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The potential dispersal of Benghal dayflower seeds by mourning doves was studied in southern Georgia, U.S.A. The gut contents (both crop and gizzard) of mourning doves harvested in the autumn months were investigated to determine if mourning doves fed on Benghal dayflower and whether seeds can survive conditions in the bird gut. Research indicated that mourning doves fed selectively on Benghal dayflower with some harvested birds containing hundreds of Benghal dayflower seeds and capsules in their guts. Further, some seeds recovered remained highly viable. Germination rates in seeds taken from bird crops were similar to controls over the first 4 wk of germination and enhanced over control treatments during the latter 16 wk of a 20-wk germination study. Ultimately, seeds extracted from dove crops had 92% germination as compared to 80% for control seeds. Seeds extracted from dove gizzards had 45% germination, about half that of controls. Benghal dayflower seeds have a structurally reinforced seed coat that probably aids in survival of mechanical damage through bird intestinal tracts. Benghal dayflower seeds exposed to 1.0 M HCl treatment for 2 h had little loss in viability, successfully germinating after such treatment. When evaluating mechanisms for the eradication of Benghal dayflower from agricultural crops, consideration needs to be given to the large number of mourning doves and other bird species that visit cropland and potentially aid in its dispersal.

Nomenclature: Benghal dayflower, *Commelina benghalensis* L. COMBE; mourning dove, *Zenaida macroura* L.
Key words: Exotic weed, Federal Noxious Weed List, frugivory, granivory, invasive species, invasive weed, seed dispersal, tropical spiderwort.

Benghal dayflower, also known as tropical spiderwort, is an introduced noxious weed that infests many agricultural crops throughout the world (Holm et al. 1977). The weed is a tenacious competitor with crop plants, becoming entrenched in agricultural fields because of its tolerance to many commonly used herbicides, particularly glyphosate (Owen and Zelaya 2005; Webster et al. 2005); its ability to propagate vegetatively from broken stem pieces (Budd et al. 1979); and its variable growth habit with negative, positive, and diagravitropic branches that produce both aerial and underground flowers, fruits, and seeds (Maheshwari and Singh 1934). Additionally, Benghal dayflower can harbor plant pathogens (Davis et al. 2006; Desaeger and Rao 2000) and has been associated with outbreaks of epidemic proportion in agricultural crops (Gibbs 2002; Kucharek et al. 1998).

Although Benghal dayflower was identified in the United States from collections as early as 1928 and was established in Florida by the 1930s (Faden 1993), only with the relatively recent introduction of genetically modified, glyphosate-resistant crop plants has this weed become problematic in agronomic crops (Culpepper et al. 2004). The evidence and opinion of agricultural experts support the fact that Benghal dayflower in the southeastern United States is becoming one of the most troublesome weeds in crops because of glyphosate-induced shifts in weed species composition (Culpepper 2006). Prior to 2001, this weed was virtually unknown as an agricultural pest in agronomic crops of the southeastern United States. To explain the rapidity of expansion of Benghal dayflower throughout the southeastern United States and to evaluate control measures, it is necessary to understand the biology of its seed dispersal as well as the invasion ecology attributed to the species' glyphosate tolerance.

Very little is known about how Benghal dayflower has explosively dispersed through many counties in Georgia, except for reports of infestations in plant nurseries distributing container ornamentals (Durham 2006). Frugivory and granivory, particularly by birds, provides a vector by which fruits and seeds can be transported to new environments. Seed passage through bird guts can enhance or inhibit germination depending on the particular type of seed and bird (Robertson et al. 2006). Mourning doves are among the top ten most abundant migratory game birds in the United States, ranging from Canada throughout the United States. These doves are found in a variety of habitats from open woodland to forest edges, grasslands, and fields, and in agricultural and suburban areas (Mirarchi and Baskett 1994). Mourning doves are common in residential areas as well as agricultural and wild habitats and appear little affected by human activity. Indeed, during hunting season, doves are known to use residential areas as refugia (Losito and Mirarchi 1991). Mourning doves are frequent visitors to row crop fields, and data show that they have relatively equal preferences for cultivated and wild fields (Best et al. 1997).

Little is known about specific feeding preferences in mourning doves except that wild mourning doves forage selectively, but with variable preferences (Hayslette and Mirarchi 2001). The present study was undertaken to determine if wild mourning doves harvested in Georgia in locations abundant with Benghal dayflower consume seeds of this weed and if Benghal dayflower seed can survive conditions in the bird gut.

Materials and Methods

Wild mourning doves were harvested during one of three legal dove seasons in the autumn of each year from 2003 to

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2006. Doves harvested from 2003 to 2005 were taken from Grady County, GA, and those from 2006 were taken either from Cook or Berrien counties, GA. In all cases, doves were hunted in locations known to have infestations of Benghal dayflower in area crops.

Gut contents from doves were removed shortly after harvest. In most cases, dove handling was not controlled until the birds reached the laboratory. In 2006, efforts were made to ensure that freshly harvested doves were placed on ice in the field for transport to the laboratory. Dove gut contents were removed in the laboratory, and the contents of crop and gizzard were combined to determine if birds ingested Benghal dayflower seeds and capsules. After extraction, the contents were rinsed with deionized water, then spread on paper towels and allowed to air dry at room temperature. Dry contents from each dove were placed into labeled specimen envelopes and stored at room temperature before observation and testing. In 2006, freshly harvested doves from Berrien County were further processed to separate gut contents into crop and gizzard contents unless those organs were damaged. Seeds of Benghal dayflower are quite distinctive (Scher 2005) and were easily identified and separated from dove gut contents. To confirm that seeds from dove contents were identified correctly, seeds extracted from the gizzard of a dove harvested in Berrien County were germinated and grown in pots to maturity, at which time voucher specimens were prepared and submitted to the Valdosta State University Herbarium (voucher specimen: U.S.A. Georgia. Berrien County: Plant grown in laboratory from seed extracted from gizzard of a mourning dove identified as *Berrien.03* harvested in Berrien County, December 6, 2006; seed planted May 1, 2007; voucher harvested August 4, 2007, *Carter 17903* [VSC]).

