

Characterization and Functions of the Whitefly Egg Pedicel

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For the silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring) (Homoptera: Aleyrodidae), scanning and transmission electron microscopic techniques were used to observe the characteristics of egg oviposition into both plant cells/tissues and artificial membranes, and to document the morphology of mature egg pedicels removed from the ovaries of females. The exterior of the distal portion of the pedicel consisted of a tangled array of fibrous structures (0.2–0.3 μm in diameter) that constituted about 20–25% of the outer diameter of the pedicel. The attachments of the fibers to the core of the pedicel suggested that the pedicel functions as the collector and conduit for water (vapor), and perhaps solute movement into the egg. Silverleaf whitefly eggs on membranes were incubated at various levels of relative humidity and the eggs were scored for egg hatch. At 98–100% rh, the percentage egg hatch was 86–98%. At lower humidity ranges of 0–20, 55–65, and 75–85% rh, none of the eggs hatched. Media (solute) uptake by silverleaf whitefly egg pedicels was determined by exposing the pedicel side of eggs oviposited on membranes to media solutions containing the high molecular weight polysaccharide, [¹⁴C]-inulin. Solute uptake by the pedicel and movement into developing silverleaf whitefly eggs were demonstrated using [2-¹⁴C]-acetate, and assaying for radioactivity in hatched nymphs. These studies, using exposure of pedicels to relative humidity and radiolabeled materials, demonstrate that whitefly egg hatch is dependent upon water uptake by the pedicel, and that the pedicel has the ability to transport solutes into the developing egg. Arch. Insect Biochem. Physiol. 49:22–33, 2002. Published 2002 Wiley-Liss, Inc.[†]

KEYWORDS: electron microscopy; morphology; ovary; artificial membrane; nymph; water uptake; solute uptake.

INTRODUCTION

The eggs of all whiteflies possess an extension of the chorion called a pedicel (Byrne and Bellows, 1991) by which the female attaches the egg to the host (Gill, 1990). For some whitefly species, the egg pedicel is inserted directly into the host plant stomata. For others, including the silverleaf whitefly, *Bemisia argentifolii* (Bellows and Perring) [=Sweet-potato whitefly, *Bemisia tabaci* (Gennadius), Biotype

B] and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), the pedicel is inserted into a slit made in the plant by the female ovipositor (Deshpande, 1936; Paulson and Beardsley, 1985). In addition to “anchoring” the egg to the host plant leaf, researchers have previously suggested that the primary function of the whitefly egg pedicel is to serve as the primary conduit through which moisture is absorbed from the host plant. As early as 1931, Weber observed that *T. vaporariorum* secrete a

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Abbreviations used: rh = relative humidity; SEM = scanning electron microscopy; TEM = transmission electron microscopy.

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Received 20 December 2000; Accepted 26 July 2001

glue-like substance around the pedicel during insertion into plant cells and suggested that water passes osmotically across the glue and enters the egg through the pedicel. Over the years, others have supported that suggestion, but without direct evidence. However, in 1990, Byrne et al., using tritiated water to irrigate the host plant, presented evidence that water is transferred from leaf tissue into the eggs of *T. vaporariorum* (Byrne et al., 1990).

In our study, microscopic analyses using SEM and TEM techniques revealed the characteristics of *B. argentifolii* oviposition into both plant cells/tissues and artificial membranes. The pedicels of *B. argentifolii* eggs oviposited on artificial membranes were exposed to radiolabeled solutions or various levels of relative humidity. SEM and TEM analyses of mature *B. argentifolii* eggs removed from ovaries were used to reveal external and internal structural features of the distal end of the pedicel. The results of this study provide a functional correlation between observed morphological characteristics of the pedicel and experimental data for the water/solute uptake by the egg pedicel.

MATERIALS AND METHODS

Materials

Inulin in the form of [methoxy- ^{14}C] inulin-methoxy (16 mCi/g) and [2- ^{14}C] acetic acid, sodium salt (54 mCi/mmol) were purchased from New England Nuclear, Boston, MA. Ecolite (liquid scintillation cocktail) was purchased from ICN Biomedicals, Costa Mesa, CA. Tissue solubilizer (0.5 M quaternary ammonium base) was purchased from Beckman Instruments, Fullerton, CA. Tousimis/Millonig's buffer (Millonig, 1961), 2.5% glutaraldehyde in Millonig's buffer and Spurr's epoxy resin were purchased from Tousimis Research Corp., Rockville, MD.

Insects

Silverleaf whiteflies, *B. argentifolii*, were initially obtained from the USDA-ARS Western Cotton Research Laboratory, Phoenix, AZ. In Fargo, a silverleaf whitefly colony was subsequently maintained on

either cotton or hibiscus plants and greenhouse whiteflies, *T. vaporariorum* (obtained from local greenhouses), were maintained on either tobacco or tomato plants. Colony insects on plants were kept in cages that were placed within a walk-in environmental chamber equipped with 485 W high-pressure sodium lamps on a cycle of 15-h light at 28°C and 9-h dark at 22°C.

Artificial Membrane Apparatus

A typical artificial membrane system was constructed using one-ounce clear styrene cups. The top of one cup (40 mm in diameter) was covered with a stretched piece of Parafilm M[®]. Approximately 5 ml of media (usually 20% sterile sucrose) was pipetted onto this inner membrane. The surface of the media was then covered with a second piece of thinly stretched (5–7 μm) Parafilm (outer membrane). The edges of the Parafilm were pressed onto the sides of the cup. To make a leak-proof seal, a styrene ring was cut from the top 3 mm of another one-ounce cup and was placed rim-side down squarely atop the outer membrane. This assembly was held together by tightly wrapping a 5 × 80 mm strip of Parafilm around the circumference of the cut ring and the top of the cup.

