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Relationship of *Schistonchus caprifici* (Aphelenchoididae) with fig inflorescences, the fig pollinator *Blastophaga psenes*, and its cleptoparasite *Philotrypesis caricae*

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Summary – The association of the nematode *Schistonchus caprifici,* the pollinator wasp *Blastophaga psenes,* and its cleptoparasite *Philotrypesis caricae* was studied in winter, spring and summer inflorescences (syconia) of caprifig trees *(Ficus carica* var. *sylvestris)* in southern Italy. This is the first report of a *Schistonchus* sp. associated with a cleptoparasite wasp. All the life stages of *S. caprifici* were found in the hemocoel of the winged females of *B. psenes* and of *P. caricae.* No association was observed with the wingless males of both wasps. For both these two fig wasps, the number of winged females transporting the nematode was greater in June than in March. However, the percentage of *P. caricae* wasps carrying *S. caprifici* was 50 % (March) and 20 % (June) smaller than that of *B. psenes.* The range of nematode number carried by each winged female was 1-3 and 1-23 for *P. caricae* and 1-4 and 1-116 for *B. psenes* in March and June, respectively. Morphometrics of entomogenous (originating from both wasps) and phytophagous females of *S. caprifici* did not differ. Histological examination of staminate caprifig florets infected by phytophagous specimens of *S. caprifici* revealed nematodes colonies inside and outside the clusters of anthers and anther filaments. Nematode feeding caused necrosis of epidermis and cortical parenchyma of anther filaments and the formation in the anthers of hypertrophied epidermal cells with darkly stained cytoplasm.

Résumé – ***Relations entre* Schistonchus caprifici *(Aphelenchoididae), les inflorescences du figuier, le pollinisateur* Blastophaga psenes *et le parasitoïde* Philotrypesis caricae** – L'association entre le nématode *Schistonchus caprifici,* la guêpe pollinisatrice *Blastophaga psenes* et le parasitoïde *Philotrypesis caricae* a été étudiée en hiver, au printemps et en automne dans les inflorescences (sycones) du figuier *(Ficus carica* var. *sylvestris)* dans le sud de l'Italie. C'est la première fois qu'est signalée l'association d'un *Schistonchus* sp. avec une guêpe parasitoïde. Tous les stades biologiques de *S. caprifici* ont été trouvés dans l'hémocoele des femelles ailées de *B. psenes* et de *P. caricae.* Aucune association n'a été observée avec les mâles aptères des deux guêpes. Pour ces deux guêpes de la figue, le nombre de femelles ailées transportant des nématodes est plus élevé en juin qu'en mars. Cependant, le pourcentage de *P. caricae* transportant *S. caprifici* est de 50 % (mars) et 20 % (juin) moins élevé que pour *B. psenes.* Le nombre de nématodes transportés par chaque femelle ailée est de 1-3 et 1-23 pour *P. caricae* et 1-14 et 1-116 pour *B. psenes* en mars et juin, respectivement. Les mensurations de femelles associées aux insectes (provenant des deux guêpes) et, de celles phytoparasites, de *S. caprifici* ne diffèrent pas. Les examens histopathologiques révèlent la présence des colonies de nématodes à l'intérieur et à l'extérieur des bouquets d'anthères et des filets. Le nématode provoque des nécroses de l'épiderme et du parenchyme cortical des filets et la formation dans les anthères de cellules épidermiques hypertrophiées, riches en cytoplasme à coloration foncée.

Key-words : Agaonidae, Aphelenchoididae, *Blastophaga psenes,* biology, Chalcididae, cleptoparasitism, nematode, *Ficus carica,* host-parasite relationships, *Philotrypesis caricae,* pollinator fig wasp, *Schistonchus caprifici.*

Phytophagous populations of the aphelenchoidid nematode *Schistonchus caprifici* (Gasperrini, 1864) Cobb, 1927 parasitize florets of caprifig trees (*Ficus carica* var. *sylvestris* L.) causing necrosis of floret cortical parenchyma (Vovlas *et al.* 1992). This nematode (Figs 1, 2) is carried in the hemocoel of female wasps, *Blastophaga psenes* L. (Hymenoptera: Agaonidae), which pollinate and parasitize pistillate (female) florets of a caprifig inflorescence (syconium). Other *Schistonchus* spp. with life cycles similar to that of *S. caprifici* have been reported by Giblin-Davis *et al.* (1995) from other fig trees such as *F. laevigata* Vahl. and *F. aurea* Nuttal and are also carried in the hemocoel of the pollinator wasps *Pegoscapus* sp. and *P. mexicanus* (Ashmead), respectively.