Seed Viability. Seed viability was tested with 1% 2,3,5-triphenyl tetrazolium chloride[1] (TZ) using the methods of Peters (2000). Prior to testing, Benghal dayflower seeds were imbibed at 32.5 C between filter paper[2] soaked with deionized water in plastic petri dishes for approximately 48 h in a plant growth chamber[3] in the dark. Imbibed seeds were bisected with a clean razor blade and placed section-side down in drops of 1% TZ in phosphate buffer (pH 7.0). Seeds were then incubated in TZ in the dark for 12 h at 32.5 C before observation. All control seeds used in TZ experiments were collected from aerial flowers from greenhouse-grown or field-collected Benghal dayflower plants in 2003. Boiled and unboiled mature seeds of Benghal dayflower were used as negative and positive controls, respectively. Staining was recorded by photographing stained, sectioned embryos.

Seed Sterilization, Scarification, and Germination. For germination tests, seeds were sown directly in sterile petri dishes onto autoclaved filter paper soaked in sterile deionized water. In all germination tests reported, 100 seeds were used and distributed into five replicates of 20 seeds each. Where noted, wild seeds were sterilized with commercial bleach solutions before testing. For this, seeds were first imbibed for 24 to 48 h in deionized water or running tap water before being placed in 10% bleach containing 0.1% polyoxyethylene sorbitan monolaurate solution[4] as a wetting agent for 30 min. Seeds were given a 15-min rinse in sterile deionized water, changing the sterile water at least one time, before processing further.

The germination of seeds extracted from several doves was tested, with more extensive tests conducted on seeds extracted from doves harvested in 2006 from Berrien County. Doves from this collection had seed extracted from separate crop and gizzard organs, and Benghal dayflower seeds were recovered in relatively large quantity. Two doves were first tested from the 2006 harvest, Berrien.02 and Berrien.03, by sowing 100 seeds from each bird's crop and gizzard onto sterile filter paper soaked with sterile deionized water. Despite high germination rates in these tests, results were variable between organ replicates, possibly because of extensive microbial growth that may have interfered with germination. Therefore, a subsequent germination test of seeds extracted from crop or gizzard from two remaining doves (Berrien.01 and Berrien.06) with substantial numbers of seeds in both crop and gizzard were tested. For this, however, 20 seeds per replicate were dispersed in a 2% solution of an antimicrobial preservative medium developed for plant tissue culture (PPM[5]) for 12 h. Seeds were dried briefly on sterile filter paper then arranged in petri dishes over filter paper soaked in sterile deionized water containing 0.2% PPM to retard microbial growth during the germination tests. Similar replicates of control seeds were also treated with PPM as above for a positive control, and an identical test without PPM treatment of wild seeds was used as a negative control. All control seeds in this test were from aerial flowers collected from greenhouse grown plants in 2006.

The need for seed scarification to break dormancy was tested in Benghal dayflower aerial seeds collected in 2006. Replicate tests (five by 20 seeds) were used for each treatment, and the entire test was repeated three times, staggering the commencement of the test in 2-wk intervals. Wild seeds tested were imbibed for 24 h in sterile deionized water then processed to surface-sterilize the seeds with bleach as described previously. Bleach-sterilized control seeds not treated in acid were sown on wetted filter paper with no further treatment. Two sets of control seeds were prepared: one set was left uncovered in the light identical to the treatments for the acid scarified seeds, and the other set was covered in two layers of foil[6] to incubate simultaneously in the dark. The dark-treated controls remained covered in the dark for 12 wk of the 20-wk germination test, when the foil was removed and the seeds were exposed to light conditions for the remaining period. Acid scarification treatments included 0.1 M HCl (equivalent to strong avian stomach acid; Welty and Baptista, 1988) for 1, 2, or 4 h; 1.0 M HCl for 1 or 2 h; or 12 M HCl for 1 h. All acid treatments included a 15-min rinse in sterile water (two changes) after acid treatment and prior to seed sowing. Each treatment was sown in sterile petri dishes with autoclave-sterilized filter paper soaked in sterile deionized water. Petri plates were sealed[7] and incubated at 32.5 C in plant growth chambers[3] on a 12 h/12 h light/dark cycle. All germination tests were monitored for 20 wk after sowing and sterile deionized water added as needed to ensure that petri plate moisture remained high. A seed was considered germinated once a radicle emerged and was clearly visible, approximately 2 mm in length. Germination data were recorded at 1, 2, 4, 8, 12, 16, and 20 wk after treatment (WAT).

Statistical Analysis. Seed germination data were analyzed at 4, 12, and 20 WAT using PROC Mixed in SAS,[8] with variances partitioned into random effects of trial and

Table 1. Summary of mourning doves harvested by year from 2003 to 2006 and the total number of doves recovered with Benghal dayflower seeds. All doves harvested in 2003 to 2005 were taken in Grady County, GA. Doves harvested in 2006 were from Cook or Berrien counties, GA as noted.

Year	Doves harvested	Doves with Benghal dayflower seeds	Total Benghal dayflower seeds recovered	Doves ingesting Benghal dayflower seeds	Benghal dayflower seeds recovered
	No.			%	No. of seed/bird
2003	6	*[a]	32	n.d.[b]	5.3
2004	11	3	116	27	10.5
2005	14	9	90	64	6.4
2006 (Cook)	32	6	209	19	6.5
2006[c] (Berrien)	7	7	2029	100	289.9

[a] Dove gut contents from all doves collected in 2003 were combined.
[b] Abbreviation: n.d., not determined.
[c] See also Table 2.

replication. Germination data were square-root transformed prior to analysis of variance. Transformed treatment means were separated using Fisher's Protected $LSD_{0.05}$ but are presented in original form for clarity.

Microscopy. All stereomicroscope photographs were taken with a stereo–dissecting microscope outfitted with a digital camera.[9] Some samples were prepared for scanning electron microscopy[10] (SEM) as follows. Air-dried seeds or gut contents were not processed further but were mounted on SEM stubs using double-stick carbon tape. Images were obtained from samples coated with gold–palladium.[11]

