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# Novel sources of resistance to *Striga hermonthica* in *Tripsacum dactyloides*, a wild relative of maize

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## Summary

- The parasitic weed *Striga hermonthica* lowers cereal yield in small-holder farms in Africa. Complete resistance in maize to *S. hermonthica* infection has not been identified. A valuable source of resistance to *S. hermonthica* may lie in the genetic potential of wild germplasm.
- The susceptibility of a wild relative of maize, *Tripsacum dactyloides* and a *Zea mays–T. dactyloides* hybrid to *S. hermonthica* infection was determined. *Striga hermonthica* development was arrested after attachment to *T. dactyloides*. Vascular continuity was established between parasite and host but there was poor primary haustorial tissue differentiation on *T. dactyloides* compared with *Z. mays*. Partial resistance was inherited in the hybrid.
- *Striga hermonthica* attached to *Z. mays* was manipulated such that different secondary haustoria could attach to different hosts. Secondary haustoria formation was inhibited on *T. dactyloides*, moreover, subsequent haustoria formation on *Z. mays* was also impaired.
- Results suggest that *T. dactyloides* produces a signal that inhibits haustorial development: this signal may be mobile within the parasite haustorial root system.

**Key words:** *Tripsacum dactyloides*, *Striga hermonthica*, parasitic plants, wild relatives, plant resistance, haustorium.



## Introduction

*Striga hermonthica* is an obligate root hemiparasite native to the semiarid tropics. Agronomically important cereals such as maize, sorghum, millet and upland rice are major hosts and infection of these crops threatens grain production for subsistence farmers in Africa. Yield losses of 5–15% are common, although locally, under severe infestations, losses can far exceed this amount, resulting in complete crop failure (Riches & Parker, 1995).

The lifecycle of *S. hermonthica* is intimately associated with that of its host to ensure survival. Seeds remain dormant in the soil until chemical signals (hydroquinones) released from the roots of potential hosts initiate seed germination (Hauck *et al.*, 1992; Sugimoto *et al.*, 1998). An array of phenolic derivatives, distinct from those signals involved in germination, have been identified that induce haustorial development in *Striga* spp. (MacQueen, 1984; Albrecht *et al.*, 1999). The haustorium is a unique infection structure that provides a physiological bridge between host and parasite, facilitating the transfer of host-derived water and solutes to the developing parasite through direct host–parasite xylem–xylem continuity (Dörr, 1997).

The influence of *S. hermonthica* on biomass allocation of its cereal host is well documented with stem and grain weight being most severely affected (Gurney *et al.*, 1999, 2002a). Losses in host productivity can occur when the biomass of the parasite is very small and a negative impact on host performance can be detected within days of infection (Frost *et al.*, 1997). Differences in dry matter accumulation between infected and uninfected cereals partly results from the role of *S. hermonthica* as a sink for host carbon and inorganic solutes but also as a result of a lowering of host carbon fixation (Frost *et al.*, 1997; Gurney *et al.*, 2002a). In addition, the parasite has a marked influence on host nitrogen metabolism, altering the free amino acid profile of host tissues (Pageau *et al.*, 2003). The possibility that *S. hermonthica* disrupts host metabolism through toxins has also been raised (Ejeta & Butler, 1993), although, there is no direct evidence to support this hypothesis.

Control of *S. hermonthica* has proved challenging, partly as a result of the intricate life-cycle of the parasite with its host, but also because of financial and practical constraints that limit the use of chemical forms of control in developing countries. Much research has focused on the development of cereals resistant to infection as a sustainable long-term control strategy. Complete resistance to *S. hermonthica* infection has not been identified for maize or sorghum, although varieties often differ in their sensitivity to infection (Gurney *et al.*, 1995, 2002a). Varieties of cowpea resistant to the parasite *Striga gesnerioides* have been identified (Lane *et al.*, 1993). At present there is no evidence of complete resistance to *Striga* spp. in cultivated maize: a valuable source of resistance to *S. hermonthica* may lie in the genetic potential of wild germplasm (Tanksley & McCouch, 1997). One possible pool of resistance genes/alleles lies in the wild relative of maize, *Tripsacum*, a small genus that occurs naturally in the Americas from latitudes 42° N to 24° S (Harlan & De Wet, 1977).

Two studies were conducted. The aim of the first study was to evaluate the susceptibility of a wild relative of maize, *Tripsacum dactyloides* and a *Zea mays–T. dactyloides* hybrid to *S. hermonthica* infection. Four stages of the host–parasite association were examined for each host genotype: (1) germination of *S. hermonthica*; (2) attachment of *S. hermonthica*, specifically primary haustoria maturation; (3) development of *S. hermonthica* post attachment; and (4) the influence of *S. hermonthica* on host growth. Differences in the development of *S. hermonthica* on *Z. mays* and *T. dactyloides* were explored further to determine whether *T. dactyloides* lacked metabolites/signals necessary for the differentiation of *S. hermonthica* haustoria or whether *T. dactyloides* produced metabolites that impaired haustorial development. Specifically, secondary haustoria were examined from individual *S. hermonthica* plants that had been attached to *Z. mays* and *T. dactyloides*. Secondary haustoria differ from primary haustoria only in that they initiate at subterminal positions on a lateral root, whereas primary haustoria differentiate from the radicle/root apex (Kuijt, 1966). This study allowed the following questions to be addressed: (1) if *T. dactyloides* lacked appropriate signals could these be supplied via attachment to susceptible *Z. mays*; (2) following attachment of a secondary haustorium on *T. dactyloides* would subsequent attachments to maize be affected, providing evidence for the movement of a metabolite/signal from *T. dactyloides* to *S. hermonthica* and even to the maize host?

## Materials and Methods

### Study 1: Evaluation of *T. dactyloides* and *T. dactyloides–Z. mays* hybrid infected by *S. hermonthica*

**Plant material** Before this study, 30 *Tripsacum* accessions were screened in western Kenya for parasite emergence. Only one of these appeared to lack parasite attachments. This accession of *T. dactyloides* was used in this study together with a hybrid maize variety (H1) derived from a cross between two CIMMYT (Mexico) inbred lines (CML 135 × CML 139) (susceptible to *S. hermonthica*), and a hybrid derived from a cross between H1 and *T. dactyloides*. Again, a preliminary study was conducted to examine the $BC_1$, $BC_2$ and $BC_3$ hybrids for resistance to *S. hermonthica*. All hybrids showed a similar level of resistance to *S. hermonthica* (i.e that it was intermediate between the susceptible parent and the resistant *T. dactyloides* parent; data not shown). Plants from the third backcross ($BC_3$-38C) were selected for detailed laboratory studies for the following reasons: (1) $BC_3$ lines were examined in the field in western Kenya and showed no *S. hermonthica* emergence; (2) $BC_3$ plants were phenotypically similar to the *Z. mays* parent and individuals showed a uniform morphology; (3) $BC_3$ plants contained a full compliment of the *Z. mays* genome (20*n*) and one-quarter of the *T. dactyloides* genome (18*n*). Further details of these plants can be found in Leblanc *et al.* (1996).

