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Occurrence of Graniferous Tracheary Elements in the Haustorium of *Cassytha filiformis* Linn., A Stem Parasite of Lauraceae

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ABSTRACT: A distinct interrupted zone is present in the haustorium of *Cassytha filiformis* a non-host specific stem parasite. Majority of the xylem elements in the vascular core are perforated. The graniferous tracheary cells were observed in the vascular core of haustorium as well as in the parasite stem for the first time. Histochemical studies confirmed the proteinaceous nature of granules. Tracheary cells containing granules were examined using scanning electron microscope. The structure in relation to function of the tracheary cells and their granules are discussed in the light of earlier work.

KEY WORDS: Graniferous tracheary elements, *Cassytha filiformis*, Haustorium, Parasite, Lauraceae.

INTRODUCTION

Graniferous tracheary elements are unique type of tracheids or vessel members containing cellular inclusions in their lumen. Tracheary elements possessing granules are characteristically associated with the haustorium of parasitic angiosperms, although current work from other laboratories indicate that these cells may also occur elsewhere in the parasite, and possibly even within the host vasculature.

These tracheary elements were first reported by Heinricher (1895) in *Lathraea* (Scrophulariaceae). Apparently similar cells were described by Benson (1910) in *Exocarpus cupressiformis* and *Thesium* of Santalaceae. Since then many other members of Santalaceae have been reported to possess graniferous inclusions in xylem elements of their haustoria (Fineran, 1963, 1974, 1985; Simpson and Fineran, 1970; Weber, 1977; Fineran *et al.*, 1978; Nietfeld *et al.*, 1983; Niranjana and Shivamurthy, 1987a, b). Outside this family such cells are also known to occur in some parasites belonging to Krameriaceae, Loranthaceae, Olacaceae, Opiliaceae and Scrophulariaceae (Fineran, 1985).

The function of graniferous tracheary elements in the haustorium, as an organ for absorbing nutrients from the host and transporting them into the parasite remains obscure, because some workers are of the opinion that they have a mechanical role rather than a metabolic role (Fineran, 1985). So far not much work has been done on the occurrence of graniferous tracheary elements in the haustorium of *Cassytha filiformis*. Therefore, the present study deals with the occurrence, nature and function of graniferous tracheary elements and some aspects of haustorial interface.

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MATERIALS AND METHODS

Haustoria at different stages of development of *Cassytha filiformis* Linn, were collected from Indralibetta near Udupi, Udupi district and Mangalore University campus, Konaje, Mangalore district, Karnataka State, India. They were immediately fixed in FAA (formalin, acetic acid and alcohol), and FPA (formalin, propionic acid and alcohol). Material for microtomy was dehydrated using a tertiary butanol series and embedded in paraffin. Sections 10-14 μm thick were cut using a rotary microtome. Northen's variations of tannic acid and ferric chloride combination and safranin and aniline blue were employed to stain the sections (Johansen, 1940). The haustoria were macerated using Jeffrey's maceration fluid and stained with safranin to study the nature of tracheary elements (Johansen, 1940). To determine the chemical nature of granules paraffin embedded sections were stained using periodic acid schiff's (PAS) reaction for insoluble polysaccharides (Jensen, 1962) and mercuric bromophenol blue (Mazia *et al.,* 1953) for proteins. For scanning electron microscope (SEM) study, haustoria of *Cassytha filiformis* were fixed in FAA and subjected to dehydration series and stored in 70% alcohol. Then the haustoria were rinsed in absolute alcohol, cut exactly in median plane and were placed on aluminum specimen stubs, air dried and later were vapour coated with gold. Then the haustoria scanned on a Hitachi-S 450 stereoscan at an accelerating voltage of 15 kV and photomicrographs were taken.

RESULTS

Cassytha filiformis Linn, is a non-host specific stem parasite of Lauraceae that can attack simultaneously many plants belonging to different taxa. It parasitizes dicotyledonous hosts like *Bridelia scandens* Gehrm, *Canthium parviflorum* Lam., *Canthium rheedii* DC., *Syzigium caryophyllaeum* Gaertn., and *Dioscorea oppositifolia,* Linn. (Figs. 1A, B).