Results and Discussion

Do Mourning Doves Eat Benghal Dayflower Seeds and Capsules? Mourning doves ingested Benghal dayflower seeds from all locations where they were harvested and in all years studied. Benghal dayflower seeds were ingested by 19 to 100% of doves collected from different locations (Table 1). In some doves, gut contents were nearly exclusively Benghal dayflower fruits and seeds, although seeds of other plant species were usually present. When Benghal dayflower was present in any dove, it was most often present in abundance regardless of other food types noted, indicating either that doves had been foraging and feeding selectively on Benghal dayflower or that there was an abundance of Benghal dayflower available relative to other food sources. Mourning doves feed mostly on seeds, with most reports identifying these birds as granivorous (Hayslette and Mirarchi 2001). Mourning doves have a stable year-round population in Georgia, with additional numbers of migratory doves populating the area during the cooler months. Nesting pairs of doves breed primarily from February through October and produce new clutchs repeatedly during the season, generally after a previous clutch has fledged (Mirarchi and Baskett 1994). Likewise, Benghal dayflower grows with a continuous emergence pattern during the summer months; it flowers and sets seeds continuously, potentially producing multiple generations a year (Webster et al. 2005). Estimates of resource allocation in Benghal dayflower show that the plant allocates 15% of its total resources to reproduction (Kaul et al. 2002). An Australian field study demonstrated that a single Benghal dayflower plant can potentially produce as many as 7,940 seeds (Walker and Evenson 1985). The fact that mourning doves eat Benghal dayflower seeds selectively in abundance indicates that they find Benghal dayflower a highly palatable food source that they actively feed on when it is available. The preference of mourning doves for Benghal dayflower seeds and their potential to disperse this weed present a potentially catastrophic situation for agroecosystems, considering the persistence of both dove and Benghal dayflower during the growing season, the prolific seed volume produced by populations of Benghal dayflower, and the potential spread of Benghal dayflower through this avian vector.

Many of the Benghal dayflower seeds taken from the doves with combined gizzard and crop contents appeared intact with varying degrees of surface scarring. When crop and gizzard contents were observed separately from doves taken in 2006, the crop of all but one bird contained more Benghal dayflower seed than the gizzard (Table 2). This observation is expected because doves feed quickly in the field, filling their crop with food and later digesting the crop contents from the safety of roosting sites (Mirarchi and Baskett 1994). The crop contents of birds positive for Benghal dayflower contained numerous dehisced or partially dehisced capsules of Benghal dayflower with up to hundreds of seeds present (Figure 1a; Table 2). Seeds present in the crop were morphologically similar to control seeds with little evidence of damage to the seed coat when viewed by light microscopy (Figure 1a inset). Once seeds and fruits entered the gizzard, however, capsules were not easily distinguished, being ground in the gizzard to smaller pieces with fibrous material intertwining the seeds and other gut contents (Figure 1b). Still, several intact seeds, including Benghal dayflower and other species, were present in the gizzard contents (Figure 1b). In general, relatively intact seeds of Benghal dayflower, often in large numbers, were obtained from the gizzard, although frequently some seed pieces were found, indicating that at least some seeds had been fragmented in the gizzard. Despite reports of the seeds of

Table 2. Number of Benghal dayflower seeds recovered separately from crop and gizzard in doves harvested in 2006 from Berrien County.

Bird Number	Organ	Seeds Recovered
Berrien.01	Crop	562
	Gizzard	123
Berrien.02	Crop	149
	Gizzard	122
Berrien.03	Crop	310
	Gizzard	104
Berrien.04 [a]		115
Berrien.05	Crop	3
	Gizzard	42
Berrien.06	Crop	271
	Gizzard	123
Berrien.07	Crop	not recovered
	Gizzard	105

[a] Crop and gizzard combined.

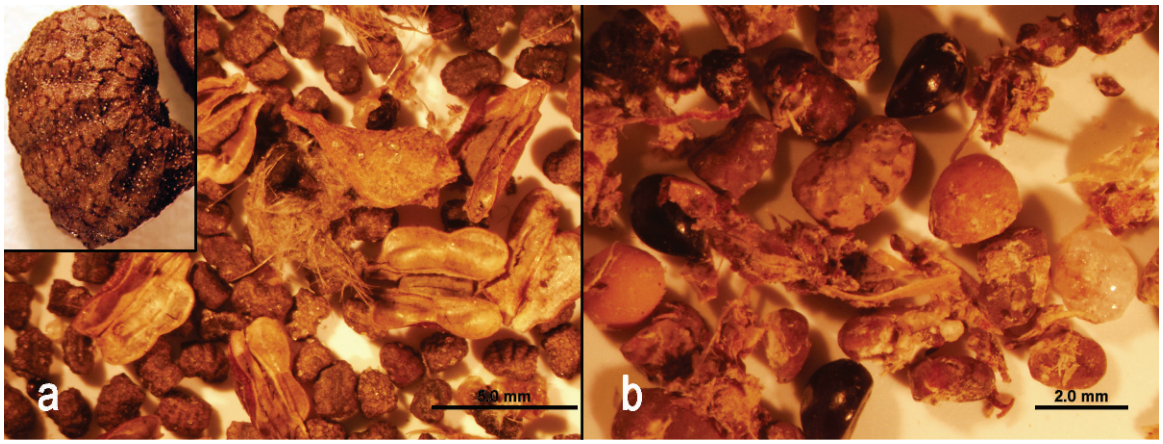

Figure 1. Crop and gizzard contents extracted from a mourning dove in 2006 identified as Berrien.01. (a) Crop contents with numerous intact dehisced capsules and seeds of Benghal dayflower are present. Inset: higher magnification of a seed taken from the crop that appears unscathed (bar = 5 mm). (b) Gizzard contents show no intact capsules or other fruits present but many intact seeds from Benghal dayflower (center) and other species (bar = 2 mm).

Commelina species having a hard seed coat that require abrasive scarification for germination (Budd et al. 1979), knowing whether these seeds can withstand the caustic and mechanical stresses of the dove intestinal tract intact is paramount to understanding whether doves or other avian species facilitate the spread of Benghal dayflower.

Seed Viability. For the initial seed viability test, data indicated a small percentage of seeds from doves taken in 2004 tested strongly positive with TZ, indicating that at least some ingested seeds were viable with staining similar to controls (Figures 2a–c). Although only 5 to 7% of the seeds taken from gut contents demonstrated strongly positive staining similar to the controls (Table 3), there were many seeds that had weak staining of the embryo but were assessed as a negative reaction. To determine further if seeds from dove gut contents could regenerate Benghal dayflower, additional germination tests were performed on seeds from different doves.