Egg Oviposition on Artificial Membranes

The fully assembled membrane cup was inverted over a 40-mm hole in the top of a cage containing leaves with emerging or young whitefly adults. Adults were obtained from either the *B. argentifolii* colony reared on cotton or hibiscus plants or the *T. vaporariorum* colony reared on tobacco. Females were lured to the membrane surface by covering the bottom of the inverted cup assembly with yellow cellophane. Illumination from above with an incandescent light made the membrane surface appear yellow; a color that is known to attract whitefly adults. After 24 h, the membrane assembly was removed from the cage and feeding adults were removed from the membrane. To access the pedicel side of the egg-laden outer membrane, the bottom two-thirds of the cup was cut away and the exposed

portion of the inner membrane was excised to expose the media. The media was then carefully removed; leaving the intact membrane surface with exposed egg pedicels. To remove residual media, the membrane surface was rinsed three times with sterile deionized water and blotted dry.

Egg Pedicel Exposure to Water Vapor

For exposure to relative humidity (rh), egg-laden membranes were placed within an air-tight plastic cabinet (9"W × 8"D × 5"H) and held at 25°C. For radiolabel uptake experiments, the pedicels of oviposited eggs on artificial membranes were submerged in test solutions, placed in the plastic cabinet, and incubated at 98–100% rh. For experiments on the effects of rh on egg development and hatch, eggs attached to membranes were exposed to various levels of relative humidity for the normal number of days (6–7) necessary for egg hatch when egg pedicels are in constant contact with water. An rh of 0–20% within the airtight cabinet was achieved by placing a layer of anhydrous calcium sulfate (Drierite®) on the bottom of the cabinet. An rh of 55–65% was achieved by normal humidity control of the reach-in environmental cabinet used in the experiments. An rh of 75–85% was achieved by placing water-dampened paper towels in the bottom of the airtight cabinet. An environment of near saturation with water vapor (98–100%) was achieved by placing an open container of water in the bottom of the cabinet and using paper toweling dipped into the water container.

Pedicel Uptake of ¹⁴C-Inulin and ¹⁴C-Acetate

For experiments involving labeled inulin or acetate uptake into whitefly egg pedicels, oviposition was restricted to small circular portions of artificial membranes. The restriction of the egg pedicels to a small target area (10 mm in diameter) was desirable for avoiding excessive dilution of radiolabeled inulin or acetate. The restricted portion was obtained by fitting the membrane side of the apparatus with a 40-mm circle of black paper with a 10-mm diameter cutout in the center. After 24 h of exposing the al-

tered membrane apparatus to ovipositing female whiteflies, the black-paper circle, the inner membrane, and the media were carefully removed.

For labeled inulin uptake, a 100–300 µl drop of ¹⁴C-inulin in 7.5% sterile sucrose (0.44–0.56 µCi/100 µl) was placed atop the 10-mm diameter cluster of protruding egg pedicels on the inner side of the outer membrane. After 48 h at 25°C and 100% rh, the ¹⁴C-inulin solution was removed from the membrane. The membrane was quickly rinsed with fresh media to remove any adhering ¹⁴C-inulin. Controls consisted of a 100–300 µl drop of ¹⁴C-inulin in media on portions of membranes without oviposited eggs. Portions of membranes with attached eggs and without eggs (controls) were placed in a 7-ml glass counting vial containing 0.5 ml of water. For homogenization of eggs, membrane pieces in water were frozen by submersion in a dry ice/ethanol bath, thawed, and the freeze/thaw procedure was repeated twice. The thawed suspension was sonicated with a Brauns Sonic Model 1510 equipped with a 4-mm needle probe. The homogenate was diluted to 6–7 ml with Ecolite liquid scintillation cocktail and assayed for ¹⁴C using a Packard (Model 2300TR) liquid scintillation analyzer.

For uptake of radiolabeled material into nymphs, egg pedicels were exposed to radiolabeled acetate (20–55 µCi/100 µl) in 15% sucrose or water for 4 days. Immediately after removal of the labeled solution, the pedicel side of the membrane was rinsed thoroughly with water and then exposed to 98–100% rh until egg hatch. Emerged nymphs were counted and transferred into 20-ml scintillation counting vials. Beckman tissue solubilizer (0.5 ml) was added to each vial. The vials were capped and heated at 60–70°C for 24 h to digest the nymphs. After cooling, 19.5 ml of Ecolite was added, and each vial assayed for ¹⁴C.

Extraction of Eggs from Membranes, Leaf Tissue, and Female Ovaries

Eggs on membranes and leaves were removed using carbon adhesive tabs attached to aluminum SEM specimen mounts. Portions of egg-laden membranes or leaves were lightly pressed against

the adhesive surface. The membrane or leaf pieces were pulled away from the mounts leaving eggs attached to the adhesive surfaces. The eggs were prepared for SEM analysis as described below. Eggs were removed from gravid female *B. argentifolii* adults. Cold-anesthetized (0–4°C) females were placed above a small drop of ice-chilled 0.1 M Millonig's buffer, pH 7.35. The ovipositor was pulled down into the dissecting buffer to expose the ovaries and the mature eggs were teased out. Dissected eggs were transferred to an ice-chilled, fixation solution of 2.5% glutaraldehyde in Tousimis/Millonig's buffer. After 24 h at 4°C, the eggs were rinsed in Tousimis/Millonig's buffer and prepared for either TEM or SEM analysis as described below.