The mature haustorium of *Cassytha filiformis* shows a simple anatomical organization. The host organ and the haustorium are held together by means of clasping folds (Fig. 1C). The mature haustorium consists of central region of tracheary elements surrounded by compactly arranged parenchymatous cells. Distinct interrupted zone is present. Endophyte is a small component of haustorium, comprising of a few tracheary and parenchymatous digitate cells which are in contact with the host stele.

Tracheary cells appear to be arranged in many seriate strands (2-8 vertical rows) (Figs. 1C, E). The actual entry of haustorium cells into the host tissues is restricted to 8-10 cells in width, but lengthwise it can be multicellular. Both xylem and parenchymatous multilobed digitate cells (Fig. 1D) intrude the host tissue establishing contact with the stele. There is a direct xylem to xylem contact between the two individuals.

Some of the tracheary elements in the haustorium showed granular inclusions. The inclusions are typically granular, but, often amorphous masses and dispersed fibrils derived from the granules may also occur (Fig. 2A). During transformation of parenchymatous cells into tracheary elements granular structures appear in the cytoplasm of these cells. These inclusions continue to persist even in mature tracheary elements. The size of granules in different tracheary cells varies from 0.5 μm to 2 μm in diameter and their number is much less in each cell. All granules of tracheary elements are of the same size. These are freely suspended in the lumen of tracheary cells and appear to align along the pit membranes. Tracheary elements containing granules and those without any inclusions may exist side by side. Some of the tracheary elements of the parasite stem adjoining the haustorium also possess granular inclusions (Figs. 2C, D).