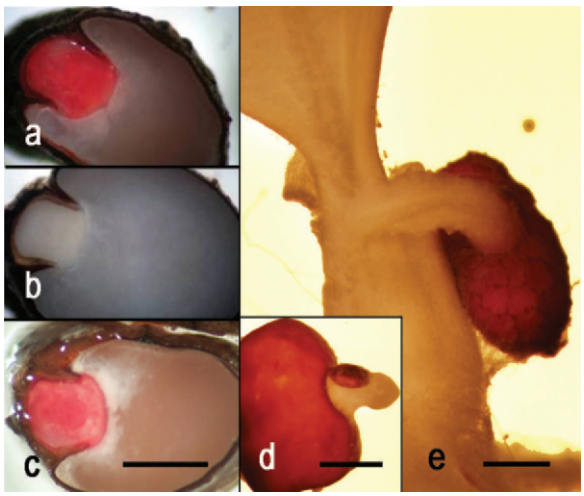

Figure 2. Seed viability and germination of Benghal dayflower. (a–c) Strong positive results of viability testing with tetrazolium (TZ). (a) Control seed that was imbibed unboiled and stained in 1% TZ demonstrates a red-stained embryo. (b) Control seed that was imbibed then boiled to kill cells of the embryo, then stained in 1% TZ demonstrates no staining of the embryo. (c) Seed extracted from gut contents, stained with 1% TZ showing a strongly positive red-stained embryo from bird 2004.01. (d–e) Germinating seeds of Benghal dayflower. (d) The embryotega is lifted as the embryo emerges while the seed coat remains intact. (e) Germinated seedling of Benghal dayflower. The still intact seed/seed coat is connected to the vertically oriented seedling by the taenia or cotyledonary stalk (bars = 0.5 mm).

Functional Morphology of *Commelina* Seeds and Seed Germination. Seeds of *Commelina* species germinate by rupturing through the micropyle region and lifting the embryotega, a callus-like covering over the micropyle that functions essentially as an operculum (Figure 2d). Even after imbibition, the seed coat never ruptures, an attribute apparently related to its strength, but the germinating seedling emerges by pushing the embryotega aside (Figure 2d). Most of the embryo is extruded and develops outside the seed coat connected by a taenia, or cotyledonary stalk, to the scutellum inside the seed (Figure 2e). Benghal dayflower seeds have a distinctive shape with a clearly visible embryotega and linear hilum (Figure 3a). The seed surface has a relief pattern with large and smaller reticulations (Figure 3b). There is a thin papery outer layer of the seed coat that covers the entire seed including the embryotega, which likely developed from the epidermis postanthesis. In unimbibed seeds, this layer is intact with no visible cracks or breaks when viewed by SEM (Figures 3b and 3c). After imbibition, small cracks can first be seen over the embryotega and linear hilum regions (Figures 3d and 3e, respectively).

In crop extracts many capsules were found still containing Benghal dayflower seeds (Figure 4a). Seeds extracted from bird crops were not identical to control seeds as perceived by light microscopy but had extensive cracks in the surface layer of the seed coat, particularly around the embryotega when viewed by SEM (Figure 4b; compare with Figure 3a). Even seeds still attached to dehisced capsules demonstrated surface

Table 3. Results of TZ[a] testing of seeds taken from the gut contents of two doves harvested in 2004.

Treatment/bird	No. of seed tested	Seed with a strong positive TZ reaction	Positive TZ reaction
	No. of seed		%
Control (boiled)	11	0	0
Control (not boiled)	22	17	77.3
2004.01	15	1	6.7
2004.03	38	2	5.3

[a] Abbreviation: TZ, tetrazolium.

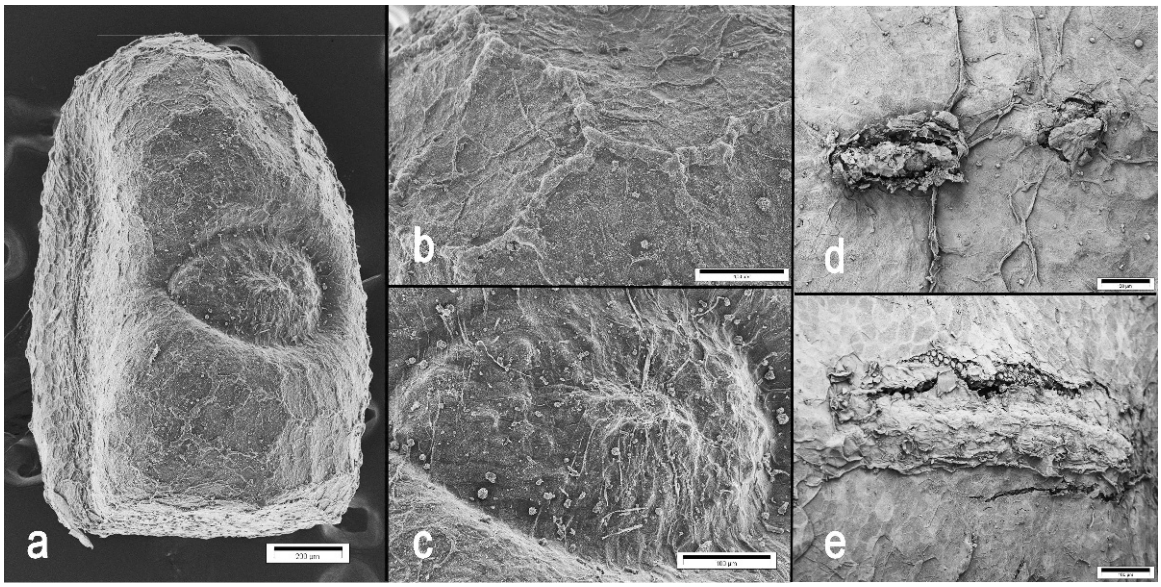

Figure 3. Surface morphology of wild, control seeds that had been imbibed or not. (a) Intact unimbibed aerial control seed collected from Benghal dayflower plants in the field. Distinctive features include the elliptical embryotega at the center and the linear hilum apparent on the flattened surface at left in the image (bar = 200 μm). (b) A distinctive feature of Benghal dayflower seeds is the raised surface over the seed coat in a reticulate pattern. Both large and smaller reticulations are clearly evident (bar = 100 μm). (c) Higher magnification image of the unimbibed control seed embryotega in Figure 3a. The embryotega is covered, with no cracks or breaks, by a layer of the seed coat that is continuous over the entire seed (bar = 100 μm). (d) In an imbibed control seed, cracks are shown in the outer surface layer of the seed coat over the embryotega (bar = 50 μm). (e) The linear hilum region of a control seed that had been imbibed also demonstrates extensive cracking of the surface layer of the seed coat (bar = 100 μm).

abrasion in the crop (Figure 4c). In many seeds extracted from the crop, the surface layer of the seed coat had already been abraded away, or chemically removed, revealing a subtending layer of cells with interconnected thick walls in a honeycomb pattern (Figure 4d). This layer of cells and the subtending wall appear to form a resilient barrier of the seed coat, which is discussed further with regard to the morphology of seeds extracted from the gizzard.