The seeds of *S. hermonthica* used in this study were collected from plants parasitizing maize in Kibos, western Kenya in 1997. *Striga hemonthica* is an obligate outcrossing species, thus populations will be genetically variable. This 'population' of *S. hermonthica* was selected because it is representative of *S. hermonthica* found in a large area of western Kenya.

**Germination study** *Striga hermonthica* seeds were preconditioned as described by Gurney *et al.* (2002b). Sterilization of *Z. mays*, *T. dactyloides* and the *Z. mays–T. dactyloides* hybrid was carried out as for *S. hermonthica* but, in addition, germination was carried out under aseptic conditions. The seeds were placed in Petri dishes containing N6 nutrient agar medium at pH 5.7 (Chu *et al.*, 1975). Petri dishes were then placed in the dark in a controlled environment room with a 30°C/20°C day/night temperature until germination had occurred (3 d). Following germination, seedlings were transferred to 25 $cm^3$ glass tubes as described by Gurney *et al.* (2002b). Seedlings were placed in a controlled environment room operating with a 12-h photoperiod and a photon flux density of 800 μmol $m^{-2}$ $s^{-1}$ at plant height. The day/night temperatures were maintained as above and relative humidity was maintained at a 50%/30% day/night regime.

The germination of *S. hermonthica* in the presence of plant root exudate was examined for all three genotypes. Twenty-four hours before testing the activity of the root exudate on *S. hermonthica* seeds the vials were emptied, rinsed and refilled with distilled water. *S. hermonthica* seeds were exposed to 200 μl of root exudate in microtitre plates, each well containing approx. 30–50 *S. hermonthica* seeds. In addition, *S. hermonthica* seeds were also exposed to 200 μl of the synthetic germination stimulant GR-24 (0.1 mg $l^{-1}$) to determine seed viability. Ten replicates of each treatment were established. The microtitre plates were sealed, wrapped in aluminium foil and placed in the controlled environment room for 24 h. The percentage of seeds that had germinated after this time was

counted under a dissecting microscope. Germination was also expressed as percentage germination of viable seed.

**Inoculation of plant material with *S. hermonthica*** Preconditioning of *S. hermonthica* seeds, plant germination and initial growth conditions were as described above. After 12 d a single seedling was transferred to a root observation chamber (rhizotron). Rhizotrons were used to observe attachment and development of *S. hermonthica* throughout the period of study (Frost *et al.*, 1997). A sheet of glass-fibre filter paper (Whatmann GF/A, BDH, Poole, UK), of 25 × 15 cm, was placed in the rhizotron on the surface of the sand and the plant roots were evenly spread out over the surface of the filter paper. The rhizotrons were drip-fed with 40% Long Ashton solution containing 1 mol $m^{-3}$ ammonium nitrate (Hewitt, 1966) at four intervals during the photoperiod to give a total volume of 200 ml $d^{-1}$. Twenty-four rhizotrons for each genotype were established.

At 25 d after planting (dap) eight plants of each genotype were infected with 20 mg of preconditioned *S. hermonthica* seeds (approximately 2000 viable seeds): seeds were suspended in 20 ml of distilled water and pipetted evenly on to the glass-fibre filter paper. A further eight plants of each genotype were infected with *S. hermonthica* with added synthetic stimulants: a germination stimulant (GR-24) and a haustorial initiation factor (HIF; syringic acid) (Macqueen, 1984). *Striga hermonthica* seeds were suspended in 20 ml of GR-24 (0.1 mg $l^{-1}$) and then pipetted onto the filter paper: after 48 h, 20 ml of syringic acid (0.5 mg $l^{-1}$) were pipetted on to the glass-fibre filter paper (before this study it had been determined that 0.5 mg $l^{-1}$ syringic acid initiated haustorial formation in 50% of germinated *S. hermonthica* seeds). This was done for two reasons: (1) to create two levels of infection; and (2) to overcome any resistance at the level of germination or attachment. The rhizotrons were returned to the controlled environment room in a complete randomised design.

**Growth analysis** Between 25 dap and 46 dap, the root systems of infected plants were observed through the perspex sheet with a binocular microscope (SUZD 338, Former USSR). The number of tubercles and subsequent numbers of *S. hermonthica* plants supported by each host were counted on the entire root system. In addition, the development of *S. hermonthica* plants was defined according to their morphological appearance as follows: stage 1, *S. hermonthica* radicle had attached to the host root and swollen to form a tubercle. The seed coat remained intact; stage 2, leaf primordia had emerged from the seed coat; stage 3, *S. hermonthica* shoots had between two and five scale leaf pairs; stage 4, *S. hermonthica* shoots had between six and 10 scale leaf pairs; stage 5, *S. hermonthica* shoots had 11 scale leaf pairs or more. Data are reported for 46 dap only.

At 90 dap biomass was determined by separating the plants into stems, leaves and roots. Roots were separated from the sand by careful washing over a 2-mm meshed sieve after which *S. hermonthica* plants were detached from the roots at the point of tubercle attachment. The plant material was oven dried at 70°C for 72 h before weighing.

**Primary haustoria: tissue processing for light microscopy** Primary haustoria of *S. hermonthica*, together with the region of infected host root, were dissected from the host root system at 37 dap and 47 dap (representing 7 d and 17 d after infection, dai). At each of these time-points the developmental stage of *S. hermonthica* was recorded for each host plant (see above) and five representative haustoria were sampled from each host plant. For each haustorium sampled, sections were cut and observed across the entire haustorium to ensure that the maximum tissue development was observed and that results were not obscured by differences in the angle and position of haustorium attachment. Specimens were fixed in 3% w : v formaldehyde in 50% (v : v) ethanol–5% (v : v) glacial acetic acid for 24 h at 21°C. Specimens were then dehydrated in an ethanol series (50, 80, 90, 100, 100%: 24 h each) and transferred to an embedding solvent (Histoclear; BDH, Poole, UK) through a histoclear–ethanol series (30%, 50%, 80%, 100%; 24 h each) and finally saturated with paraffin (paraplast Xtra; Sigma, St. Louis, USA). Sections (5 μm) were cut with a microtome (Reichert, Osterreich, Austria) and attached to adhesive-treated microscope slides (polysine slides; SLS, Nottingham, UK). After the removal of paraffin, slides were stained with Safranin O (1% w : v in 30% v : v ethanol, 5 min) and Astra blue (0.5% w : v in 2% w : v tartaric acid, 10 s). Sections were dried on a hot plate at 45°C for 1 h and mounted with DePeX (BDH). Sections were observed using a transmission microscope (Olympus BX51; Olympus Optical Ltd, London, UK) and photographed using a digital camera (Olympus DP11).