Light and Electron Microscopy

Samples of either cotton leaves with attached whitefly eggs or eggs from ovaries, were prepared for TEM by fixation in 2.5% glutaraldehyde in Tousimis/Millonig's buffer. The samples were post fixed in 2% osmium tetroxide in the same buffer. Following fixation, samples were dehydrated in a graded series of acetone (30, 50, 70% (saturated with uranyl acetate), 90, 100%). The tissue was embedded in Spurr's epoxy resin and polymerized at 70°C for 8 h. Thin sections were made with a RMC MTXL ultramicrotome and placed on formvar-carbon films on copper slot grids prior to staining with lead citrate. Sections were examined and photographed using a JEOL JEM 100CX transmission electron microscope. Thick sections (1 µm) were cut from the same blocks with the ultramicrotome and stained with toluidine blue, then examined and photographed using an Olympus BH2 compound light microscope.

For SEM, eggs removed from ovaries were fixed in 2.5% glutaraldehyde in Millonig's buffer, rinsed in the same buffer, dehydrated in ethanol, and critical point dried. For eggs attached to either leaf tissue or membranes and for eggs extricated from leaves and membranes, the samples were examined without prior fixation. Prior to SEM analysis, all samples were mounted on carbon adhesive tabs

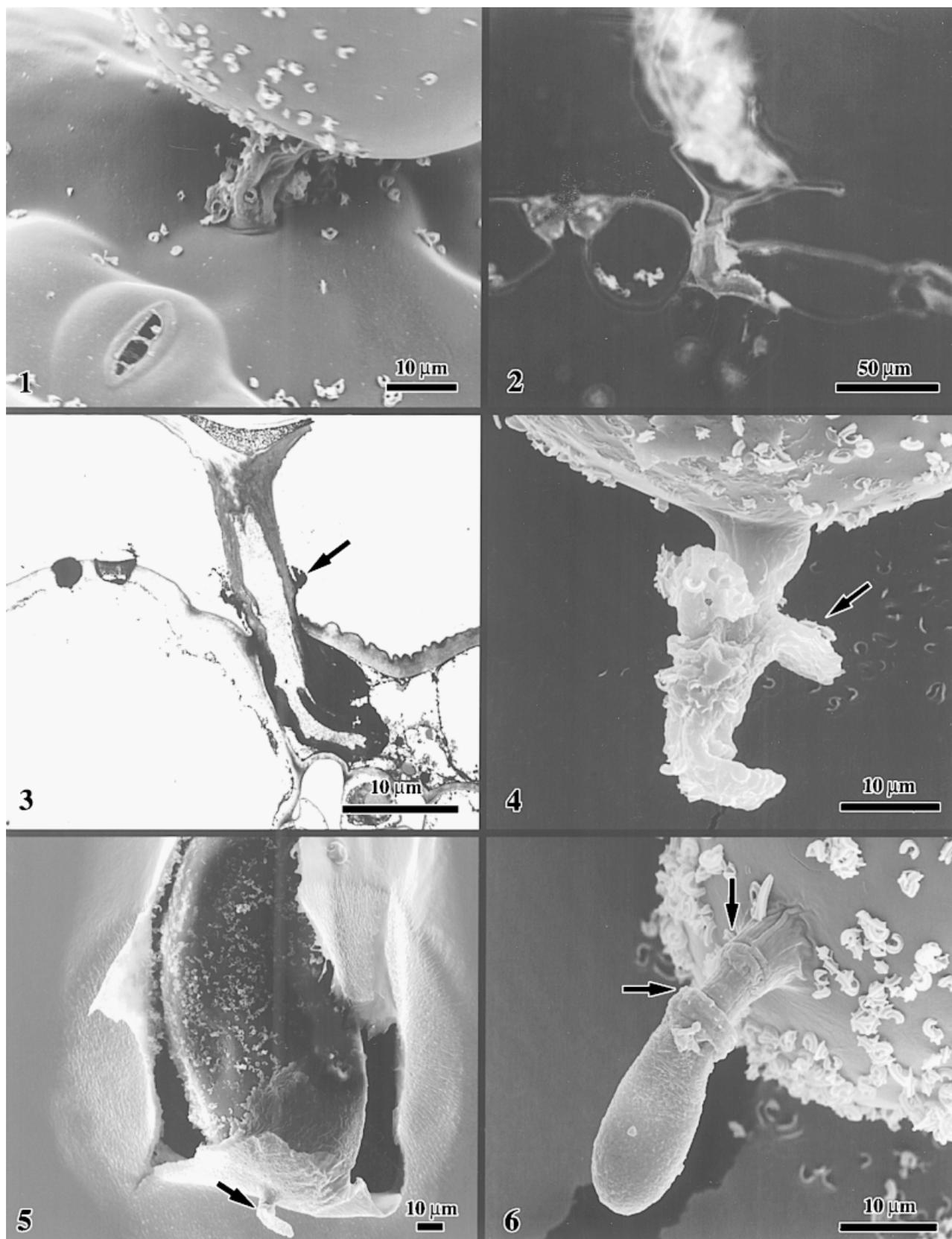
attached to aluminum specimen mounts and sputter coated with gold/palladium (60:40).