Some seeds present in the gizzard had been stripped of surface layers of the seed coat, revealing the embryotega completely (Figure 5a). Other seeds extracted from the gizzard were encrusted with debris (Figures 5b and 5c) but with their papillate embryotega revealed (Figure 5c). Large sections of the outer layer of the seed coat were removed in the gizzard revealing the honeycombed network of cells seen in seeds from the bird crop (Figure 5d). The surface reticulations

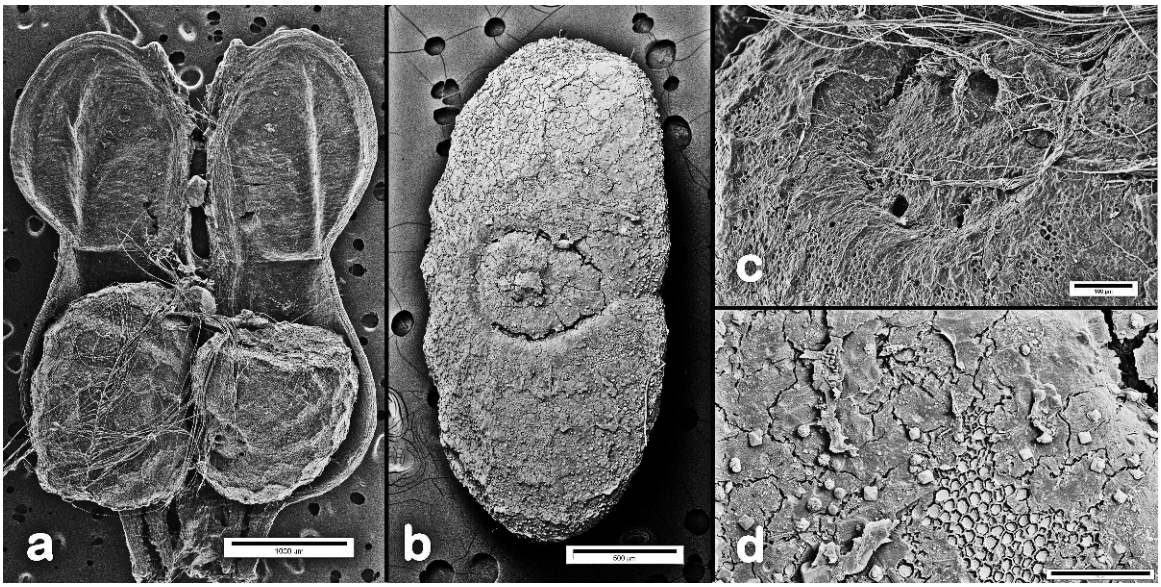

Figure 4. Morphology of seeds extracted from dove crops. (a) Image of an intact dehisced capsule with two seeds in place extracted from a dove crop (bar = 1 mm). (b) Backscatter electron image of a whole seed extracted from dove crop. The outer surface layer of the seed coat is extensively cracked, particularly around the edge of the embryotega (bar = 0.5 mm). (c) High-magnification image of one of the seeds present in Figure 4a, from dove crop. Surface of the seed (shown near the embryotega) is already cracked and abraded revealing underlying cells in a seed that has not yet been mechanically dislodged from the fruit (bar = 100 μm). (d) Higher magnification backscatter-electron scanning electron micrograph of the seed surface from a bird crop showing parts of the outer layer of the seed coat already fully removed revealing a tightly packed layer of cells whose walls form a honeycomb pattern (bar = 100 μm).