**Primary haustoria: tissue processing for transmission electron microscopy (TEM)** Primary haustoria were collected from *Z. mays* and *T. dactyloides* as above and fixed in 5% (v : v) glutaraldehyde–4% (w : v) paraformaldehyde (pH 7.2) at 0°C for 2 h, and washed in sucrose (10%) in 0.1 M sodium cacodylate buffer. Secondary fixation was carried out in 2% (w : v) osmium tetroxide at room temperature: 1 h. The specimens were dehydrated in an ethanol series (75, 95, 100%, and 100% dried over anhydrous $CuSO_4$; 15 min each) followed by propylene oxide (two changes, 15 min each). Specimens were placed in 50 : 50 mixture of propylene oxide–Spurrs epoxy resin (Agar Scientific Ltd., Stansted, UK) followed by full-strength Spurrs epoxy resin for 6 h. Specimens were then embedded in fresh resin at 60°C for 48 h. Semithin sections (1 μm) were cut with glass knives using a Reichert Ultracut E ultramicrotome, stained with toluidine blue and observed with a Nikon microscope (Nikon Corporation, Kawasaki, Japan). Once maximum tissue differentiation was observed for each haustorium, ultrathin sections (70–90 nm) were cut using a diamond knife and were collected on copper grids.

Post-staining was achieved with 3% uranyl acetate in 50% ethanol followed by staining with Reynolds lead citrate (Reynolds, 1963). Observations were carried out using a Philips CM10 transmission electron microscope (Philips, Eindhoven, The Netherlands) at an accelerating voltage of 80 Kv.

### Study 2: Evaluation of the ability of *T. dactyloides* to impair haustoria development

**Manipulation of secondary haustoria** *Zea mays* plants were established in the rhizotron system and grown for 14 d. Concurrently, *T. dactyloides* seedlings were grown in water culture (as described for study 1). The following steps were then performed (Fig. 1).

Step 1: *Z. mays* was infected by an individual *S. hermonthica*. Approximately 14 dai the primary haustorium produced lateral roots. A *T. dactyloides* seedling was introduced as a second host for the same *S. hermonthica* plant. A root from *T. dactyloides* was placed in front of a developing lateral root to allow a secondary haustorium to form (T in Fig. 1).

Step 2: Subsequently, secondary haustoria were left to develop on the original *Z. mays* host (Z in Fig. 1) after the introduction of *T. dactyloides*. The rhizotrons were returned to the growth room for 10 d.

Control rhizotrons were established at the same time where the second introduced host was either (1) a second *Z. mays* plant, H1: CML 135 × CML 139 (identical genotype to the first host) or (2) *Sorghum bicolor* (L.) Moench, var. CSH-1, a known *S. hermonthica*-susceptible variety from India (Gurney *et al.*, 1999). Ten rhizotrons were established for each host genotype introduced into the system.

***Secondary haustoria*: tissue processing for light microscopy** Two haustoria from each rhizotron were dissected and sectioned from the host root systems (as described for study 1): the secondary haustorium attached to the second introduced host, *T. dactyloides* (T in Fig. 1); and the secondary haustorium left to attach to the original *Z. mays* host after the addition of *T. dactyloides* (Z in Fig. 1). The same two haustoria types were sectioned when the second introduced host was either *Z. mays* or *S. bicolor*.

### Statistical analyses

The influence of *S. hermonthica* on the biomass of its host, parasite biomass supported by each host and the development of the parasite were analysed using analysis of variance procedures for a randomised design (Minitab statistical package, version 10.2, Minitab Inc., Pensylvania, USA). Tukey's multiple comparison tests were carried out on the original data (Zar, 1999). Proportional data were analysed using analysis of variance procedures following arcsin $\sqrt{x}$ transformation of the actual data. The numbers of attached *S. hermonthica* per host plant were analysed using a non-parametric Kruskal–Wallis test (Minitab) followed by multiple comparison procedures (Zar, 1999).

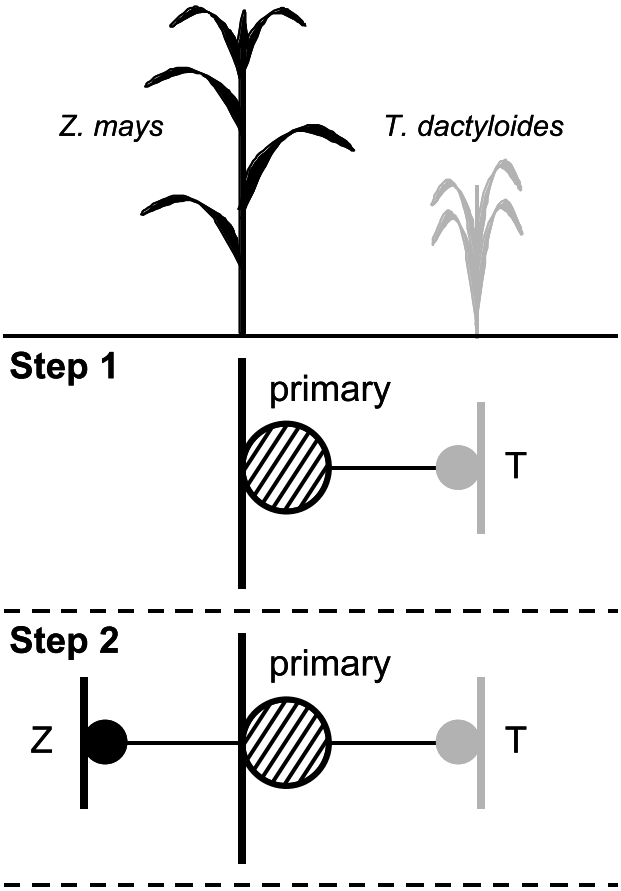



**Fig. 1** Diagram of an experiment to examine secondary haustoria from an individual *Striga hermonthica* plant attached to two hosts. Step 1: *Zea mays* is infected by the primary haustorium of *S. hermonthica*. Lateral roots produced by *S. hermonthica* are allowed to attach and form a secondary haustorium on a second host, *Tripsacum dactyloides* (T) (a different *Z. mays* or *Sorghum bicolor* may also be introduced as a second host). Step 2: following attachment of a secondary haustorium to *T. dactyloides*, a different secondary haustorium is allowed to form on the original *Z. mays* host (Z). Two haustoria types are sampled: T, secondary haustorium on a second host, *T. dactyloides* (or *Z. mays*, or *S. bicolor*) and Z, secondary haustorium on the original *Z. mays* after the introduction of *T. dactyloides*.

## Results

### Study 1: Evaluation of *T. dactyloides* and *T. dactyloides–Z. mays* hybrid infected by *S. hermonthica*

***Striga hermonthica* germination, attachment and development** *Striga hermonthica* seed germinated in the presence of root exudate from all three genotypes examined; *Z. mays*, *T. dactyloides* and *Z. mays–T. dactyloides* hybrid (Table 1). High