RESULTS

Egg Pedicel Morphology and Penetration Characteristics

B. argentifolii females usually oviposit eggs into the epidermal cells on the abaxial surface of host plant leaves in an upright position (Fig. 1). The egg pedicel is inserted into a slit made by the ovipositor. The tip of the pedicel usually remains within the epidermal plant cell and seldom penetrates through the basal cell wall and into the cells or intercellular spaces of the spongy parenchyma (Fig. 2). TEM techniques were employed to show a cross-sectional view of the distal end of an egg pedicel penetrating the epidermis of a cotton leaf (Fig. 3). The median longitudinal section revealed the ultrastructure of the entire pedicel including the stainable external wall and the central longitudinal core. The curved pedicel tip remained within an epidermal cell and surrounding amounts of cement (arrow) were observed. When eggs were extricated from the leaves, it was common to observe plant tissues adhering to the glue-like material that surrounded the egg pedicel (Fig. 4).

The artificial membrane apparatus with thinly stretched Parafilm was successfully used to study adult feeding, oviposition, and egg hatch for both *B. argentifolii* and *T. vaporariorum*. Comparisons were made using different media preparations: water, 7.5% sucrose, 15% sucrose, 20% sucrose, and 30% sucrose (data not shown). Adults would feed on any of the media preparations, but more eggs were oviposited at the higher sucrose concentrations (20 and 30%). However, a higher percentage of egg development and hatch was observed in either water, 7.5% sucrose, or 15% sucrose. After exposure to adults for 24–48 h, membranes were recovered with oviposited eggs attached. Microscopic examinations revealed that the egg pedicels were pushed through the 5–8-µm-thick membrane and extended into the media side of the membrane (Fig. 5). SEM techniques were used to observe eggs



Figs. 1-6. (Legend on facing page)

removed from membranes. The pedicels of oviposited *B. argentifolii* eggs extend 30–40 μm from the basal end of the egg, and the distal ends of pedicels have bulbous shapes with usually a smooth surface texture. Observations of the stalk of the pedicel often showed the presence of surrounding amounts of the glue-like material that marked the outer and inner surface of the membrane (Fig. 6).

To examine the *B. argentifolii* egg pedicel before the oviposition process, mature eggs were removed from gravid females. The proximal half of the egg pedicel had a smooth surface that appeared to be

an extension of the egg chorion layer (Fig. 7). At high magnification, the surfaces of the distal portion of the pedicel appeared as tangled arrays of fibers, 0.2–0.3 μm in diameter (Fig. 8). In longitudinal (Fig. 9) and cross-sectional (Fig. 10) views of *B. argentifolii* egg pedicels, the array of fibers comprised the outer 20–25% of the pedicel diameter. The fibers appear to attach to the core of the pedicel as strands that parallel the longitudinal axis of the pedicel (Fig. 11).

Egg Pedicel Uptake of Water/Solutes

Eggs oviposited on artificial membranes were used to study the possibility that the whitefly egg pedicel functions as a conduit for water/solute movement into the egg. To demonstrate solute movement into the egg pedicel, solutions of the radiolabeled polysaccharide, inulin, were placed in contact with exposed pedicels on the media side of artificial membranes. For ^{14}C -inulin experiments, the quantities of radiolabeled material remaining on areas of membrane without oviposited eggs were only 4–10% of the amounts of ^{14}C on areas of membrane with eggs (Table 1). In two experiments with 69 and 127 eggs clustered on the artificial membranes, the net uptake of ^{14}C , during the 48-h exposure to radiolabel, ranged from 521–976 dpm (7.6–7.7 dpm/egg). Although not shown in Table 1, similar uptake quantities were shown for an experiment with *T. vaporariorum* eggs. The pedicels of 125 *T. vaporariorum* eggs were exposed to ^{14}C -inulin in water for 48 h and an uptake of 6.6 dpm/egg was determined. Based on the solute concentrations in the media, the rate of volume uptake for both *B. argentifolii* and *T. vaporariorum* eggs would be 0.6–0.7 nl/egg.

To demonstrate the movement of a solute into the developing *B. argentifolii* egg, the pedicels of eggs on membranes were exposed to solutions of ^{14}C -acetate. After exposure to label for 4 days, the pedicels were rinsed free of label and then exposed to 98–100% rh until egg hatch. The amounts of ^{14}C were determined for collected nymphs. The collected data from three experiments indicated that substantial quantities of label were taken up

Fig. 1. A SEM micrograph of a silverleaf whitefly egg oviposited on the abaxial surface of a cotton leaf. The pedicel of the egg is inserted into a slit or puncture hole made by the ovipositor of the female. The insertion site is usually within an epidermal cell and not within a stomata.