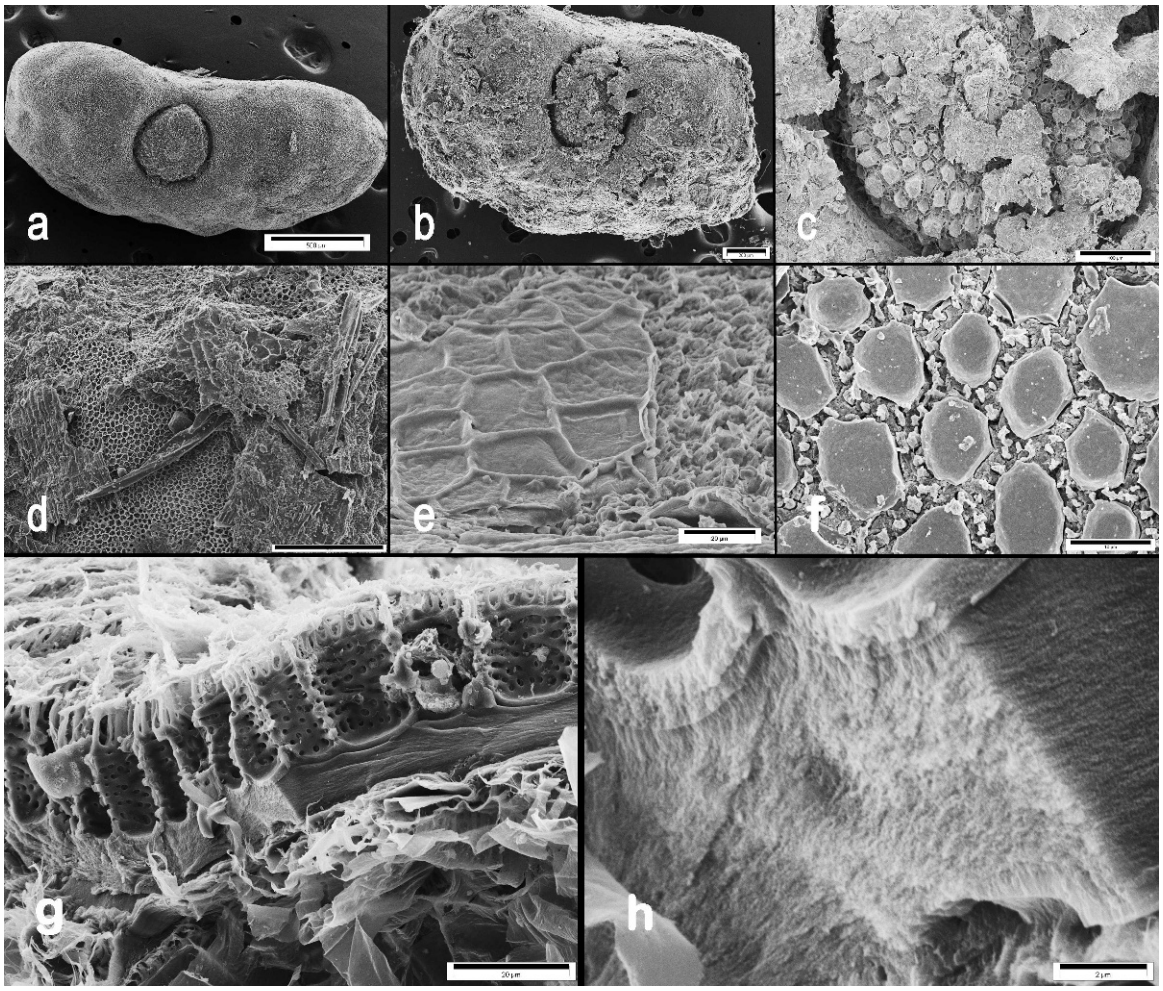

Figure 5. Morphology of Benghal dayflower seeds extracted from dove gizzard. (a) Whole seed with the surface covering fully removed and exposing the embryotega covering the micropyle (bar = 0.5 mm). (b) Common appearance of seeds in the gizzard demonstrates debris covering the surface with most of the seed coat covering abraded, revealing underlying layers of the seed coat (bar = 200 μm). (c) Higher magnification scanning electron micrograph (SEM) of embryotega with papillate cells (bar = 100 μm). (d) SEM image of the seed surface with debris or seed coat layers partially occluding the continuous layer of cells forming the underlying surface of the seed coat (bar = 200 μm). (e) SEM image of a portion of the seed surface with a remnant of the outer layer of the seed coat with minor reticulations. Minor reticulations are larger than the underlying cells (bar = 20 μm). (f) SEM image of the layer of cells underlying the seed coat removed in the gizzard. Lateral walls have projecting spires that give them a punctate appearance when seen in surface view. Lateral walls are closely appressed between cells and provide no identifiable intercellular spaces between cells. Cells are covered with a lid-like structure (bar = 10 μm). (g–h) Lateral view of a seed coat cell layer (similar to surface view of the same layer in Figure 5f) in a seed fractured with a razor blade prior to processing for SEM. (g) Surface cells have no intercellular spaces and trabeculate lateral walls, open at the surface. All surface cells are appressed to a continuous tangential wall layer forming part of the seed coat (bar = 20 μm). (h) High magnification image of the tangential wall at the base of the seed coat (seen in Figure 5g; bar = 2 μm).

noted in intact seeds (Figure 3b) are often not apparent in seeds extracted from the gizzard or are present only as dislodged fragments of seed coat surface material among other debris in the gizzard (Figures 5d and 5e). These fragments appear to be exclusively a part of the outer layer that is removed during digestion.

The underlying cell layer of the seed coat has complex lateral cell walls that form a honeycomb pattern and have little or no intercellular spaces (Figure 5f). In one seed that was forcibly fractured with a razor blade during preparation, the outer honeycomb-patterned cell layer of the seed coat was revealed in cross-section (Figure 5g). In the fractured seed coat layer, the cell lumen is exposed, revealing thick, almost trabeculate walls with a subtending continuous tangentially oriented wall layer (Figure 5g). This subtending wall layer is microfibrillar in structure and forms a barrier beneath the honeycomb-patterned cells upon which each cell in the layer is continuous (Figure 5h). The Benghal dayflower seed coat appears adapted for strength and rigidity. Although the honeycombed network of cells in this layer appears to provide structural reinforcement, this layer of cells is sometimes scraped away in the gizzard (Figures 6a and 6b) revealing only the subtending continuous tangential wall layer (Figure 6b). The honeycomb pattern of the abraded cells is still apparent on the surface that is revealed (Figure 6b). Few seeds were found that were cracked or in pieces relative to the number of intact seeds.

The Benghal dayflower seed coat appears at least two-layered with a rigid thick inner layer and a thin outer covering. From the morphological data acquired in this study and the germination data presented subsequently, it appears that maintaining the integrity of the outer covering of the seed coat is necessary to preserve seed dormancy. The inner layer of the seed coat appears to be structural in nature and may protect the seed from harsh mechanical perturbation in the bird gizzard. The question remains whether seeds that pass

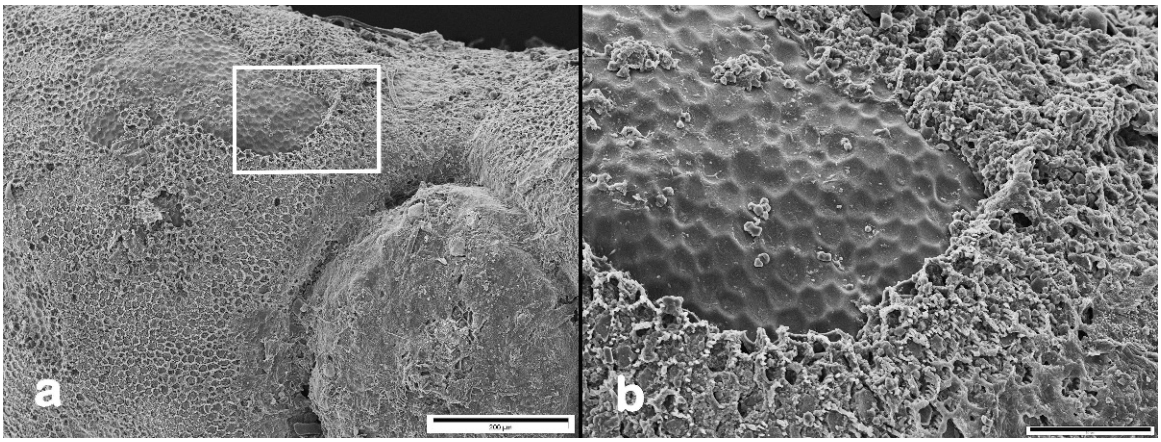

Figure 6. Scanning electron micrograph of the surface of a seed extracted from dove gizzard. (a) Parts of the trabeculate cell layer have been abraded and removed exposing the continuous tangential cell layer (bar = 200 μm). (b) Detail of the portion of Figure 6a outlined by the white box. Seed coat is not cracked even with the surface layer of cells abraded away. Tangential wall that remains shows clearly the honeycombed pattern of the cell walls that were removed (bar = 50 μm).

through dove digestive tracts remain intact and viable, germinating after passing, a test not accomplished in this study.