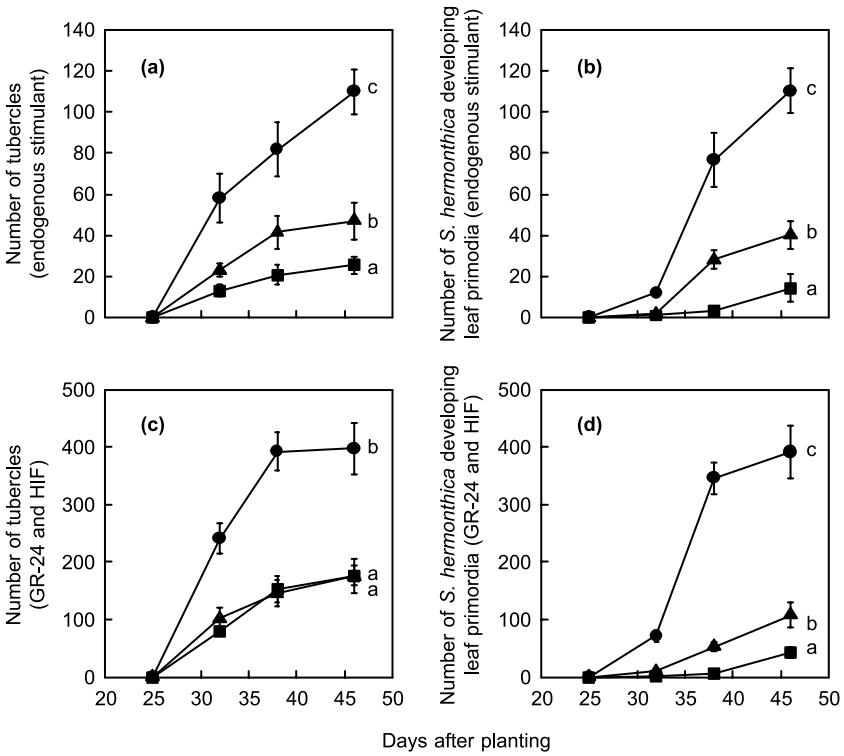



**Fig. 2** Number of tubercles (a,c) and number of *Striga hermonthica* that develop leaf primordia (b,d) attached to the roots of *Zea mays* (circles), *Tripsacum dactyloides* (squares) and *Z. mays–T. dactyloides* hybrid (triangles). Plants were infected with *S. hermonthica* with either no added stimulant (a,b) or with added GR-24 and syringic acid (c,d). Data are means ± SE, $n = 6$. Means at 46 dap not sharing the same letter within each graph are significantly different ($P \leq 0.05$). Note the different scale on the *y*-axis.

**Table 1** Germination of *Striga hermonthica* in the presence of root exudate collected from 12-d-old plants and in the presence of GR-24 (0.1 mg $l^{-1}$)

| Genotype | % *Striga* germination |
|---|---|
| *Zea mays* | $81.4 \pm 1.90^{c}$ (94.7) |
| *Tripsacum d*actyloides | $30.6 \pm 1.75^{a}$ (35.6) |
| *Z. mays–T. dactyloides* hybrid | $65.6 \pm 2.13^{b}$ (76.4) |
| GR-24 | 85.9 ± 0.82 (100) |

Data are expressed as percentage germination in the original media (means ± SE, $n = 8$). Data analysed using ANOVA procedures following arcsin $\sqrt{x}$ transformation of the actual data. Means not sharing the same superscript letter within each column are significantly different ($P \leq 0.05$). Data in parenthesis show germination as a percentage of viable seed.

rates of germination were observed for all three species when corrected for differences in root biomass.

The first visible sign of *S. hermonthica* attachment occurred at 30 dap for all three genotypes (Fig. 2). By 46 dap the greatest number of attachments were observed on *Z. mays* (110 attachments); 4.4 times greater than with *T. dactyloides* (25 attachments). The *Z. mays–T. dactyloides* hybrid was intermediate in response (48 attachments) (Fig. 2a). All *S. hermonthica* attached to *Z. mays* successfully developed leaf primordia. In marked contrast, only 56% of attachments on *T. dactyloides* developed leaf primordia. The *Z. mays–T. dactyloides* hybrid was again intermediate between parent genotypes with 85% of attachments developing leaf primordia (Fig. 2b). Addition of GR-24 and syringic acid resulted in a dramatic increase in the number of attachments to the roots of all host genotypes (Fig. 2c) with a three-, seven- and five-fold increase for *Z. mays*, *T. dactyloides* and the *Z. mays–T. dactyloides* hybrid, respectively, compared with no added stimulant. By 46 dap, *Z. mays*, *T. dactyloides* and *Z. mays–T. dactyloides* hybrid supported 350, 176 and 175 attachments, respectively. Again, all attachments on *Z. mays* developed leaf primordia. In marked contrast, 24% and 60% of attachments on *T. dactyloides* and *Z. mays–T. dactyloides* hybrid developed leaf primordia (Fig. 2d).

After initiation of leaf primordia development of *S. hermonthica* on *Z. mays* was rapid and by 46 dap 91% of shoots had 11 scale leaf pairs or more (development stage 5) (Fig. 3). By contrast, the growth of *S. hermonthica* on *T. dactyloides* was arrested at an early stage of development: 53% developed leaf primordia (stage 2) and only 3% produced between two and five scale leaf pairs (stage 3). Development of *S. hermonthica* on the *Z. mays–T. dactyloides* hybrid was greater than that of its *T. dactyloides* parent: 45% of *S. hermonthica* developed to stage 3, and like the *Z. mays* parent, a number of parasites showed good development; 26% and 9% reached stage 4 and stage 5, respectively. Addition of stimulants increased the numbers of *S. hermonthica* plants at every development stage for each genotype compared with plants without added stimulants (Fig. 3). The pattern of development of *S. hermonthica* from stages 2–5 for all host genotypes was not affected, although, the number of *S. hermonthica* that arrested at attachment (stage 1) was greatly increased on the *T. dactyloides* and *T. dactyloides–Z. mays* hybrid.

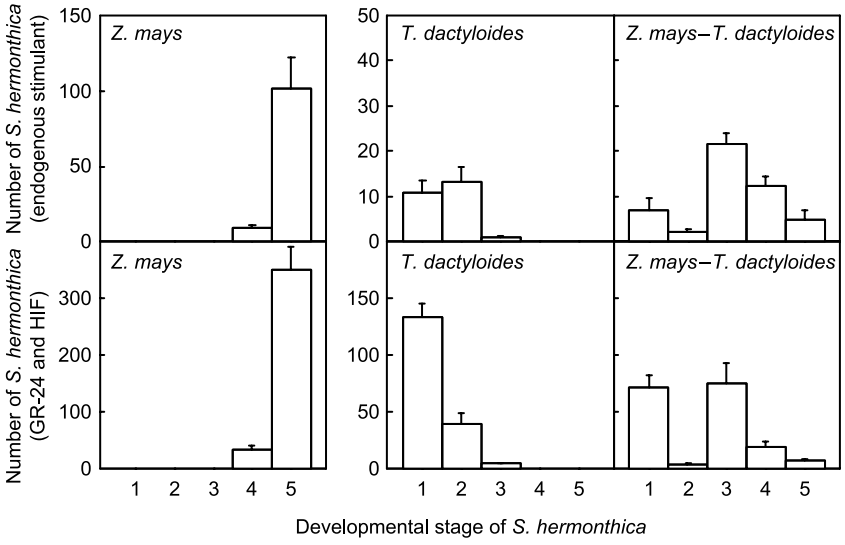



**Fig. 3** Development of *Striga hermonthica* plants attached to the roots of *Zea mays*, *Tripsacum dactyloides* and *Z. mays–T. dactyloides* hybrid at 46 dap. Plants were infected with *S. hermonthica* with either no added stimulant (upper graphs) or with added GR-24 and syringic acid (HIF) (lower graphs). Data are means ± SE, $n = 6$. Development of *S. hermonthica* plants were defined according to their morphological appearance; 1, *Striga* attachment, seed coat is intact and a tubercle is evident; 2, emergence of leaf primordia; 3, *Striga* shoots with between 2 and 5 scale leaf pairs; 4, *Striga* shoots with between 6 and 10 scale leaf pairs; 5, *Striga* shoots with more than 10 scale leaf pairs. Note the different scales on the *y*-axis.

