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Haustorial morphology of *Cronartium conigenum* in naturally infected cones of three *Pinus* species from Guatemala[1]

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Summary

Haustorial morphology of the cone-rust pathogen, *C. conigenum*, was investigated among naturally infected female strobili of three Guatemalan pines, *P. maximinoi, P. pseudostrobus,* and *P. oocarpa.* Among the three pine species, haustorial shapes and sizes were more variable in *P. maximinoi* and *P. oocarpa* than in *P. pseudostrobus.* The haustorial shapes and sizes were more variable in parenchyma cells of the cortex, xylem, and pith than in the cells in the phloem, xylem rays, and tracheids. The haustoria were also present in larger numbers in the parenchyma cells of the cortex, xylem, and pith than in phloem parenchyma cells and tracheids. In living cells, the tips of some haustoria were appressed to the host nuclei.

1 Introduction

Cronartium conigenum (Pat.) Hedgc. & Hunt causes a serious disease in Mexico and Central America on the cones of many *Pinus* species, including *Pinus chihuahuana* Engelm., *P. oocarpa* Schiede, *P. montezumae* Lamb., *P. caribaea* Morelet, *P. pseudostrobus* Lindley, and several *Pinus* species in New Mexico and Arizona. *Pinus elliottii* Engelm. var. *elliottii* from the southeastern US is also susceptible to this pathogen (GIBSON 1979). Several species of the genera *Quercus, Castanea, Castanopsis,* and *Lithocarpus* serve as primary hosts for this pathogen (HEDGCOCK and SIGGERS 1949).

Studies of the haustorial morphology of some rust fungi at different stages of their life cycle have been reported (HARDER 1978; BORLAND and MIMS 1980). However, comparative morphology of pycnial and aecial haustoria among tissue types of different host species, especially the pine-cone rust diseases, has not been described. Considerable literature exists on the haustorial characteristics of other pine-stem rusts (HEDGCOCK and SIGGERS 1949; JACKSON and PARKER 1958; MATTHEWS 1960; JEWELL et al. 1962; WALKINSHAW 1978). Despite considerable economic importance, there is very little information on *C. conigenum,* largely confined to general reports on the severity of cone infection in certain geographical areas (HEDGCOCK and HUNT 1922; SCHIEBER 1967; ANONYMOUS 1977). In view of the ability of *C. conigenum* to infect female strobili of many pine species from Central America to southern Arizona and New Mexico (HEDGCOCK and HUNT 1922; SCHIEBER 1967; QUINARD and MARTINEZ 1987; RAYACHHETRY et al. 1995), its pathogenic and environmental plasticity is evident.

The purpose of this study was to compare the haustorial morphology and host-parasite relationship of *C. conigenum* in naturally infected cones of three economically important pine species from Guatemala.

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2 Materials and methods

A cone with signs and symptoms characteristic of those arising from *C. conigenum* infection was collected in Guatemala from each of three trees of *P. pseudostrobus*, *P. oocarpa* (from the central highlands), and *P. maximinoi* H. E. Moore (from the highland region near Guatemala City). Cone materials were fixed, segments prepared, (from the umbo, scale base, and axis), dehydrated, infiltrated, embedded, sectioned, and stained using a previously described method (RAYACHHETRY 1987, 1995). Measurements of haustorial structures and photomicrographs were taken using a light microscope (Nikon UFX-II Optiphot, Nippon Kogaku, NY, USA).

A total of 25 haustoria in the cells of the cortex, phloem, xylem (parenchyma and tracheids), and pith in scales and axes, representing sections from three cones (one from each of three trees) of each species, were measured to assess haustorial variation among cell types. The diameter of the haustoria were measured at the broadest point. To determine the percentage of simple, lobed, and branched haustoria, more than 100 randomly selected haustoria from the cells of each cone-tissue type of all three pine species were measured. When notches were shorter than 50% of the total haustorial length, the haustoria were considered lobed, and, when the notches exceeded 50% of the haustorial length, they were considered to be branched.