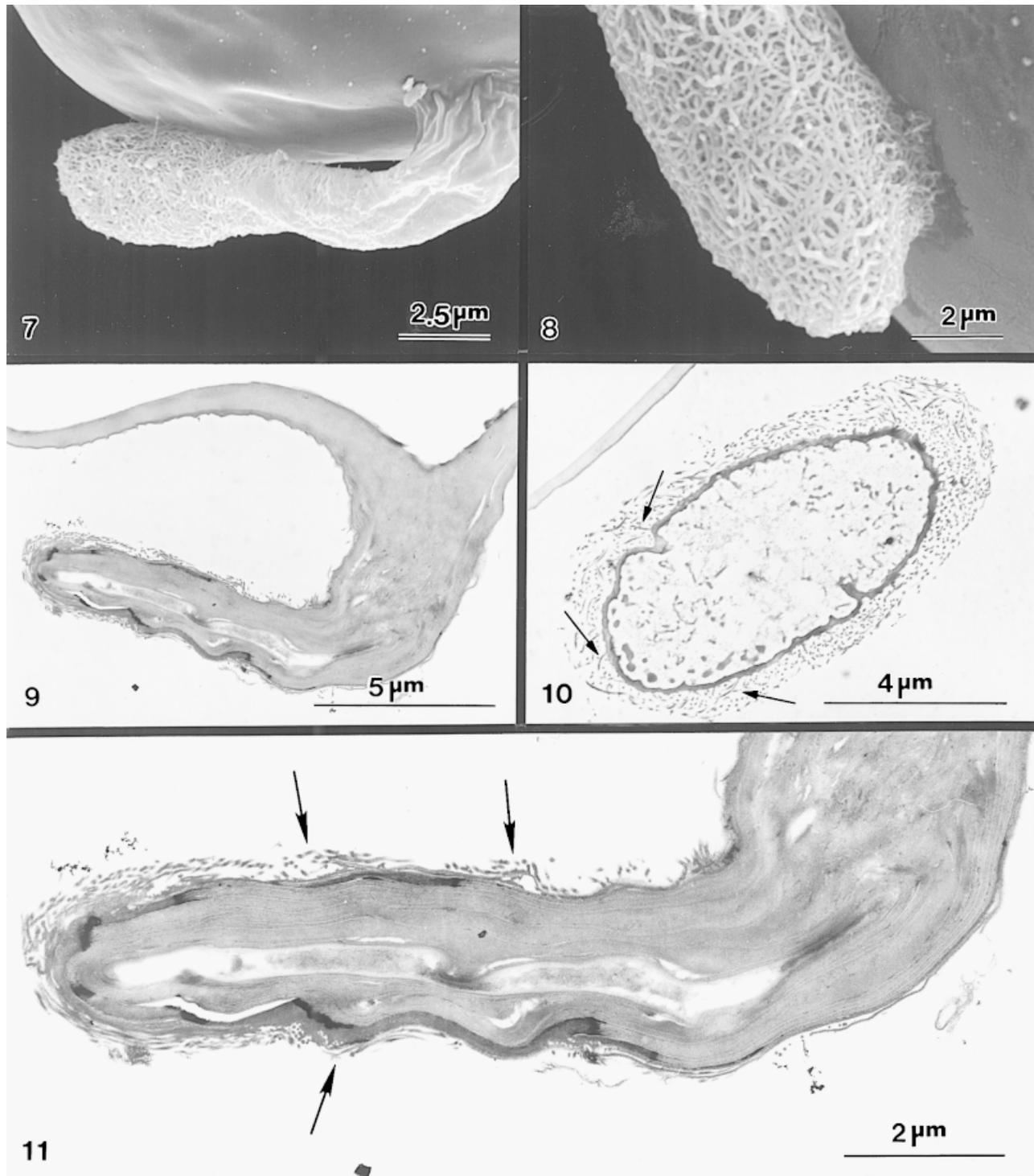
Fig. 2. A light micrograph showing a cross section of an egg oviposited into the abaxial surface of a cotton leaf. The distal portion of the pedicel is usually curved and commonly contained within a single epidermal cell.

Fig. 3. A TEM micrograph of a silverleaf whitefly egg attached to the abaxial surface of a cotton leaf. This median longitudinal section shows the ultrastructure of the entire pedicel and the presence of the glue-like material (arrow) that is associated with the process of egg insertion.

Fig. 4. A SEM micrograph of an oviposited silverleaf whitefly egg removed from a cotton leaf. Some eggs extricated from leaves show portions of leaf epidermis (arrow) cemented to the pedicel. As also shown in Figures 2 and 3, many of the pedicel tips within epidermal cells were curved or hooked.

Fig. 5. A SEM micrograph of a silverleaf whitefly egg oviposited on an artificial membrane. The membrane was mounted on the specimen stub with the egg side of the membrane attached to the stub surface. The micrograph shows that the egg has ruptured the thinly stretched Parafilm membrane. The majority of the length of the pedicel extends through the membrane and into the media side of the membrane (arrow).

Fig. 6. A SEM micrograph of an oviposited egg removed from an artificial membrane. Arrows mark the presence of cement on the pedicel and the distance between the arrows indicates the position and thickness of the artificial membrane. Circular waxy particles from adult whiteflies adhere to the egg surface.



Figs. 7-11. (Legend on facing page)

by the pedicel and into the hatched nymphs (Table 2). In three experiments with 198, 290, and 503 eggs on membranes, egg hatch ranged between 91 and 100%, and uptakes of ^{14}C were

339, 349, and 672 dpm/nymph, respectively. Based on the concentration of label in the media, the volume uptake per nymph ranged between 0.8 and 1.8 nl.

TABLE 1. Uptake of [^{14}C]-Inulin by Whitefly Eggs Oviposited on an Artificial Membrane*

Experimental conditions	Inulin (dpm/nl)	No. of eggs	Uptake		
			Total dpm	Net dpm/egg	nl/egg ^a
<i>B. argentifolii</i>					
Experiment 1	12.40	127			
Control			117		
Test			1,093	7.7	0.62
Experiment 2	12.70	69			
Control			21		
Test			542	7.6	0.59

*Methods for obtaining eggs on membranes, exposure of egg pedicels to labeled inulin for 48 h, and assays for radioactivity are described in Materials and Methods. The media consisted of ^{14}C -inulin diluted in 7.5% sucrose. Controls consisted of areas of membrane without whitefly eggs that were exposed to media containing ^{14}C -inulin. Total dpm = dpm of the sample minus the solvent blank (6 ml Ecolite + 1 ml water). Net dpm = Test dpm minus Control dpm.