Germination Studies. Seeds from several doves, harvested between 2003 through 2005, germinated in our tests (data not shown). Because the gut contents of these birds contained combined crop and gizzard contents, the enhancement or inhibitory effects of each organ on the germination rate was in question. We therefore took seeds separated from these organs from doves harvested in Berrien County in 2006 that had large numbers of seeds extracted from both organs (Table 2). In initial tests, seeds were not subjected to surface sterilization to reduce the chance of artificial enhancement or reduction in seed germination, but these petri plates developed extensive fungal growth that may have inhibited seed germination. Each petri plate in this first test had different microbial flora populations, probably resulting from different foraging habits of individual doves, and replicate treatments were inconsistent with each other. The antimicrobial solution PPM used in subsequent tests eliminated most microbial growth and only minor fungal growth appeared on test plates (data not shown). The results obtained from the second controlled test are presented in Table 4. We subjected the PPM control tests to statistical comparison with the bleach-sterilized control seeds in the acid treatments. Data analysis indicated there were no differences between these controls except at 20 WAT when the bleach-treated seeds had greater germination (92%) than the PPM control (80%). Control tests of seeds left untreated in PPM had extensive fungal and bacterial contaminants. Comparison between the PPM-treated control seeds and untreated control seeds was highly significant at all times (Table 4), indicating that microbial competition or pathogenesis can greatly reduce seed germination in Benghal dayflower seeds.

Table 4. Summary results of seed germination in seeds extracted from bird crop and gizzard in birds Berrien.01 and Berrien.06 harvested in 2006, and between PPM[a]-treated control vs. no PPM treatment.

	4 wk	12 wk	20 wk
	% seed germination		
Organ of seed recovery			
Crop	49	87	93
Gizzard	37	45	45
F-value	3.11	76.93	79.88
P-value	0.099	< 0.0001	< 0.0001
Control treatment			
PPM	50	79	80
No PPM	24	37	51
F-value	15.23	89.62	47.74
P-value	0.0169	0.0007	0.0023

[a] Abbreviation: PPM, plant preservative medium.

Seeds taken from the crops of birds Berrien.01 and Berrien.06 germinated at rates similar to controls up to 4 WAT, but ultimately their germination rate exceeded that of the control test with PPM at 12 and 20 WAT (Table 4). At 20 WAT, seeds extracted from bird crops had 93% germination, greater than the 80% germination in the control. This enhancement in germination rate may be due to the cracking and seed coat scarification we observed with SEM in seeds from dove crops (e.g., Figures 4b–d). Seeds recovered from the gizzards of the birds had 45% germination, less than half the germination of seeds from the control and seeds from crops (Table 4). Benghal dayflower seeds are released under strong dormancy, presumably because of their hard seed coat (Budd et al. 1979). Physical or chemical seed scarification is required to increase Benghal dayflower germination (Budd et al. 1979; Kim et al. 1990). Birds tend to ingest grit and small stones that collect in the gizzard that help to fragment ingested food. Passage of seeds or other foods to the gizzard from the crop move through a proventriculus or glandular stomach that secretes acid and digestive enzymes that range in pH from 0.7 to 2.5, before grinding in the gizzard and passing to the intestine (Welty and Baptista, 1988). Our data indicate that exposure to bird crop conditions enhanced germination. In contrast, seed from the gizzard germinated at significantly reduced rates likely because of their exposure to the harsher conditions present there. It is unlikely that acid treatment alone reduces the germination of Benghal dayflower seeds extracted from the gizzard when the effects of acid treatment are considered (below). It is more likely that the mechanical grinding in the gizzard ultimately disrupts the integrity of seeds. We observed variation in the amount and size of grit and small stones in the dove gizzard. This variation may present an opportunity for some seeds to

Table 5. Summary results of percentage of seed germination at 4, 12, or 20 wk after planting, in different acid treatments on wild seed. Analysis of variance was performed using PROC Mixed in SAS, with variances partitioned into random effects of trial and replication. Transformed treatment means were separated using Fisher's Protected $LSD_{0.05}$.

Treatment	4 wk		12 wk		20 wk	
	% seed germination[a]					
Light control	54	a	79	ab	92	a
Dark control	12	d	30	c	87	b
0.1 M HCl for 1 h	41	bc	84	a	93	a
0.1 M HCl for 2 h	37	c	75	ab	93	a
0.1 M HCl for 4 h	49	ab	79	ab	92	a
1 M HCl for 1 h	40	c	70	b	84	b
1 M HCl for 2 h	41	bc	70	b	82	b
12 M HCl for 1 h	0	e	0	d	0	c

[a] Means with different letters within a column are significantly different.

pass relatively unscathed through birds with decreased grit in their gizzard.

Many birds are known to regurgitate some of the seeds or food that they eat; they particularly regurgitate larger seeds whereas smaller seeds are processed and defecated (Murray et al. 1994). The crop of mourning doves is a glandular organ that produces crop milk for feeding young. Both male and female doves regurgitate crop milk and seed for their young during brooding that is often continuous from February through October and sometimes occurs year-round (Mirarchi and Baskett 1994). It is possible that regurgitated seeds from the dove crop would have an enhanced germination rate and that some seeds of Benghal dayflower eaten by mourning doves might be dispersed from field to field or field to nest by regurgitation, retaining the potential to germinate and establish new Benghal dayflower populations.

Although we could not evaluate the viability of seeds that fully pass through dove guts with our methods, the high rate of germination recorded from seeds extracted from the gizzard and crop indicates that some Benghal dayflower seeds may be surviving complete passage and would be passed to new territory as the dove travels. Increased time under mechanical and acidic stress may reduce germination, and this hypothesis should be tested in the future. It should be noted that our results were obtained from wild birds and that seed retention time was not controlled. Although this results in considerable variation, significant germination was obtained from seeds extracted from all bird crops and gizzards tested. This might indicate a high probability that Benghal dayflower seeds remain viable in the dove gut and could potentially germinate when passed, spreading this weed. Retention time is a key factor affecting viability of seeds dispersed endozoically by birds (Traveset et al. 2001a) with shorter retention generally resulting in greater viability. The results of one previous study based upon unempirical visual estimates suggested that mourning doves had a seed retention time of approximately 4 h (Blockstein et al. 1987). Other bird species, particularly those eating fleshy fruits, have documented retention times from only 12 to 75 min (Barnea et al. 1991; Bartuszevige and Gorchov 2006; Murray et al. 1994), sometimes being regurgitated and other times defecated. Also, larger seeds tend to be regurgitated in some birds or pass through the gut more quickly than smaller seeds, increasing their chance of survival and subsequent germination (Murray et al. 1994; Traveset et al. 2001a). This is somewhat significant because Benghal dayflower produces dimorphic seeds of variable size from aerial and underground flowers. Differences have been reported for large and small seeds from aerial and underground flowers, particularly with respect to germination success (Kim et al. 1990; Matsuo et al. 2004). Further research using captive birds to determine the retention time of Benghal dayflower capsules and seeds in doves and the viability of defecated and regurgitated seeds should be done to understand fully whether the dove and Benghal dayflower have developed a mutualistic relationship promoting seed dispersal.

