

Haustorial Initiation and Non-host Penetration in Witchweed (*Striga asiatica*)

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Striga asiatica (L.) Kuntze (= *S. lutea* Lour.) (Scrophulariaceae) is a well-known root parasite and a serious pathogen of gramineous crops in several areas of the world. Since its discovery in the United States in the Carolinas in the 1950s a federal control and quarantine programme has been employed to restrict this parasite.

The factors affecting the germination of witchweed seeds have received a great deal of attention. It is now known that in order for witchweed seeds to germinate they must be pretreated in excess water (not equivalent to 100 per cent r. h.) at 30 °C for 10-14 days and be exposed to a germination stimulant (either derived directly from host roots or synthesized). The germination stimulant strigol, originally isolated from cotton, has been synthesized (see Heather, Mittal & Sih, 1974). Other compounds are also known to stimulate germination (Johnson, Rosebery and Parker, 1976).

Little information is available on the morphogenesis of the primary haustorium of witchweed. This structure has been defined as a modified root apex which develops directly into a haustorium (Kuijt, 1969). Despite the importance of the primary haustorium in the life history of this parasite, little is known about the factors affecting its development.

The recent discovery by one of us (Riopel) that an alcoholic extract of gum tragacanth (GT) stimulates the initiation of haustoria in *Agalinis purpurea*, also a root parasite in the Scrophulariaceae but producing only secondary (lateral) haustoria, raised the question of the possible effect of this extract on the development of primary haustoria. This paper reports the effects of GT on witchweed radicles and their attachment to and penetration of host roots.

Two methods of culturing the parasite were used: microtitre plates and glass tubes.

Disposable plastic microtitre plates each with 24 wells of 1 ml were chemically sterilized in a 3 per cent commercial bleach solution and rinsed several times in distilled water. These plates were then transferred to 20 × 100 mm sterile petri dishes.

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Enough pretreated seeds to cover the bottom of a 50 ml Erlenmeyer flask were soaked in water overnight. Next, the floating seeds were discarded leaving 10 ml of water with seeds. These seeds were poured on to a 25 mm diameter nylon mesh circle (160 μm mesh) and another circle placed on the first to form a sandwich containing seeds. This mesh circle was then placed in a 25 mm Swin-Lok membrane holder and the entire apparatus connected to a sterile 10 ml Luer-Lok syringe. Seeds were sterilized by drawing the following solutions into the syringe and evacuating about 50 times each:

- (1) four drops of 20 per cent Tween-20/200 ml distilled water;
- (2) two changes of distilled water;
- (3) 3 per cent commercial bleach solution (0.17 per cent NaOCl; time in bleach solution not to exceed 5 min);
- (4) two changes of distilled water.

This technique was developed to sterilize the extremely small seeds (c. 0.2 mm in length). The mesh circles containing the seeds were then put in a sterile 15 \times 60 mm Petri dish and placed in an oven at 45–50 $^{\circ}\text{C}$ for 12 h. Dried seeds were rinsed off the mesh with distilled water and placed in a petri dish. This was then incubated at 30 $^{\circ}\text{C}$ for 10–14 days to effect pretreatment.

Five to ten pre-treated seeds were placed in each of the 24 wells of the microtitre plates. Two replications of each treatment (i.e. two microtitre plates) were used as well as water controls. One ml of germination stimulant was pipetted into all the wells containing seeds. Three germination stimulants were used: (1) strigol (10^{-4} p.p.m.); (2) unconcentrated maize root exudate consisting of water solution from hydroponically grown maize; and (3) an extract of maize roots homogenized in distilled water. Stimulant was added to all wells (including controls) since seedlings were needed for later treatments with GT. Germination rates of 80–90 per cent resulted after 24 h at which time excess stimulant was removed and the GT was applied at a concentration of 0.2 mg ml $^{-1}$. An equal volume of water was added to the control plates.