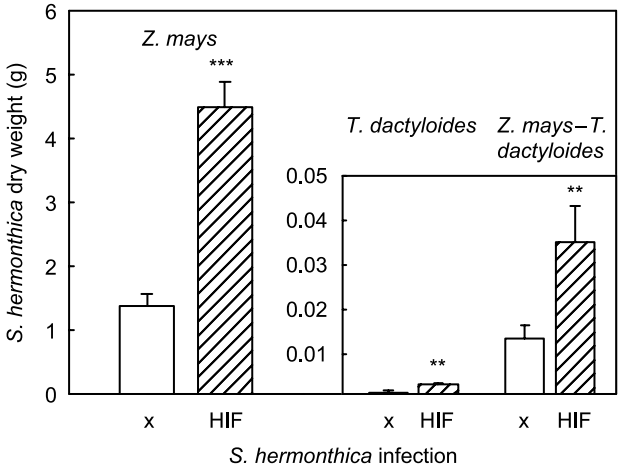



**Fig. 4** Dry weight of *Striga hermonthica* supported by *Zea mays*, *Tripsacum dactyloides* and *Z. mays–T. dactyloides* hybrid. Plants were infected with *S. hermonthica* with either no added stimulant (x) or with added GR-24 and syringic acid (HIF). Data are means ± SE, $n = 6$. Asterisks denote significant differences between *S. hermonthica* dry weight with no added stimulant and with added GR-24 and syringic acid for each host genotype (**, $P \leq 0.01$; ***, $P \leq 0.001$).

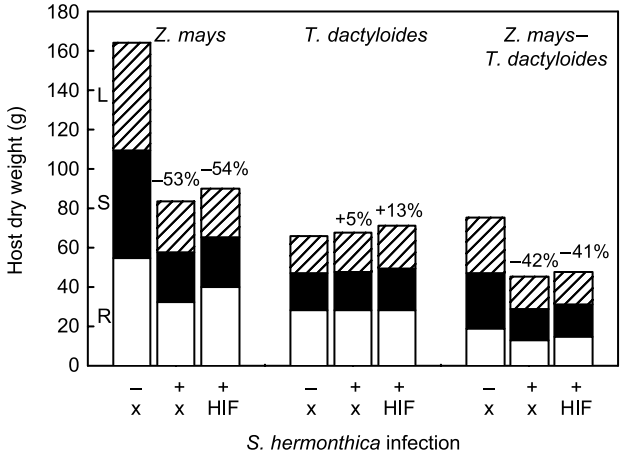



**Fig. 5** Dry weight partitioning in *Zea mays*, *Tripsacum dactyloides* and *Z. mays–T. dactyloides* hybrid. Plants were grown in the absence (–) or presence (+) of *Striga hermonthica*. Plants were infected with *S. hermonthica* with either no added stimulant (x) or with added GR-24 and syringic acid (HIF). Plants were separated into root (R), stem (S) and leaf (L) components. Data are means of 6 replicates. Data inset expresses the stem biomass of infected plants as percentage biomass not gained or gained compared with the stem biomass of respective control plants.

**Plant growth and biomass accumulation** At harvest, *Z. mays* supported the greatest parasite biomass of 1.4 g *S. hermonthica* per plant. *Tripsacum dactyloides* and *Z. mays–T. dactyloides* hybrid supported levels two to three orders of magnitude lower (1.3 mg and 10 mg *S. hermonthica*/plant for hosts with no added stimulant, respectively) (Fig. 4). Addition of stimulants resulted in a 3.5- and 4-fold increase in parasite biomass for *Z. mays* and *Z. mays–T. dactyloides* hybrid, respectively, compared with infected plants where no stimulant was added. *T. dactyloides* was least affected with only a twofold increase in parasite biomass (Fig. 4). Greater parasite biomass with the addition of stimulants was a consequence of increased numbers of attached parasites as no significant increase in the dry weight of individual parasites was observed (data not shown).

*Striga hermonthica* infection had a negative impact on total plant weight and dry weight partitioning for *Z. mays* and *Z. mays–T. dactyloides* hosts (Fig. 5). Infected plants accumulated 45% and 33% less total biomass, respectively, compared with control plants ($P \leq 0.01$). Biomass allocation to stem and leaf components was most severely affected. *Striga hermonthica* had a similar effect on each host regardless of the level of parasite infection. Total plant biomass accumulation