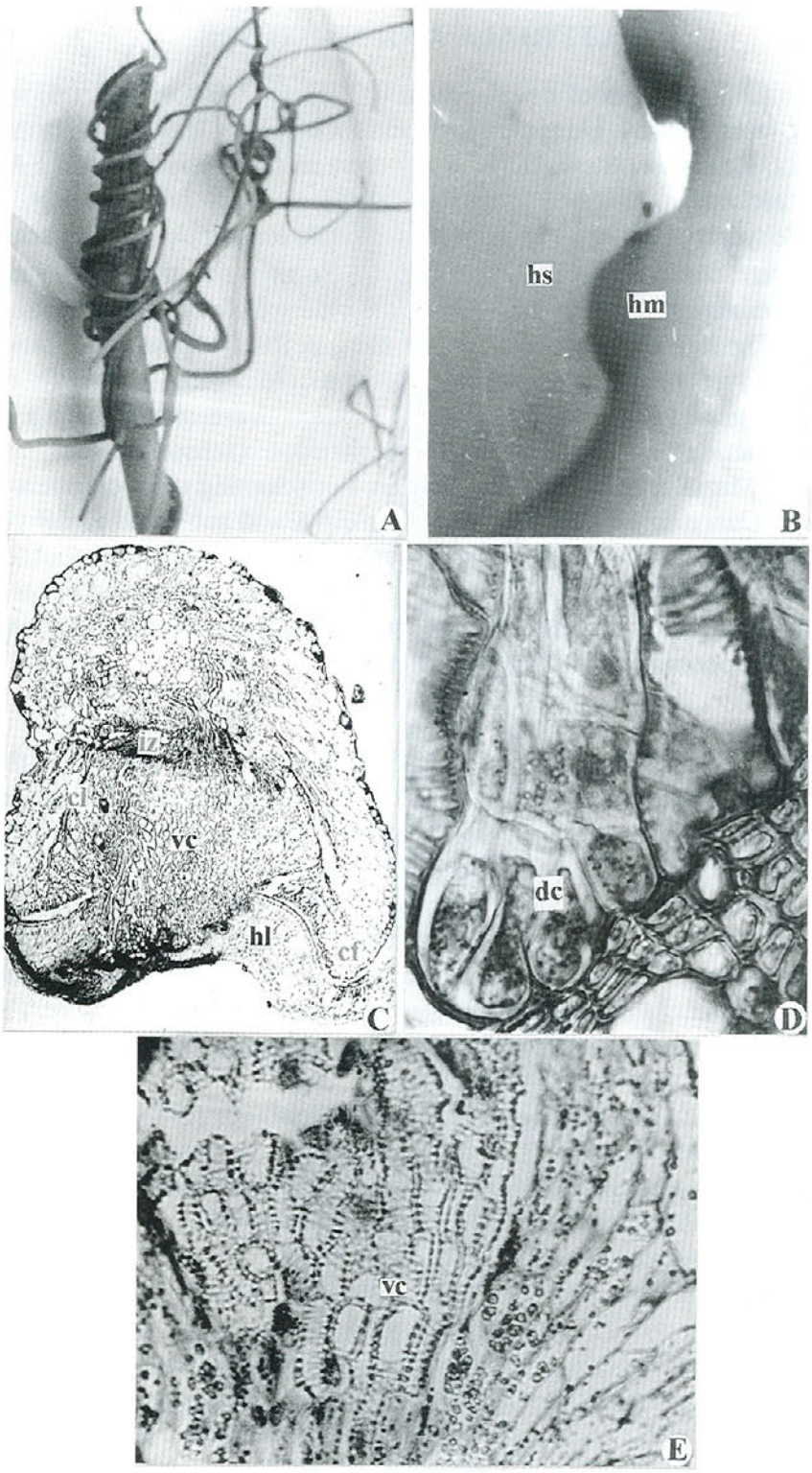

Fig. 1. A. *Cassytha filiformis* parasitizing stem of Dioscorea x 4. B. Magnified view of *C. filiformis* haustorium (hm) with host stem (hs) x 100. C. Vertical section of haustorium on the leaf of *Canthium rheedii* (host leaf, hl). Note the presence of vascular core (vc), interrupted zone (iz), collapsed layer (cl) and clasping folds (cf) x 965. D. Magnified view of the multilobed digitate cells (dc) in contact with host xylem x 1300. E. Portion of Fig.C enlarged to show the vascular core (vc) x 1200.