3 Results and discussion

Variation in haustorial shapes was observed for *C. conigenum* in cones of *P. maximinoi, P. pseudostrobus*, and *P. oocarpa*. In the various cone tissues of *P. maximinoi*, 24 haustorial types were identified (Fig. 1). Almost all of these types were also present in the corresponding tissues of *P. oocarpa*, but only types A, F, G, K, L, P, S, T, and W were observed in *P. pseudostrobus*. The percentages of branched haustoria were 34, 15, and 7% for *P. maximinoi, P. oocarpa*, and *P. pseudostrobus*, respectively (Table 1). MATTHEWS (1960) also reported both branched and simple haustoria for the southern cone-rust fungus *C. strobilinum* (Arthur) Hedgc. & Hahn in cones of *P. elliottii* var. *elliottii*. Similar variation of haustorial attributes among other *Cronartium* species has been reported for different host species and ages of both the infected host tissue and pathogen (JACKSON and PARKER 1958; JEWELL et al. 1962). JEWELL et al. (1962) described various haustorial shapes with occasional branching for *C. quercuum* (Berk.) Miyabe ex Shirai f.sp. *fusiforme* in *P. taeda* L. Variation in haustorial shapes also has been shown for *Peridermium pini* (Pers.) Lev. in parenchymatous cells of the axenic callus of *P. sylvestris* L. and *P. nigra* var. *maritima* (Aiton) Melville (PEI 1989).

A large proportion (55–79%) of haustoria in the cells of phloem tissues, tracheids, and ray parenchyma of the cone scale and axis were consistently simple in the cones of the three pine species investigated (Fig. 1; Table 1). JACKSON and PARKER (1958) described allantoid, monokaryotic, and unbranched haustoria of *C. quercuum* f.sp. *fusiforme* in *P. taeda*. WOO

Table 1. **Percentages of simple, lobed, and branched haustoria of *Cronartium conigenum* in infected cone tissues of three Guatemalan pine species**

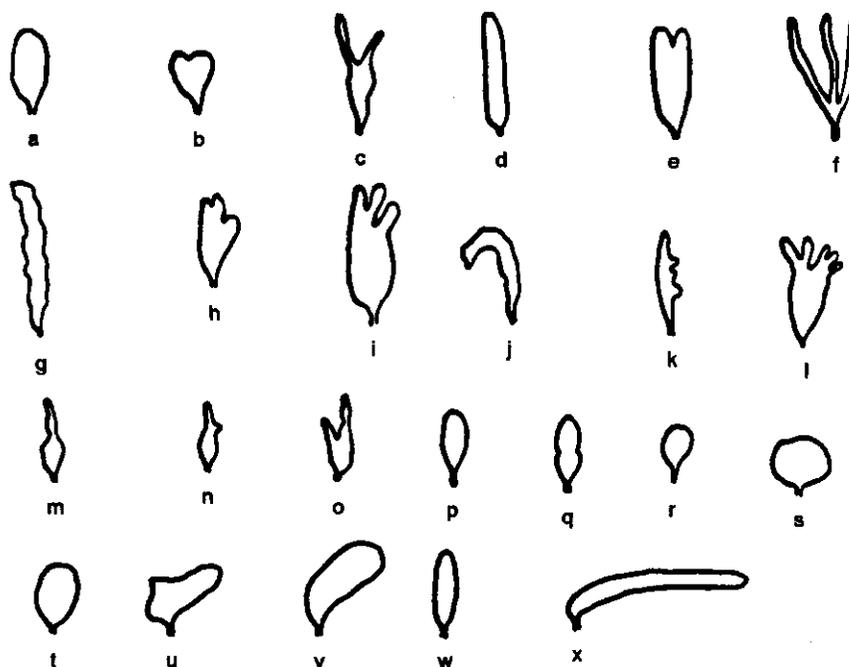| Host	Haustoria (%)			
	Simple	Lobed	Branched	Sample size
P. maximinoi	55	11	34	172
P. oocarpa	73	12	15	182
P. pseudostrobus	79	14	7	175