^aCalculations for nl/egg uptake were based on the starting concentration of ^{14}C -inulin and the assumption that water was taken up at the same rate as ^{14}C -inulin.

TABLE 2. Uptake of [^{14}C]-Acetate by *B. argentifolii* Egg Pedicels and Radioactivity Levels in Nymphs*

Experiment condition	Acetate (dpm/nl)	No. of eggs	No. of hatched nymphs	Uptake	
				dpm/nymph	nl/nymph ^a
Experiment 1	1,190	503	488		
15% sucrose				672	0.56
Experiment 2	539	198	181		
Water				339	0.63
Experiment 3	432	290	290		
Water				349	0.81

*Methods for obtaining eggs on membranes, exposure to egg pedicels to labeled acetate, and assays for radioactivity in nymphs are described in Materials and Methods. The media consisted of ^{14}C -acetate diluted in either 15% sucrose or water.

^aCalculations for nl/nymph uptake were based on the starting concentration of ^{14}C -acetate and the assumption that water was taken up at the same rate as ^{14}C -acetate.

Fig. 7. A SEM micrograph of a silverleaf whitefly egg removed from the female ovary. The proximal portion of the pedicel is characterized by an extension of the smooth-surfaced egg chorion and the surfaces of the distal end have a fibrous appearance.

Fig. 8. A SEM micrograph of an egg removed from the female ovary at high magnification. The distal end of the pedicel is covered with an array of tangled-like fibers, 0.2–0.3 μm in diameter.

Fig. 9. A TEM micrograph of a longitudinal sectional view of the basal portion of a *B. argentifolii* egg removed from the female ovary. The outer perimeters of the pedicel tip consist of the array of fibers that surround the central core of the pedicel.

Fig. 10. A TEM micrograph of a cross-sectional view of the distal end of the pedicel of a *B. argentifolii* egg removed from the female ovary. The array of fibers completely surrounds the core of the pedicel. Arrows indicate points of fiber attachment to the pedicel core.

Fig. 11. At higher magnification of the distal end of the pedicel the ends of the fibers are attached to the core of the pedicel as strands that parallel the longitudinal axis of the pedicel (arrows).

B. argentifolii eggs on membranes were incubated at various levels of relative humidity and egg hatch percentages were determined. The hatch rate for eggs exposed to water-saturated air (98–100% rh) for 6–7 days ranged from 86 to 98% (Table 3). At the lower rh incubation levels of 75–85, 55–65, and 0–20%, none of the eggs hatched. At 0–20% rh, the eggs collapsed and showed obvious signs of desiccation. The hatch rate for *B. argentifolii* eggs on membranes where the exposed pedicels were in constant contact with water (87–98%) was similar to the hatch rate for eggs in saturated air (Table 3).

Experiments were designed to demonstrate that water-saturated air (98–100% rh) was taken into the developing *B. argentifolii* egg via the pedicel and not through the shell of the egg. Normal egg hatch was observed in experiments in which the pedicel sides of membranes were exposed to water-saturated air and, at the same time, the egg sides were exposed to lower rh (Table 3). Egg hatch was not observed in reverse experiments with the egg sides at high rh and pedicel sides at lower rh. We also observed that it

TABLE 3. Effects of Water and Relative Humidity (rh) on the Development and Hatch of *B. argentifolii* Eggs Oviposited on an Artificial Membrane

Conditions		Number of eggs	% Hatch
Sterile water ^a	Experiment		
	1	152	86.8
	2	184	98.3
98–100% rh ^b	Experiment		
	1	242	86.0
	2	210	98.5
	3	134	97.0
	4	109	98.1
	5	40	90.0
	6	171	95.3
75–85% rh ^b	Experiment		
	1	218	0.0
	2	256	0.0
55–65% rh ^b	Experiment		
	1	76	0.0
	2	65	0.0
0–20% rh ^b	Experiment		
	1	74	0.0
	2	54	0.0
	3	94	0.0
	4	158	0.0
Pedicel side at 98–100% rh and egg side at 55–65% rh ^c	Experiment		
	1	233	85.0
	2	39	94.9
Egg side at 98–100% rh and pedicel side at 55–65% rh ^c	Experiment		
	1	378	0.0
	2	89	0.0

^aThe pedicel side of each egg-laden membrane was in continual contact with water, whereas the egg side of the membrane was exposed to 55–65% rh.

^bBoth sides of each egg-laden membrane were exposed to the indicated range of rh.

^cExperiments 1 and 2 were conducted at different times. For each experiment, two egg-laden membranes were obtained from exposure to the same group of adult whiteflies. Atop two holes cut in the lid of a plastic containers (6" in diameter × 2.5" high), the two egg-laden membranes were placed. One membrane was oriented with the pedicel side facing the inside the container and the other membrane oriented with the pedicel side facing the outside of the container. The inside of the container was kept at 98–100% rh and the outside held at 55–65% rh.

was most important for egg pedicels to be in contact with water or water vapor during the early stages of egg development. Egg hatch was normal for eggs on membranes that were held at 98–100% rh for 5–6 days and then transferred to 55–65% rh for the last 2 days (data not shown).