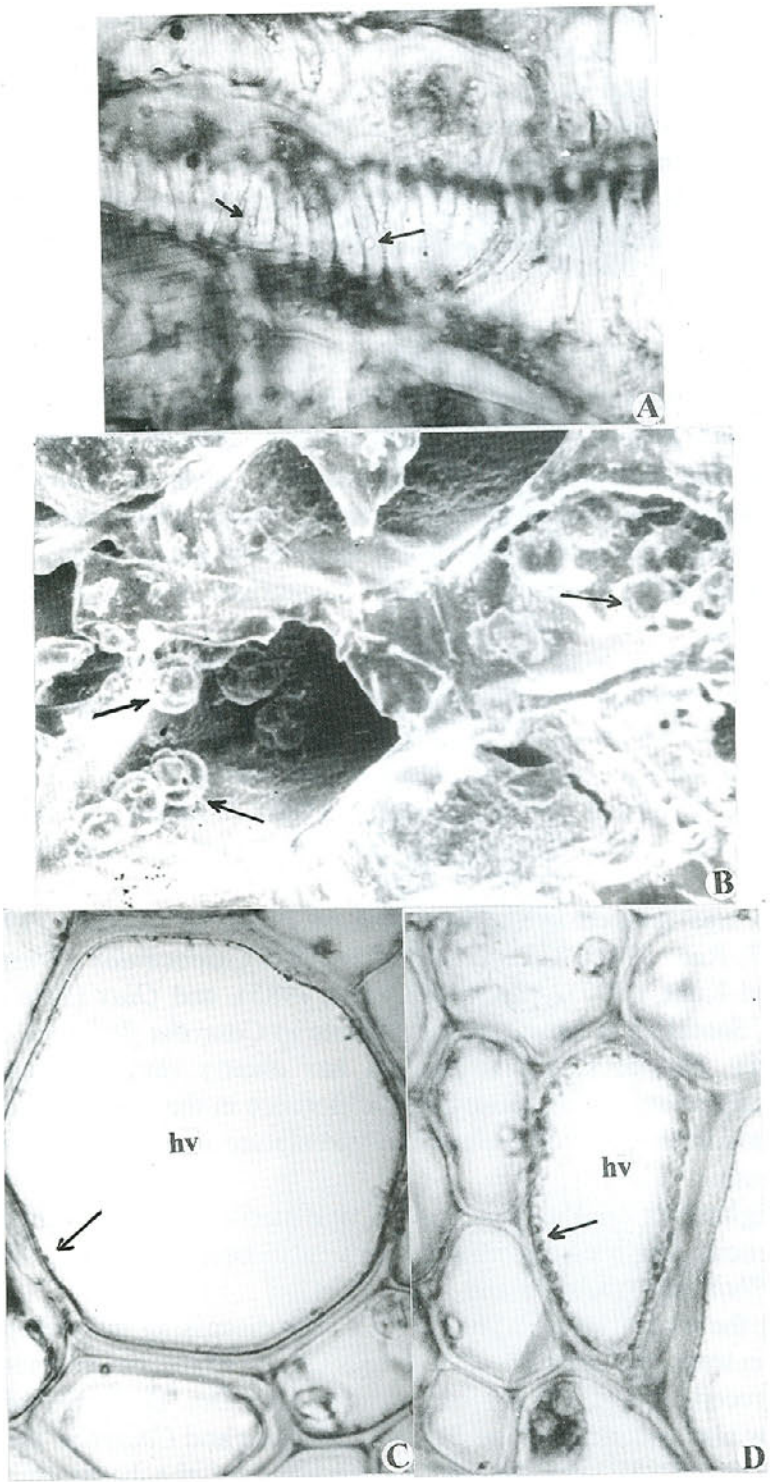

Fig. 2. A. Transverse section of vascular core of the haustorium showing granules (arrows heads) in tracheary cells (arrows heads) x 980. B. SEM (higher magnification) of a portion of the vascular core showing tracheary elements with granules in their lumen (arrow heads) x 1000. C and D. Graniferous tracheary elements of parent stem. Note the presence of granules (arrow heads) very near to the wall of host vessel element (hv) x 1200.

Histochemical studies reveal that granules stain green and blue when fast green and aniline blue were employed as counterstains respectively. Histochemically, granules respond positively to mercuric bromophenol blue and ninhydrin Schiff's reagent indicating their proteinaceous composition and respond negatively to PAS, I_2KI and phloroglucinol tests revealing the absence of insoluble polysaccharides, starch, and lignin, respectively.

SEM studies showed that the granules lie in clumps in the lumen of tracheary cells and they are spherical in shape. At lower magnification the granules exhibit a reasonably smooth surface, but at higher magnification they appear to be somewhat rugulose (Fig. 2B).

DISCUSSION

The mature haustorium of *Cassytha filiformis* is simple in its anatomical structure and organization. The host organ and the haustorium are held together by means of clasping folds or mantle—a consistent feature of Santalalean haustoria. Mantle has been reported to be absent in some of the members of Scrophulariaceae such as *Euphrasia, Lathraea, Melampyrum* and *Pedicularis* (Kuijt, 1977). Collapsed layers which appear in the cortical region of the haustorium in *Cassytha filiformis* (of the present study) extended from the distal portion of clasping folds to the lower portion of the haustorium. In *Cuscuta* (another odd stem parasite) they are absent.

Another interesting feature to be noted with reference to the formation of collapsed layer is its appearance coinciding with the differentiation of the gland as in most of the members of Santalales. However, the two processes *i.e.*, formation of collapsed layers and differentiation of gland are not associated with each other in the haustorium of *Viscum minimum*, where the gland is considered to be absent (Olsen and Kuijt, 1986). Whereas the gland formation is completely absent *in Cassytha filiformis*, but collapsed layers are seen in the developing haustorium.

Interrupted zone was noticed in the haustoria of *Buckleya* (Kusano, 1902), *Santalum album* (Barber, 1907; Rao, 1942), *Cansjera* (Barber, 1908), *Geocaulon* (Warrington, 1970), *Comandra* (Toth and Kuijt, 1977), *Nuytsia* (Fineran, 1983), and *Olax* (Pate *et al.,* 1990) a consistent feature of Santalacean haustoria, also visible in *Cassytha filiformis* only in vertical sections of haustoria. The present investigation has clearly revealed that the apparent interruption of vasculature appears because of the difference in the origin and development of the tracheary elements in the haustorium and the vasculature running from the parasite root towards the haustorium.

Endophyte is originated from the mitotically active meristematic cells of the haustorium. This type of endogenous origin of the endophyte has also been reported in *Santalum album* (Barber, 1906) and *Phthirusa* (Dobbins and Kuijt, 1974).