The GT extract was prepared in the following way. Thirty grams of gum tragacanth was relaxed with 500 ml of 95 per cent ethanol for two periods totaling 12 h. After filtering through a no. 5 Whatman filter and a 0.65 μm Millipore filter, the extract was evaporated in a rotary evaporator under vacuum to almost dryness and then resuspended in 50 ml of water. The mixture was then dried in an evaporating dish at 75 $^{\circ}\text{C}$. Thirty grams of gum tragacanth yielded 1.719 g of alcohol extract.

All Petri dishes with microtitre plates were placed in a humid 30 $^{\circ}\text{C}$ germinator and results recorded after 24 h. A positive result was scored if the seedling radicle showed root hair formation (i.e. the first stage in haustorial development).

Host plants were grown in glass tubes similar to those described by Visser, Dörr and Kollmann (1977) in order to observe witchweed parasitism. In our set-up, the glass tube was lined with filter paper and filled with sterile vermiculite. Host seeds were germinated between the inner glass wall and the filter paper and the tube was then placed in a growth chamber maintained at a 12 day/night cycle (22 $^{\circ}\text{C}$ day, 18.4 $^{\circ}\text{C}$ night). The hosts were allowed to develop extensive root systems before the witchweed seeds were added.

The following documented hosts of witchweed were used: sweet corn (*Zea mays*); oats (*Avena sativa*); and sorghum (*Sorghum vulgare*). The following non-host plants were used: cotton (*Gossypium hirsutum*); soyabean (*Glycine max*); garden pea (*Pisum sativum*); green bean (*Phaseolus vulgaris*); and lima bean (*P. limensis*). Witchweed seeds germinated in strigol and treated with GT were added to both groups.

Root hairs (haustorial initials) were seen on all seedlings treated with GT, regardless of the germination stimulant used. GT alone did not cause germination (at least at the attempted 0.2 mg ml concentration). Although strigol, maize root extracts, and maize root exudates did cause significant rates of germination, none induced haustoria without the addition of GT.

Fig. 1(a) shows a strigol germinated seedling apex devoid of root hairs. Fig. 1(b) shows an early stage of hair formation (epidermal cell swelling?) just 8 h after the addition of GT. A later stage of development shown in Fig. 1(d) shows a seedling after 24 h exposure to GT. Notice the continuation of apical growth. When young seedlings (less