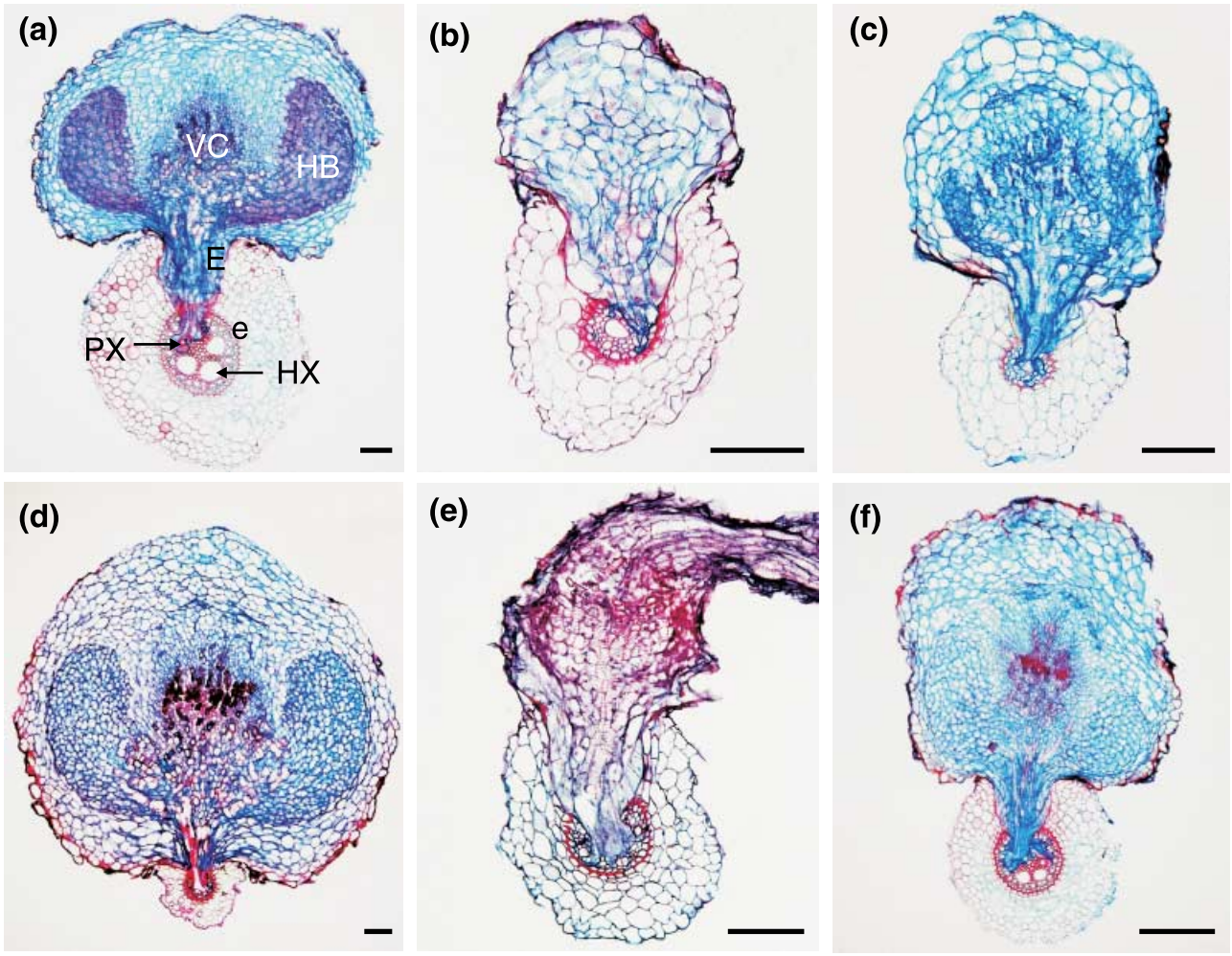



**Fig. 6** Light micrographs of longitudinal sections of haustoria attached to *Zea mays* (a,d), *Tripsacum dactyloides* (b,e) and *Z. mays–T. dactyloides* hybrid (c,f). Haustoria were sampled at 7 d (a,b,c) and 17 d (d,e,f) after infection. E, endophyte; **e**, endodermis; HB, hyaline body; HX, host xylem; PX, parasite xylem; VC, haustorium vascular core. Bar, 0.1 mm.

in infected *T. dactyloides* plants showed no detrimental response to *S. hermonthica* infection.

**Primary haustorial development** At 7 dai, longitudinal sections of the haustorium on the roots of *Z. mays* revealed three clearly defined regions, the vascular core (VC), the hyaline body (HB) and the endophyte (E) (Fig. 6a). The vascular core comprised xylem tracheary elements intermingled with parenchyma cells: tracheary elements traversed the haustorium to form a xylem bridge. Encircling the vascular core was a well-defined hyaline body. This densely stained region showed an abundance of cell organelles (Fig. 7a). The endophyte penetrated the *Z. mays* root and traversed the cortex tissue: compressed host cells were evident in this region and lignification of cells surrounding the endophyte was observed. Penetration of the root endodermis was achieved despite heavy lignification of these cells. Finally the parasite xylem (PX) penetrated the host endodermis (Fig. 6e) into the host xylem tissue (HX). At this point a functional continuum was established between the *Z. mays* and *S. hermonthica* xylem vascular systems. Similarly, haustoria attached to *T. dactyloides* (Fig. 6b) and the *Z. mays–T. dactyloides* hybrid (Fig. 6c) demonstrated successful host–parasite xylem–xylem connections, despite heavy lignification of the host stele. In marked contrast to *Z. mays*, the haustorium attached to *T. dactyloides* showed poor tissue differentiation. This was most evident for the hyaline body, which also showed a lack of organelle-rich cells (Fig. 7b). The haustorium formed on the *Z. mays–T. dactyloides* hybrid also showed a lack of tissue differentiation compared with *Z. mays*. In addition, the overall size of the haustorium formed on *T. dactyloides* and the *Z. mays–T. dactyloides* hybrid was less than half the size of that on *Z. mays*. By 17 dai, the organization and structure of the haustorium formed on *Z. mays* was similar to those examined earlier, although, the overall size of the haustorium was much greater (Fig. 6d). The haustorium formed on *T. dactyloides* showed no further development than

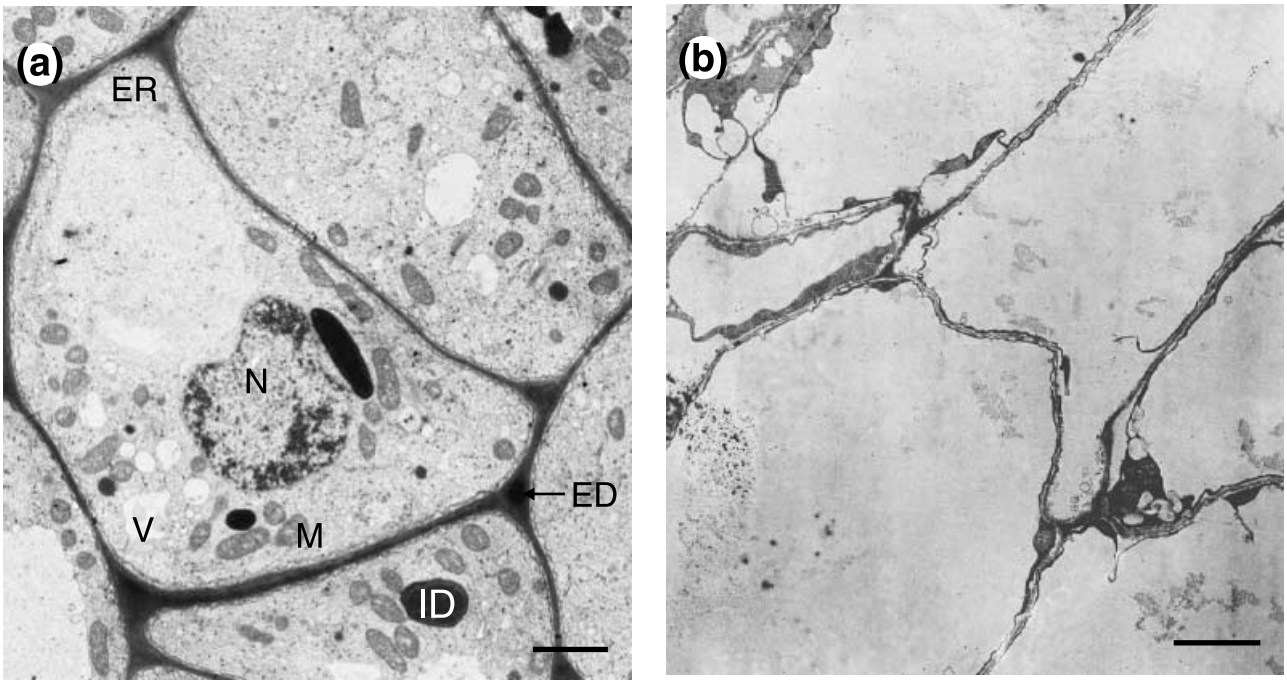



**Fig. 7** Electron micrographs of the hyaline body cells of *Zea mays* (a) and *Tripsacum dactyloides* (b) at 17 d after infection. ER, endoplasmic reticulum; ED, extracellular deposits; ID, intracellular deposits; M, mitochondria; N, nucleus; V, vacuole. Bar, 2 μm.

that observed at 7 dai (Fig. 6e), despite substantial xylem–xylem connections between host and parasite. By contrast, the haustorium attached to the *Z. mays*–*T. dactyloides* hybrid showed greater development with more clearly defined regions, particularly the xylem core and hyaline body (Fig. 6f).

