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. **27**, 137a–138a.

KELLEY, R., DEKKER, R., BLUEMINK, J., 1975: Thiocarbohydrazide-mediated osmium binding. In: Principles and Techniques in Scanning Electron Microscopy. Biological Applications, Vol. 4 (HAYAT, M., ed.), pp. 34–44. New York: Van Nostrand Reinhold Co.

KIRBY, E., ROBERTS, R., 1971: The localized incorporation of ^{3}H-L-fucose into cell-wall polysaccharides of the cap and epidermis of corn roots. Planta **99**, 211–221.

KROH, M., KNUIMAN, B., 1982: Ultrastructure of cell wall and plugs of tobacco pollen tubes after chemical extraction of polysaccharides. Planta **154**, 241–250.

KUIJT, J., 1969: The Biology of Parasitic Flowering Plants. Berkeley: Univ. Calif. Press.

– 1977: Haustoria of phanerogamic parasites. Ann. Rev. Phytopathol. **17**, 91–118.

LAEMMLI, W., 1970: Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (London) **227**, 680–685.

LAPP, M., SKOROPAD, W., 1978: Nature of the adhesive material of *Colletotrichum graminicola* appressoria. Trans. Br. mycol. Soc. **70**, 221–223.

LYNN, D., STEFFENS, J., KAMAT, V., GRADEN, D., SHABANOWITZ, J., RIOPEL, J., 1981: Isolation and characterization of the first host recognition substance for parasitic angiosperms. J. Am. chem. Soc. **103**, 1868–1870.

MARCHASE, R., VOSBECK, K., ROTH, S., 1978: Intercellular adhesive specificity. Biochim. biophys. Acta **457**, 385–416.

MINASSIAN, H., HUANG, S., 1979: Effects of sodium azide on the ultrastructural preservation of tissues. J. Microsc. **117**, 243–253.

MURASHIGE, T., SKOOG, F., 1962: A revised medium for rapid growth and bio assays with tobacco culture. Physiol. Plant. **15**, 473–497.

MUSSELMAN, L., 1980: The biology of *Striga*, *Orobanche*, and other root-parasitic weeds. Ann. Rev. Phytopathol. **18**, 463–489.

– DICKISON, W., 1975: The structure and development of the haustorium in parasitic *Scrophulariaceae*. Bot. J. Linn. Soc. **70**, 183–212.

NISHITANI, K., MASUDA, Y., 1982: Roles of auxin and gibberellic acid in growth and maturation of epicotyls of *Vigna angularis*: Cell wall changes. Physiol. Plant. **56**, 38–45.

PAULL, R., JOHNSON, C., JONES, R., 1975: Studies on the secretion of maize root cap slime. I. Some properties of the secreted polymer. Plant Physiol. **56**, 300–306.

RAY, P., BAKER, D., 1965: The effect of auxin on synthesis of oat coleoptile cell wall constituents. Plant Physiol. **40**, 353–360.

REID, J., WILKIE, K., 1969: Polysaccharides of the oat plant in relationship to plant growth. Phytochem. **8**, 2045–2051.

REISERT, P., 1981: Plant cell wall structure and recognition phenomena with reference to symbioses. Int. Rev. Cytol., Suppl. **12**, 71–112.

REYNOLDS, J., ROBERTS, R., 1976: Cytochemical analysis of callose localization in *Lilium longiflorum* pollen tubes. Ann. Bot. **40**, 409–416.

RIOPEL, J., 1979: Experimental studies on induction of haustoria in *Agalinis purpurea*. In: Proceedings of the 2nd International Symposium on Parasitic Weeds (MUSELMAN, L., WORSHAM, D., EPLEE, R., eds.), pp. 165–173. North Carolina State University, Raleigh, North Carolina.

– MUSSELMAN, L., 1979: Experimental initiation of haustoria in *Agalinis purpurea* (*Scrophulariaceae*). Amer. J. Bot. **66**, 570–575.

ROUGIER, M., 1981: Secretory activity of the root cap. In: Encyclopedia of Plant Physiology, Vol. 13B (TANNER, W., LOEWUS, F., eds.), pp. 542–574. New York: Springer.

STEFFENS, J., LYNN, D., KAMAT, V., RIOPEL, J., 1982: Molecular specificity of haustorial induction in *Agalinis purpurea* (L.) Raf. (*Scrophulariaceae*). Ann. Bot. **50**, 1–8.

TOTH, R., KUIJT, J., 1977: Anatomy and ultrastructure of the haustorium in *Comandra* (*Santalaceae*). Can. J. Bot. **55**, 455–469.

TSIVION, Y., 1979: Physiological concepts of the association between parasitic angiosperms and their hosts – A review. Israel J. Bot. **27**, 103 – 121.

TURNER, R., JR., 1978: Sponge cell adhesion. In: Receptors and Recognition. Series B, Vol. 4 (GARROD, D., ed.), pp. 199 – 232. New York: Chapman & Hall Press.

VITHANAGE, H., GLEESON, P., CLARKE, A., 1980: The nature of callose produced during self-pollination in *Secale cereale*. Planta **148**, 498 – 510.

WEBER, H., 1981: Untersuchungen an parasitischen Scrophulariaceen (Rhinanthoideen) in Kultur. I. Keimung und Entwicklungsweise. Flora **171**, 23 – 28.

– UHLARZ, H., 1976: Die Kontaktaufnahme parasitischer Rachenblütler mit den Wurzeln ihrer Wirtspflanzen. Naturwissenschaften **63**, 296.

WERTH, C., RIOPEL, J., 1979: A study of the host range of *Aureolaria pedicularia* (L.) Raf. (*Scrophulariaceae*). Amer. Midl. Nat. **102**, 300 – 306.

WRIGHT, K., NORTHCOTE, D., 1974: The relationship of root cap slimes to pectins. Biochem. J. **139**, 525 – 534.