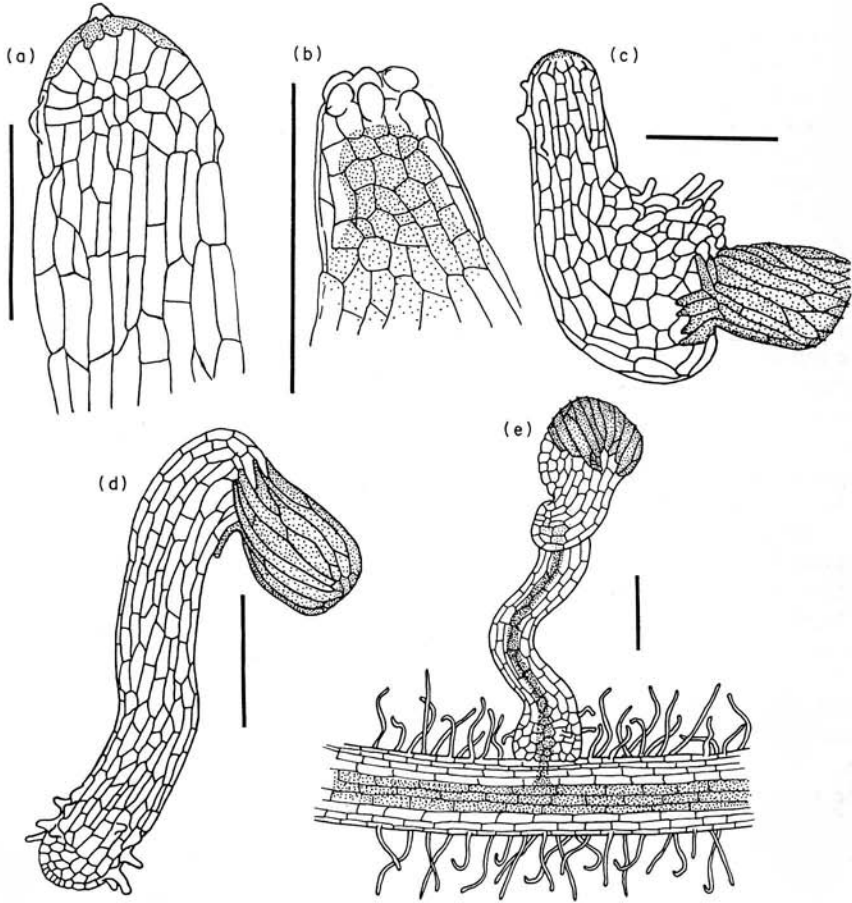


FIG. 1. (a) *Striga* radicle apex on seedling germinated with strigol. Note incipient hair formation. (b) One-day-old *Striga* radicle exposed to gum tragacanth extract for 8 h. Note swollen epidermal cells and densely cytoplasmic cortical cells. (c) Eight-hour-old *Striga* radicle exposed to GT extract for 12 h. Note lateral and subapical hair formation with continuing apical growth. (d) Seedling 1 day old exposed to GT extract for 12 h. (e) Seedling 1 day old exposed to GT extract for 12 h to initiate hair formation. Seedling was then transferred to culture tube to contact roots of *Phaseolus vulgaris*. Drawing shows primary haustorium attachment and penetration (optical section) to host stele. Note remaining hairs along haustorium. All lines equal 200 μm .

than 8 h after germination) are exposed to GT, hairs form, but they are left in a lateral position due to continued apical growth [see Fig. 1(c)].

All stages of attachment and penetration of both monocot and dicot hosts were noted in the glass tubes. Seedlings with root hairs when placed in contact with host roots became appressed to the host epidermal cells. Cortical cell proliferation in the radicle apex also occurred as the young haustorium matured. Early stages of endophyte invasion were

seen as well as later stages showing penetration to the host stele. Fig. 1(e) shows such penetration of a dicot, *Phaseolus vulgaris*.

Primary and secondary haustoria are the two general types found in parasitic Scrophulariaceae. Secondary haustoria are more widespread and are produced laterally on the roots of the parasite. Primary haustoria are known from only a few genera and are considered to be an advanced and specialized feature (Kuijt, 1969). Riopel has found that secondary haustoria can be routinely induced in *Agalinis purpurea* by treatment with 0.2 mg ml⁻¹ GT. The present work clearly indicates that a similar process of haustorial induction may occur in the primary haustorium of *Striga asiatica*.

Germination and hair formation, which we consider the early stages of haustorial development, are apparently two distinct processes mediated by separate chemical controls. This is evidenced by the fact that GT alone cannot cause witchweed seed to germinate, and in addition, none of the germination stimulants tested induced hair formation. Williams (1961) induced radicular hair formation in *Striga asiatica* with the addition of kinetin. Our work has shown that these hairs are in fact present in the natural system. Parasite seeds germinated adjacent to host roots normally show hair development prior to attachment (Williams, 1961; Nickrent and Musselman, unpublished). These hairs are inconspicuous and tend to cluster along portions of the parasite radicle to the host root.

We report here the attachment to and penetration of non-host roots. It appears that this stage of parasite development may represent one phase where host specificity is determined.

The significance of haustorial promotion by GT is currently under study. Species of *Striga* are considered physiologically advanced members of the Scrophulariaceae in that they have a narrow host range. Four stages in the life cycle of *S. asiatica* may exercise some control in host selection. (1) Witchweed has specific germination requirements which can be satisfied by such compounds as corn root exudates or strigol. (2) Haustorial initiation does not occur unless the parasite is in close proximity to a host root. (3) A compatible host substrate must be present to allow successful attachment and penetration of the haustorium. Our observations suggest that following haustorial initiation, attachment is indiscriminate and therefore may not be critical in host selection. (4) Witchweed has not been observed to flower without attachment to a monocot host. These stages suggest that host selection in the parasite may be operating at several levels in the life cycle.

The observation that GT treatment will permit the attachment of *S. asiatica* to a broad range of plants provides a basis for several lines of study in furthering our understanding of the biology of host selection in this important parasite.

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