### Study 2: Evaluation of the ability of *T. dactyloides* to impair haustorial development

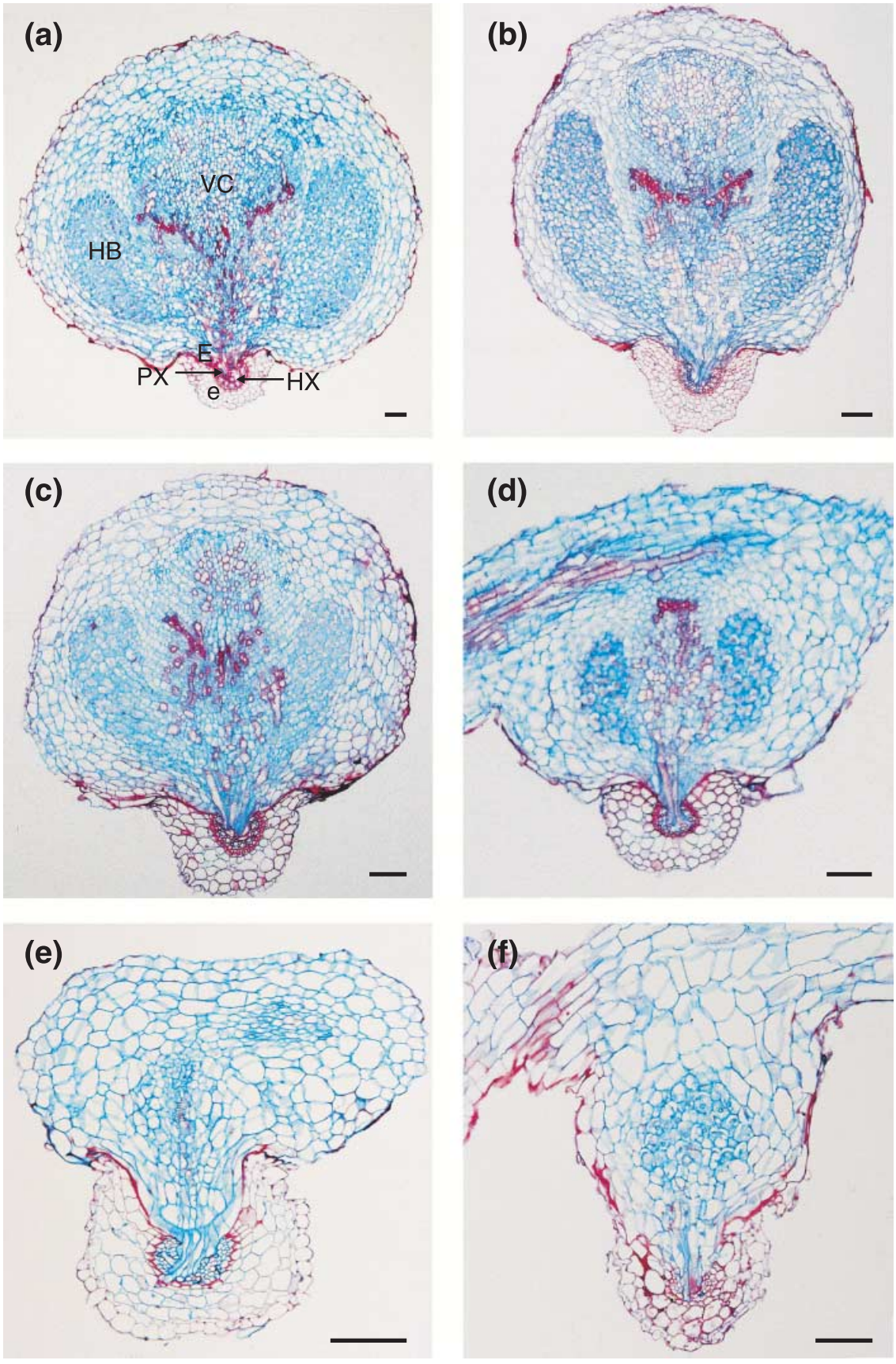
**Secondary haustorial development** Following infection of *Z. mays* by a single *S. hermonthica* plant a second host was introduced for the same parasite (see Fig. 1). When *Z. mays* or *S. bicolor* was introduced as a second host for *S. hermonthica*, the secondary haustorium that developed on each of these roots showed a mature and highly differentiated structure (a and c, respectively, in Fig. 8). After the introduction of *Z. mays* or *S. bicolor* as a second host the secondary haustoria that subsequently developed on the roots of the original *Z. mays* host also showed mature tissue differentiation (Fig. 8b and d, respectively). A markedly different response was observed with the addition of *T. dactyloides* as a second host. In this instance development of a secondary haustorium on *T. dactyloides* was severely impaired and, despite successful host–parasite xylem–xylem continuity, poor internal tissue differentiation was observed (e in Fig. 8). Moreover, the secondary haustorium that subsequently attempted to develop on the original *Z. mays* host after the introduction of *T. dactyloides* now also showed poorly differentiated structures (Fig. 8f). The hyaline body was virtually absent, as was observed for secondary haustoria formed on *T. dactyloides*.

## Discussion

### Host resistance to parasite infestation

Host resistance mechanisms have rarely been reported in parasitic angiosperm–host associations but there are marked exceptions to this. A hypersensitive response has been observed in the *Striga gesnerioides*–cowpea (Lane *et al.*, 1993) and the *Orobanche aegyptiaca*–vetch association (Goldwasser *et al.*, 2000): necrotic areas appeared at the site of parasite attachment due to localized cell death of the host tissue, resulting in degeneration of the parasite. Physical barriers to infection have been illustrated for the *Orobanche cumana*–sunflower/vetch association with the production of an encapsulation layer halting progress of the invading endophyte and increased lignification of the

**Fig. 8** Light micrographs of longitudinal sections of secondary haustoria. Following attachment of *Striga hermonthica* to *Zea mays*, secondary haustoria were manipulated such that they could attach to a different second host. Structures of the first secondary haustorium on the introduced hosts *Z. mays* (a) or *Sorghum bicolor* (c) or *Tripsacum dactyloides* (e) are shown. Structures of the subsequent secondary haustoria, all on the original *Zea mays* host, are shown after the first secondary haustorium attached to *Z. mays* (b), *S. bicolor* (d) and *T. dactyloides* (F). E, endophyte; e, endodermis; HB, hyaline body; HX, host xylem; PX, parasite xylem; VC, haustorium vascular core. Bar, 0.1 mm.


(a)
VC
HB
E
PX
HX
e
(b)
(c)
(d)
(e)
(f)

endodermal cell walls (Dörr *et al.*, 1994; Labrousse *et al.*, 2001). Chemical barriers to infection can be secreted at the host–parasite interface including phenolic compounds (Goldwasser *et al.*, 1999) and induced phytoalexins (Wegmann *et al.*, 1991; Jorrin *et al.*, 1996). Moreover, induction of the synthesis of pathogenesis-related proteins has been observed in *Orobanche*-infected tobacco (Joel & Portnoy, 1998; Westwood *et al.*, 1998).