DISCUSSION

Light and electron microscopic techniques were successfully used to view the pedicels of *B. ar-*

gentifolii whitefly eggs that had been oviposited into plant tissue, oviposited into and through stretched Parafilm membranes, and un-oviposited eggs removed from female ovaries. Egg-laden cotton and hibiscus leaves from *B. argentifolii* colony cages and cotton leaves collected from fields in Phoenix, AZ, were examined for oviposition sites on leaf surfaces. The majority of the *B. argentifolii* eggs were inserted into epidermal cells. A low number of eggs were inserted between cells and rarely was an egg pedicel inserted into a stomatal opening. These findings were consistent with earlier reports by others indicating that whitefly species tend to be specific in regard to stomatal or nonstomatal pedicel placement. For the whitefly species, *B. tabaci* (Avidov, 1956) and *T. vaporariorum* (Lloyd, 1922), the pedicel is inserted into a slit made in the plant leaf by the ovipositor. For the whitefly, *Aleurocybotus occiduus* (Russell), found on grasses and sedges, the pedicel was directly inserted into a stoma (Poinar, 1965). The author suggested that placement in a stoma is an adaptive response to the high amounts of epidermal silica and lignin that make it difficult for the whitefly to slit or penetrate the epidermis during oviposition. Paulson and Beardsley (1985) examined the point of pedicel insertion into host plant tissues for 14 whitefly species using SEM. As in earlier findings (Lloyd, 1922; Avidov, 1956), the pedicels of *T. vaporariorum* and *B. tabaci* were inserted directly into plant tissue, but for the other 12 species, pedicel placement was in the stoma.

For the greenhouse whitefly, *T. vaporariorum*, Weber (1931) observed that the female secretes a glue-like substance around the pedicel during oviposition. A similar substance, referred to as "cement," was observed surrounding the egg pedicel of *B. tabaci* (Gameel, 1974). The author indicated that during oviposition the female "injects the fluid by the cement gland, and this flows round the base of the stalk" and that "the quantity of the cement is greater when the stalk is implanted through the stomata or intercellular rather than intracellular." In our study with *B. argentifolii*, the glue-like material was observed attached to the pedicels of eggs oviposited into plant tissue (Fig. 3) and for eggs

removed from either plant tissue (Fig. 4) or artificial membranes (Fig. 6). Furthermore, the glue-like material was not observed on the pedicels of mature eggs removed from ovaries (Figs. 7 and 8). Thus, these findings provide the first direct evidence that the "cement" was added to the egg pedicel during the ovipositing process. Furthermore, our uptake studies with oviposited *B. argentifolii* eggs indicate that the presence of any glue-like material on the pedicel does not interfere with the uptake process of water and solutes. In addition to anchoring eggs, the cement could provide a "seal" for efficient water absorption.

As reviewed by Byrne and Bellows (1991), whitefly eggs generally are pyriform or ovoid and possess a pedicel that is a peg-like extension of the chorion. Our SEM observations of oviposited eggs removed from artificial membranes revealed differences in the surface characteristics of the proximal and distal portions of the pedicel (Fig. 6). The chorion layer appears to extend from the base of the egg to about halfway down the pedicel stalk. The distal end of the pedicel is bulbous in shape with a porous-appearing surface structure. The morphology of the distal end of the egg pedicel was even more revealing for mature eggs extricated from the ovaries of *B. argentifolii* females. SEM analyses revealed that the tip of the pedicel that normally extends into plant tissue has a highly porous surface of fibrous strands, 0.2–0.3 μm in diameter (Figs. 7 and 8). Similar SEM observations were made for mature eggs extricated from the ovaries of *T. vaporariorum* females (data not shown). The distal ends of their pedicels were fibrous, but with finer fibers, 0.1 μm in diameter. Longitudinal and cross-sectional TEM observations of the egg pedicels from *B. argentifolii* ovaries revealed that the array of fibers on the distal end constituted the outer 20–25% of the pedicel diameter (Figs. 9–11). The arrangement of the fibers and the nature of their connection to the core of the pedicel suggest that the fibers could function to collect and transport water (vapor), and perhaps solutes, into the egg. Thus, the whitefly egg with its anchoring pedicel appears to satisfy two of the several evolutionary hurdles discussed by Southwood (1973) that are

associated with insect survival on a host plant: attachment ("holding on") and desiccation.

Our artificial membrane apparatus was used to study adult feeding, oviposition, and egg hatch for both *B. argentifolii* and *T. vaporariorum*. Our findings that egg development and hatch were better when the pedicels were incubated in either water, 7.5% sucrose, or 15% sucrose as compared to higher concentrations of sucrose were similar to a study with detached *T. vaporariorum* eggs (Castane and Save, 1993). In that study, eggs were able to hatch in low and medium solute concentrations, but at higher concentrations eclosion was delayed and significant mortality was observed.

For many insect species, water uptake by the egg occurs during normal development (Edney, 1977). For some insects, the eggshell serves as a semipermeable membrane for the influx of water, and for others, specialized organs are involved in water absorption. For several acridids, including *Melanoplus* and *Locustana*, the hydropyle at the posterior pole has been thought to absorb water. Weber (1931) described the eggs of Aleurodids as having a stalk (pedicel) that bears a thin-walled terminal bladder that is inserted into a leaf. This pedicel was speculated to extract water from the plant and so make up for loss of water from the egg surface (Weber, 1931; Wigglesworth, 1965). A similar description of the whitefly pedicel as a thin-walled bladder and a function for maintaining water balance was reported for *B. tabaci* eggs oviposited into cotton leaf tissue (Gameel, 1974).