Fig. 1. Diagrammatic sketches of the haustorial types of *C. conigenum* observed in cells of different tissues of *P. maximinoi.* Note haustorial types in cortical parenchyma cells of scale and axis (a–o), sieve and phloem parenchyma cells (p–r), tracheids (s–v), phloem and xylem ray parenchyma cells (w, x), parenchyma cells of metaxylem (a–d, p, w, x), and parenchyma cells of protoxylem and pith (a–p, w, x). Also, note simple (a, b, d, g, j, m, p, x), lobed (e, h, k, n, i, l), and branched (c, f, o) haustorial types

and MARTIN (1981) found haploid, hypha-like haustoria of *C. ribicola* J. C. Fisch. ex. Rabenh. in bark cells of *P. monticola* Dougl. In this study, simple haustoria were the predominant type (Table 1) but were not restricted to any particular cell or tissue types. However, the more complex types (lobed and branched) of haustoria occurred only in the cortical-, xylem-, and pith-parenchyma cells.

The majority of *C. conigenum* haustoria in the cells of storage tissues, i.e. cortical and xylem parenchyma, were branched and larger in dimension than those observed in the cells in phloem tissue which transports water-soluble photosynthetic products. It is assumed that the haustorial dimensions and branching intensity is related either to the status of water-soluble nutrients or to the distribution pattern of cytoplasmic strands and vacuoles in the host cells.

The haustorial dimensions of *C. conigenum* in different cell types of *P. maximinoi* is presented in Table 2. In general, the haustorial dimensions of this fungus in corresponding cell types of *P. pseudostrobus* and *P. oocarpa* were comparable to *P. maximinoi.* Haustoria were longest in the cortical parenchyma cells, and shortest in the cells in the phloem and tracheids (Table 2).

A diameter range of 3.01–7.0 μm (average 5.4 μm) and a length of 13.0–17.0 μm (longest 35 μm) has been reported for typical haustoria of *C. strobilinum* (Arthur) Hedgc. & Hahn in *P. elliottii* var. *elliottii* cones (MATTHEWS 1960). JACKSON and PARKER (1958) reported haustorial lengths of 20–30 μm for *C. quercuum* f. sp. *fusiforme* in 2–3-year-old galls of *P. taeda.* JEWELL et al. (1962) reported the diameter and length ranges of haustoria to be 3.1–5.1 μm (average 3.5 μm) and 5.1–27.2 μm (average 13.1 μm), respectively, for the same

Table 2. Dimensions (length × diameter (μm) of haustoria of *Cronartium conigenum* in cells of infected cone tissues of *Cronartium maximinoi* from Guatemala. Average = average of 25 haustoria; – = no pith in umbo and scale base

Cell types	Parts of cones		
	Umbo	Scale base	Axis
Cortical parenchyma			
Minimum	8.9 × 3.4	3.9 × 2.3	5.8 × 2.9
Average	19.1 × 5.0	16.2 × 4.8	17.8 × 4.8
Maximum	31.9 × 6.0	22.4 × 7.0	22.1 × 6.7
Phloem parenchyma			
Minimum	3.9 × 2.8	5.2 × 2.4	5.3 × 3.4
Average	6.2 × 3.7	6.5 × 3.3	8.7 × 4.7
Maximum	8.4 × 5.1	9.5 × 5.6	11.5 × 5.8
Xylem parenchyma			
Minimum	3.9 × 2.8	4.5 × 2.8	7.7 × 2.9
Average	15.7 × 4.8	16.1 × 4.2	12.2 × 4.5
Maximum	21.3 × 6.2	24.1 × 5.8	26.8 × 6.7
Tracheids			
Minimum	2.8 × 2.8	2.8 × 2.3	4.8 × 2.9
Average	5.1 × 3.6	6.0 × 3.6	8.1 × 4.7
Maximum	11.2 × 5.6	16.8 × 5.0	14.4 × 6.7
Pith parenchyma			
Minimum	–	–	7.7 × 4.8
Average	–	–	18.3 × 5.8
Maximum	–	–	27.8 × 7.0