Resistance to *Striga* spp. has also been examined for the nonhost, marigold (Gowda *et al.*, 1999). Necrosis of root cortical cells around the penetrating endophyte and cell wall thickening at the *Striga*–nonhost interface was exhibited. The authors suggested that this response was similar to a hypersensitive response observed in some plant–pathogen interactions. Changes in gene expression occurred (designated NRSA-1), directly associated with the nonhost response to infection. The authors postulated the involvement of NRSA-1 in the activation/regulation of downstream responses.

For all these associations resistance was expressed in the host root before vascular continuity was established between host and parasite. Lignification of the host stele and tissues surrounding the penetrating endophyte was evident in our study for both resistant and susceptible genotypes in response to the invading endophyte. In *T. dactyloides* resistance was expressed after penetration and establishment of host-parasite xylem-xylem connections.

### Why is haustorial development impaired on *T. dactyloides?*

The success of parasitic plants results largely from strategies that tightly couple developmental transitions with host recognition signals. In the absence of specific signals and/or the production of inhibitory compounds by a potential host, successful infestation by the parasite is impaired. Haustorial initiation occurs in response to specific xenognosins produced by a potential host and is under tight redox control (Albrecht *et al.*, 1999; Keyes *et al.*, 2000). From our study, it is evident that *T. dactyloides* does produce primary HIF(s), as a small number of parasites initiated haustorial formation. However, the addition of germination and haustorial stimulants demonstrated a marked increase in the number of tubercles formed on *T. dactyloides*. These results suggest that *T. dactyloides* produces low concentrations of primary HIF(s). Studies with the root parasites *S. asiatica* and *Tryphysaria versicolor* suggested that different parasitic species may respond to different HIFs (Albrecht *et al.*, 1999; Keyes *et al.*, 2000). The question arises as to whether this is true for different races or populations of parasitic species. The *S. hermonthica* seed used in our study is likely to be a mixed population and only a small number of seeds may have recognised *T. dactyloides*-produced compounds. However, poor haustorial differentiation on *T. dactyloides* (even in the presence of syringic acid) and a failure of the parasites to develop, strongly indicates a fundamental incompatibility between host and parasite. This raises the possibility that *T. dactyloides* either lacks key specific primary/secondary metabolites/signals necessary for haustorial differentiation or that the presence of a *T. dactyloides*-specific metabolite(s) prevents haustorial development. Secondary haustoria (developed from a primary haustorium on maize) failed to develop normally when attached to the roots of *T. dactyloides*. This suggests that: (1) signals controlling haustorium development in *Z. mays* were not mobile within the roots of *S. hermonthica* connecting the primary and secondary haustoria; (2) HIFs were not released in *Z. mays* root exudate, and thus not perceived by secondary haustoria attaching to *T. dactyloides*; or (3) *T. dactyloides* produced metabolites that inhibited haustorial formation. After attachment of a secondary haustorium to *T. dactyloides* all subsequent attachments of secondary haustoria to the susceptible maize host failed to develop internal tissue structures. This key observation strongly supports the hypothesis that *T. dactyloides* produces a signal that prevents the development of haustoria, and can be transported to act even on a normally susceptible host. The inhibitory compound(s) may be mobile within the *S. hermonthica*-cereal root system or it may be released by host root exudate.

### Structure and function of the haustorium

The structure of the haustorium of *S. hermonthica* is well documented (Dörr, 1997; Neumann *et al.*, 1999), although, the role of specific cell types in the host–parasite association is less defined. Elegant studies have demonstrated the movement of solutes from host to parasite (Calladine & Pate, 2000; Haupt *et al.*, 2001), moreover, the haustorium may also play a significant role in nutrient accumulation and/or the metabolism of host-derived nutrients. The organelle-rich cells of the hyaline body (formed on *Z. mays*), indicates that this region may be involved in active nutrient synthesis and starch storage (see Visser *et al.*, 1984; Maiti *et al.*, 1984). Evidence for protein modification was revealed in the xylem-tapping hemiparasite *Olax phyllanthi* (Pate *et al.*, 1994; Pate, 2001). The haustoria of these parasites demonstrated high activity of nitrogen-assimilating enzymes and the authors postulated that haustoria could utilise and synthesise new amino acids. If the haustorium, or more specifically the hyaline body, is crucial for the regulation/modification of host-derived nutrients as these studies suggest, the poor differentiation of the hyaline body as observed on *T. dactyloides* is likely to have serious implications for parasite nutrition and the young parasites supported by *T. dactyloides* may effectively be nutrient starved. This may partly explain the arrest of parasite growth.

### How useful is *T. dactyloides* as a source of resistance for the control of *Striga?*

The impairment of haustorial development on *T. dactyloides* demonstrates resistance in a wild relative of maize. Furthermore, our results imply that *T. dactyloides* can influence subsequent

development even on a susceptible host. An allelopathic effect of a plant on a parasitic angiosperm has also been demonstrated by the 'push-pull' *Z. mays–Desmodium uncinatum* system that has successfully lowered *S. hermonthica* infestations on maize in field trials (Khan *et al.*, 2002). The authors demonstrated that this was not due to the suppression of *S. hermonthica* germination but suggested this was a result of radicle or haustorium inhibition. The inhibitory compound/signal was exuded by the legume roots. In light of this study it would be of great interest to determine whether a *T. dactyloides*-specific signal is also present in plant root exudate. However, a question arises as to whether *T. dactyloides*-based resistance would be useful if transferred to maize. Maize is considered to be the product of domestication from its wild progenitor, teosinte (*Z. mays* ssp. *mexicana*) (Matsuoka *et al.*, 2002). Preliminary studies demonstrated that different accessions of teosinte were susceptible to *S. hermonthica* infection and growth of infected plants was severely impaired (unpubl. data from this laboratory). However, it has been hypothesised that *Tripsacum* spp. also played a role in the evolution of maize (Eubanks, 2001). Early studies showed that hybridization of maize and *T. dactyloides* can occur and gene transfer is achieved (Mangelsdorf & Reeves, 1931; Bernard & Jewell, 1985). Studies have demonstrated that *T. dactyloides* can donate valuable traits to maize such as insect resistance (Moellenbeck *et al.*, 1995) and an apomictic mode of reproduction (Leblanc *et al.*, 1996; Grimanelli *et al.*, 1998). Furthermore, *T. dactyloides*-based resistance to corn rust (*Puccinia sorghi*) was transferred to maize (Berquist, 1981).

From our studies it was evident that the level of resistance to the parasite in the *Z. mays–T. dactyloides* hybrid was intermediate between the parental genotypes. The intermediate phenotype of the hybrid, together with the fact that segregation of resistance and susceptibility was not seen in the $BC_1$ and $BC_2$ hybrids suggests that the resistance trait is polygenic. However, as the parasites still developed to some extent on the hybrid they had a negative impact on host biomass. Previous studies have shown that only a small amount of parasite biomass is required to cause a large effect on host biomass accumulation (Gurney *et al.*, 1999). This, together with the fact that it is difficult to use *T. dactyloides* in conventional breeding programmes may limit the usefulness of this resistance.

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