Acid Scarification Effect on Germination. For acid scarification studies, we used hydrochloric acid to most closely imitate the stomach environment. For the control and acid-treated seeds, total germination increased over an extended period, indicating a slow release from seed dormancy and substantial variation between individual seeds. Treating sterile control seeds with acid (up to 1.0 M HCl) resulted in little difference in the rate of germination compared to the control (Table 5). Using 0.1 M HCl, simulating the pH conditions in an avian stomach, for variable times of up to 4 h (estimated time of food retention in doves) revealed no reduction in seed germination relative to the light control at 12 and 20 WAT (Table 5). When treating in acid conditions 10-fold stronger (1.0 M HCl) than typically encountered in avian digestive tracts for 1 or 2 h, there still was little difference from the controls. Only with exposure to 12M HCl for 1 h (100-fold greater acidity than avian digestive tract) is germination totally inhibited. Seeds from this latter treatment were extremely soft at the end of the germination test and none demonstrated positive TZ results (data not shown). Additionally, light promoted germination increasing germination levels considerably when compared to dark-germinated controls (Table 5), similar to results of other studies (Matsuo et al. 2004). In comparing light- and dark-treated control seeds, the difference in germination rates is highly significant at 4 and 12 WAT with greatly decreased germination in the dark. Dark-grown control seeds reached only a 30% germination rate as compared to 79% germination in controls in the light at the end of 12 WAT. Dark-treated seeds were returned to the light at the end of 12 WAT. Germination in these formerly dark-treated seeds increased to 87% at 20 WAT, slightly lower than the light-treated controls (92% germination; Table 5).

Just as seeds in the dove gizzard need to withstand severe mechanical stress in order to survive, they also must be able to withstand substantial acidic environments for any possibility of remaining viable after excretion. Previous studies have scarified Benghal dayflower seeds by mechanical means (Budd et al. 1979) and by temperature or chemical means including

concentrated sulfuric acid (no more than 2 min), dry heat or hot water, or bleach treatments (Kim et al. 1990). All scarification treatments produce some or significant increase in germination of the seeds.

The potential for mourning doves to disperse weed seeds has been studied previously with respect to the dispersal of leafy spurge (*Euphorbia esula* L.)(Blockstein et al. 1987). This study concluded that leafy spurge seed did not survive conditions through the mourning dove gut, being crushed in the gizzard. Therefore dispersal of leafy spurge was not an issue with respect to mourning dove ingestion. Although we did not use doves in captivity, our study indicates that Benghal dayflower seeds are structurally reinforced against the mechanical stresses of the dove gizzard and, at the least, survive in highly acidic environments. In other studies with different plants and birds, individual birds have been found to have no effect, an enhancement effect, or an inhibitory effect on seed germination depending on the bird and the species of plant seed the bird is ingesting (Barnea et al. 1991; Bartuszevige and Gorchov 2006; Samuels and Levey 2005; Traveset et al. 2001b). Likewise, evidence points to increasing seed viability and germination for seeds that are retained in the bird gut for shorter times rather than longer periods (Barnea et al. 1991). The ability of exotic plants to incorporate native animal species in mutualistic interactions, such as seed dispersal, is often a key factor facilitating invasion (Richardson et al. 2000).

Control of Benghal dayflower is a complex problem. No attempt was undertaken in this study to survey other avian visitors to agricultural fields in southern Georgia, but it is quite likely that other bird species are ingesting Benghal dayflower. In another study of six Midwestern states, up to 48 different bird species were surveyed in row crop fields (Best et al. 1997). Thus, where infestations of Benghal dayflower have been established, it is likely that a variety of bird vectors are dispersing Benghal dayflower seed. Our study shows that mourning doves eat Benghal dayflower seeds and that seeds from this plant have a high potential for survival in the mourning dove gut. Clearly, if we are concerned about the prevalence and control of Benghal dayflower in agricultural crops, we need to determine if other birds forage freely on Benghal dayflower. From a behavioral standpoint, any bird foraging on Benghal dayflower seed may deposit viable seed in a natural area or another agricultural field. The potential for reservoir populations of Benghal dayflower in natural areas is a definite concern with respect to the eradication of Benghal dayflower. From any perspective, aggressive control of Benghal dayflower is necessary in all known newly infested agricultural and natural environments to minimize the potential for exponential spread and impact of this weed.

Sources of Materials

[1] 2,3,5-Triphenyl tetrazolium chloride, Sigma-Aldrich, St. Louis, MO 63103.

[2] Whatman No. 1 filter paper was used in all experiments noting use of filter paper. Fisher Scientific, Pittsburgh, PA 15275.

[3] Plant growth chambers used were either a Percival E30b or RE-9 plant growth chamber, Percival Scientific, Inc., Perry, IA 50220.

[4] Polyoxyethylene (20) sorbitan monolaurate solution 70% in H_2O (Tween 20), Sigma-Aldrich, St. Louis, MO 63103.

[5] Plant preservative medium, Plant Cell Technology, Inc., Washington, DC 20036.

[6] Reynolds 655 standard foil (approx. 0.02 mm thick) was used for dark-treated plates. Fisher Scientific, Pittsburgh, PA 15275.

[7] Parafilm® M laboratory sealing film was used as a barrier to inhibit water vapor loss and promote gas exchange in seed germination experiments. Fisher Scientific, Pittsburgh, PA 15275.

[8] The data analysis for this paper was generated using SAS software version 9.1 copyright, SAS Institute Inc., Cary, NC.

[9] Stereo dissection microscope used was either an Olympus SZ-6045 stereo-dissecting microscope with phototube and Kodak DC-290 digital zoom camera, Eastman Kodak Scientific Imaging Systems, 4 Science Park West, New Haven, CT 06511, or an Olympus SZX-12 stereo-dissecting microscope outfitted with an Olympus DP-71 digital camera, Olympus America, Center Valley, PA 18034.

[10] JEOL 6480LV scanning electron microscope, JEOL USA, Inc., 11 Dearborn Rd., Peabody, MA 01960.

[11] Specimens were coated for SEM using a Denton Desk IV sputter coater, Denton Vacuum USA, 1259 North Church St., Moorestown, NJ 08057.

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