In a biology study of Aleurodidae, Deshpande (1936) observed that egg hatch was lower for whitefly eggs on dry leaves than those on living plants. Poinar (1965) suggested that the egg stalk (pedicel) of the whitefly, *A. occiduus*, absorbed moisture from the plant, keeping the egg in a viable, turgid state. This indirect evidence was based on the observation that when *A. occiduus* eggs were removed from the leaf and placed next to their point of attachment they soon dried up. Eggs of the homopteran, *Cardiaspina densitexta*, that were removed from the host leaf the day after oviposition, developed satisfactorily providing they had access to water through their pedicels (White,

1968). In experiments with the garden chafer, *Phyllopertha horticola* L., Laughlin (1957) stated that newly laid eggs hatched in "saturated air," but not at either 92 or 98% relative humidity. Hinton (1981) stated that most eggs that require water die under conditions of 98% relative humidity. Direct evidence for water uptake by the pedicel of a whitefly egg was first reported by Byrne et al. (1990). Movement of water into the pedicel of *T. vaporariorum* eggs was demonstrated by using tritiated water to irrigate plants supporting whitefly eggs and later measuring tritiated water activity in the eggs.

In our studies, the positioning of *B. argentifolii* egg pedicel stalk through the stretched Parafilm membrane allowed for exposing the distal end of the pedicel to water, water vapor, and various solutes dissolved in water. In experiments with exposure of egg-laden membranes to various levels of relative humidity, normal egg hatch was observed only in those experiments where the pedicels were in contact with water-saturated air (see Table 3). These results confirm the findings of Byrne et al. (1990) that the whitefly egg pedicel serves a conduit for water movement into the egg. Furthermore, our findings that correlate exposure of eggs to water vapor with egg hatch, provided direct evidence that water was necessary for normal embryonic development and egg hatch.

We observed that it was more important that the egg pedicel be in contact with water or water vapor during the early stages of egg development than during the last day or two before egg hatch. Eggs on membranes could be transferred from an environment of 98–100% rh to one at 55–65% rh after 5–6 days and the percentage hatch rate was normal (data not shown). Similar findings were reported by Jancovich et al. (1997) for the determination of egg hatch for *B. argentifolii* eggs washed off cabbage, cotton, bean, or zucchini leaves with water and sterilized with bleach. Egg hatch varied widely and appeared to depend on age of the egg at harvest and humidity. The researchers observed that eggs that were close to hatching and held at high humidity provided the best result.

Inulin, a naturally occurring polysaccharide, was chosen to demonstrate the uptake of a high mo-

lecular weight, water-soluble compound into the pedicel of *B. argentifolii* eggs (Table 1). Since inulin cannot pass through a biological membrane, it was assumed that the labeled inulin resided in the pedicel and not within the egg. To demonstrate solute uptake by the pedicel and movement into the developing egg, radiolabeled acetate was used. Rather than assay eggs for radiolabel, the eggs were allowed to hatch and the amounts of radioactivity in the emerged nymphs were determined (Table 2). These findings provided definitive evidence that the pedicel of whitefly eggs can act as a conduit for the movement of a water-soluble, membrane-permeable compound into developing eggs. Even though there is no guarantee that solutes and water are taken up at the same rate, the uptake data for inulin and acetate, as solutes, were used to calculate uptake volumes (see Tables 1 and 2). The values were relatively consistent, 0.62 and 0.59 nl/egg for inulin uptake and 0.56–0.81 nl/nymph for acetate uptake. Assuming a cylindrical shape and considering the variable sizes of eggs, we estimated the volume of each *B. argentifolii* egg to range between 1.2 and 1.6 nl. Therefore, if water were taken up at the same rate as solute, the amount of water taken into the egg in the 48-h incubation period (0.6–0.8 nl) would be about one-half the volume of an egg. In their study of tritiated-water uptake by the egg pedicel of the greenhouse whitefly, *T. vaporariorum*, Byrne et al. (1990) estimated that water taken from plant tissue made up 54% of the mass of the eggs exposed to tritiated water for 48 h.

Therefore, it is apparent that whitefly eggs, attached via their pedicel to leaves, must receive either water or water vapor from the plant to survive. Our experiments with labeled inulin and acetate (Tables 2 and 3) indicated that the egg pedicel was capable of water-soluble solute uptake and movement from the pedicel into the developing egg was clearly demonstrated. We observed that the majority of the eggs oviposited by *B. argentifolii* were inserted into abaxial epidermal cells of plant leaves and, therefore, the pedicels would be in direct contact with the cytoplasm of the cells. Obviously, the egg needs water from the plant cell, but any utili-

zation of cytoplasmic solutes has not yet been determined. The other locations for pedicel insertion include the intercellular spaces beyond the epidermal cells and the stomatal openings. Obviously, egg pedicels of all whitefly spp. can obtain sufficient amounts of water from the local environment associated with those locations.

ACKNOWLEDGMENTS

The technical assistance of Carlee O'Dell is appreciated, and at the Electron Microscope Center at North Dakota State University, we thank Kathy Iverson, Scott Payne, and LaRae Ewert for sample preparations, microscopic analyses, and micrograph presentations. The authors also thank Drs. John Barker and Heldur Hakk for critical review of the manuscript.



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