fungus in 9–12-month galls on *P. elliottii* var. *elliottii* seedlings. In general, the *C. conigenum* haustoria (Table 2) fell within the range of the dimensions of haustoria reported for other *Cronartium* species (MATTHEWS 1960; JACKSON and PARKER 1958; JEWELL et al. 1962).

The distribution of haustoria varied among different cone tissues and cellular contents, both within and among the three host species. In general, haustoria were present in large numbers in lightly stained cells of the parenchymatous tissues, though hyphal accumulation was greater in the similar tissues containing starch and darkly stained substances (Fig. 2a, b). Similar accumulations of fungal hyphae have also been reported in the cortical parenchyma cells of the stem galls caused by *C. quercuum* f. sp. *fusiforme* on slash pine (WALKINSHAW and ROLAND 1990).

Haustoria were present in the majority of parenchyma cells in the cortex, phloem, xylem, and pith of the infected cones of *P. maximinoi*, with the greatest density in the vascular-ray cells. Compared to *P. maximinoi*, fewer haustoria were present among corresponding cell types in *P. oocarpa* and *P. pseudostrobus*. The presence of fewer and smaller haustoria in the phloem cells of cone scales and axes in *P. maximinoi*, *P. oocarpa*, and *P. pseudostrobus* is consistent with MATTHEWS' (1960) descriptions of *C. strobilinum* in *P. elliottii* var. *elliottii* cones. By contrast, the hyphae in the phloem tissue were consistently larger than in the cortex of all three pine species. This observation reveals that *C. conigenum* may produce larger and more-abundant hyphae (Fig. 2c) rather than haustoria as a means of absorbing photosynthetic products in the phloem tissues. A similar opinion has been expressed for the autoecious rusts *Puccinia punctiformis* (Str.) Rohl and *P. lagenophorae* Cooke (BAKA and LOSEL 1992) that colonize vascular tissues of angiospermic hosts.

Some apparently necrotic haustoria, devoid of contents, were embedded in ergastic substances in the cone-tissue cells of all three pine species. This phenomenon was very

Fig. 2. Photomicrographs showing mycelia and haustoria of *C. conigenum* in different tissues of female strobili from three pine species. a. Cross sectional view of the cortical parenchymatous cells from cone scale of *P. maximinoi*. Note intercellular mycelial mat (arrow heads) and haustoria (arrows); b. A haustorium (arrow) in the lightly stained cortical parenchyma cell in cone scale of *P. maximinoi* closely appressed to a host nucleus (arrow head); c. Longitudinal section through the phloem of *P. maximinoi* cone scale, note hyphal ramification between cells (arrow heads) and haustoria (arrows); d. Note apparently empty haustoria (arrows), one of them partially embedded in darkly stained substance of cortical parenchyma cell in the cone scale of *P. oocarpa*; e. Cross sectional view through inner cortex of the cone axis of *P. pseudostrobus*. Note haustoria (arrows) whose tips are in close proximity to the host nuclei (arrow heads); f. Haustoria (arrows) with its arms around a host nucleus (arrow head) in xylem parenchyma cell of *P. maximinoi*; g. Cross sectional view of the tracheids in the cone scales of *P. maximinoi*, note haustoria (arrows) encased in the secondary-wall components (arrow heads) of tracheidal cells

conspicuous in *P. oocarpa* cones (Fig. 2d), where the abundant accumulation of a darkly stained substance was prevalent in all tissue types beyond approximately 5 mm depth from the cone surface. Deposition of tanninoid substances have also been found in cultured *P. monticola* Dougl. cells challenged by *C. ribicola* J. C. Fischer (ROBB et al. 1975). WALKINSHAW (1978) described similar depositions in cells of seedlings of *P. taeda, P. palustris* Mill., and *P. elliottii* var. *elliottii*, infected by *C. quercuum* f.sp. *fusiforme* as a normal host reaction to the presence of the pathogen.