While reviewing the ultrastructure of haustoria and in discussing the presence of granules in the tracheary elements of *Lathraea,* Kuijt and Toth (1976) commented that "In the meantime we have received personal communications from Drs. D. R. Dobbins and I. Dorr that xylary inclusions also occur in the haustoria of *Krameria* and *Cassytha*, although sparsely in the latter". Fineran (1985) made a brief examination of the haustorium of *Cassytha ciliolata* Nees, by using transmission electron microscope, no granular material in the tracheary elements was observed, but in the present investigation the presence of granules in the tracheary cells has been noticed in the haustorium of *Cassytha filiformis*.

The existence of graniferous tracheary elements in the haustoria of Rhinanthoideae of Scrophulariaceae was first reported by Heinricher (1895) in *Lathraea.* This was followed by

similar observations in 25 species belonging to 12 genera of the Santalaceae (Fineran, 1985). Outside this family such cells have also been known to occur in some parasites belonging to Krameriaceae, Loranthaceae, Olacaceae and Scrophulariaceae (Fineran, 1985). The occurrence of granules in the tracheary elements of *Cassytha filiformis* is being reported here for the first time.

Graniferous tracheary elements are of two distinct types in terms of composition and origin; those with granules composed of protein and those with starch grains (Weber and Hildenbrand, 1978; Fineran, 1985). In the present study cytochemical tests revealed that granules are proteinaceous. Most of the Santalacean members studied so far also possess proteinaceous granules. However, granules in the haustoria of some parasitic members of the Scrophulariaceae are found to be polysaccharide in nature, while in Olacaceae both proteinaceous and starch granules occur (Fineran, 1985). However, in *Cansjera rheedii* Weber and Hildenbrand (1978) reported lignin rich granules.

Recent work based on light and scanning electron microscopy revealed the occurrence of graniferous tracheary elements elsewhere in the parasite, especially in the stems of some mistletoes (Weber, 1984, Weber and Neitfeld, 1984). Nevertheless, during the present investigation granule containing tracheary cells were also observed both in the xylem elements of the haustorium and in the parasite stem.

Knowledge regarding the exact function of graniferous tracheary elements in the haustorium of parasitic angiosperms is yet to be properly explained. Heinricher (1895, 1901) assumed that these cells in *Lathraea* were storage tracheids. Benson (1910) named them as phloeotracheids believing that they combined the function of xylem and phloem. Fineran and Bullock (1979) and Fineran (1985) noted that the presence of granules in the tracheary cells might create a mechanical drag on the flow of xylem sap passing between the host and parasite. Again, Fineran (1985) was of the opinion that, in contrast to the normal course of formation of more open xylem pathway for conduction, there is a reverse trend in xylem evolution in the haustorium with the secondary formation of short tracheids and appearance of granules in their lumens which hinder free conduction. Presence of a larger proportion of perforate xylem cells were observed in the haustorium of *Osyris arborea* (Niranjana and Shivamurthy, 1987a). However, our studies on *Cassytha filiformis* have revealed the presence of a larger proportion of perforate xylem cells in the haustorium. If the tracheary cells in the haustorium were to be perforated, granules lying free in the lumen would presumably be carried out of the haustorium along with the sap. Further, in *Cansjera rheedii* the granules have been reported to be attached to the wall (Weber and Hildenbrand, 1978). Many of these parasitic angiosperms are non-host specific and are considered to be poor taxonomists. They produce haustoria on any species they come in contact with and develop haustoria on many host plants of both monocotyledons and dicotyledons simultaneously. It is likely that osmotic potential of these host plants and the parasite vary greatly, these granules probably act as valves in order to prevent the back flow of nutrients from parasite to the host. In general it may be said that granule containing tracheary cells have a mechanical role. However, other functions cannot be ruled out.

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無根草(樟科植物莖寄生)吸器內粒狀導管之發生

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摘　　要

本文報導無根草(樟科)的吸器內一明顯的中斷區。無根草為一種對寄主不具專一性的莖寄生植物，其維管束中大部分的導管有穿孔。粒狀導管首次在吸器的維管束和寄生莖中被觀察。由組織化學的研究確認這些顆粒具有蛋白質的特性。在掃描電子顯微鏡下觀察到含有顆粒的導管。早期的研究者曾討論過導管和它的顆粒的構造與其功能的關係。

關鍵詞：粒狀導管，無根草，吸器，寄生植物，樟科。

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