In the light-microscopic investigations in this study, some haustorial tips of *C. conigenum* were observed to be oriented towards host nuclei, regardless of their origin in the cells (Fig. 2b, e, f). An association between host nuclei and fungal haustoria, and even indentation of nuclei, has been reported (RICE 1927; MATTHEWS 1960; JEWELL et al. 1962; ROBB 1975). SULLIVAN et al. (1974) found that such association does not lend any survival qualities to the fungal haustoria. MATTHEWS (1960) hypothesized that this relationship is a delicate host-parasite relationship involving no apparent damage to the host nucleus.

In several instances, *C. conigenum* haustoria were observed closely appressed to the host nuclei (Fig. 2b, e, f), and the host nuclei appeared almost semilunar. Several cases were also observed where haustorial branches encircled the host nucleus (Fig. 2f). Early investigators described such proximal association between fungal haustoria and host nuclei as a possible defence mechanism of a host that facilitates callose deposition on haustoria, or as some type of positive tropism (RICE 1927). However, ROBB et al. (1975) did not observe haustoria actually penetrating the host nuclei. In general, the nuclei in the living cells are highly mobile. The nuclei may migrate with the cytoplasmic stream to the proximity of the tips of growing haustoria to facilitate some kind of chemical defence against intruding foreign bodies in the cell. This possibility is further supported by the condition illustrated in Fig. 2b, where the haustorial envelope touching a host nucleus is densely stained.

Haustoria were often abundant in tracheids adjacent to the xylem parenchyma cells. Where the tracheidal cells were penetrated, those haustoria were encased in lignified materials from the cell wall (Fig. 2g). Similar encasement of haustoria within the lignified wall of vessels has also been reported for other rust fungi on angiospermic hosts (BAKA and LOSEL 1992).

Acknowledgements

Résumé

Morphologie des haustoria de Cronartium conigenum chez les cônes naturellement infectés de trois espèces de pin au Guatemala

Le travail a porté sur *Pinus maximinoi, P. pseudostrobus* et *P. oocarpa*. La forme et les dimensions des haustoria étaient plus variables chez *P. maximinoi* et *P. oocarpa* que chez *P. pseudostrobus*. A l'intérieur de chaque espèce de pin, les formes et dimensions des haustoria étaient plus variables dans les cellules du parenchyme du cortex, du xylème et de la moelle que dans les cellules du phloème, des rayons ligueux et des trachéides. Les haustoria étaient aussi plus nombreux dans les cellules du parenchyme du cortex, du xylème et de la moelle que dans celles du phloème et des trachéides. Dans les cellules vivantes, l'extrémité de certains haustoria était apprimée contre le noyau de l'hôte.

Zusammenfassung

Haustorienmorphologie von Cronartium conigenum in natürlich infizierten Zapfen von drei Pinusarten in Guatemala

Die Morphologie der Haustorien des Zapfenrostes *C. conigenum* wurde in natürlich infizierten weiblichen Zapfen von drei Kiefernarten aus Guatemala (*Pinus maximinoi, P. pseudostrobus, P. oocarpa*)

untersucht. Die Form und Grösse der Haustorien waren in *P. maximinoi* und *P. oocarpa* variabler als in *P. pseudostrobus*. Innerhalb der Kiefernarten variierten Form und Größe der Haustorien in den Parenchymzellen von Cortex, Xylem und Mark stärker als in Phloem, Xylem, Markstrahlen und Tracheiden. Auch waren mehr Haustorien im Cortex, Xylem und Markparenchym vorhanden als im Phloemparenchym und in den Tracheiden. In lebenden Zellen legten sich die Spitzen einiger Haustorien an den Zellkern an.

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