In southern Italy, another wasp, *Philotrypesis caricae* Hass (Hymenoptera: Chalcididae) also parasitizes pistillate florets of caprifig syconia (Grandi, 1985). *Philotrypesis caricae* female wasps, which have cleptoparasitic habits, oviposit at the outside of the syconium of florets which have been already parasitized and transformed into a gall with a *B. psenes* larva inside. The voracious larva of *P. caricae* emerges from the egg and develops rapidly by feeding on the hyperplastic tissues inside the floret gall. The *B. psenes* larva is not able to compete for food with the cohabiting cleptoparasitic larva of *P. caricae* and dies (Grandi, 1985). The larva of *P. caricae* completes its development, pupates, and emerges from the floret gall as a wingless male or a fertilized winged

female wasp. Female wasps leave the syconium in search of new generation syconia inhabited by *B. psenes,* oviposit at the outside of the syconium and start another cycle which plays no role in the pollination of *Ficus carica* (Grandi, 1985) (Fig. 1).

Nothing is known about the association between *P. caricae* and the nematode *S. caprifici.* The purpose of this study was to elucidate the association between *S. caprifici* and *P. caricae,* examine the anatomical alterations induced by *S. caprifici* parasitism in florets of a caprifig syconium, and expand the observations, previously reported by Vovlas *et al.* (1992), concerning the relationships between the fig wasp *B. psenes,* the fig nematode *S. caprifici,* and their common host: caprifig flowers.

Materials and methods

Association between *P. caricae, B. psenes,* and *S. caprifici*

To determine the ability of *S. caprifici* to utilize *P. caricae* as a host wasp, specimens of winter, spring, and summer syconia (see Grandi, 1985) inhabited with *B. psenes, P. caricae,* and *S. caprifici* were collected early February, late March, and late June 1994. Syconia were opened in distilled water in Petri dishes under a stereomicroscope and emerging insects and nematodes were collected. No attempt was made to detect nematode presence in the hemocoel of *P. caricae* and *B. psenes* preadult stages. Live male and female wasps of *P. caricae* and *B. psenes* (n=100 per each species and sex) were washed in distilled water and dissected under a stereomicroscope for observations. Nematode load is defined as number of nematodes per wasp. At each sampling date, receptacles and florets of syconia were incubated in water to extract nematodes from fig tissues. Nematodes from caprifig tissues and wasp bodies were fixed in 4 % formaldehyde + 1 % propionic acid and processed in glycerin (Seinhorst, 1966) to determine morphometric differences among the entomogenous and phytophagous specimens.

Histopathology

To study the anatomical alterations induced by *S. caprifici* in floret tissues of syconia, group of florets were removed from nematode infected caprifig syconia and fixed in formaldehyde-acetic acid-alcohol (8:7:85) solution. Then, they were dehydrated in a tertiary butyl alcohol series and embedded in 55-58 °C melting point Parawax. Embedded material was sectioned 10-12 µm thick, stained with safranin and fast green, mounted in xylen resin, and observed under a compound microscope (Johansen, 1940).

Results and discussion

Association between *P. caricae, B. psenes,* and *S. caprifici*

Blastophaga psenes winged females enter the syconium cavity (Fig. 1 A) and pollinate pistillate florets, whereas *P. caricae* wasps do not enter the syconium cavity and do not pollinate florets. The life cycle of *B. psenes* inside the syconium cavity starts with a wasp which lays an egg and releases phytophagous specimens of *S. caprifici* inside a pistillate floret (Fig. 1 B). Phytophagous nematodes invade, feed, and reproduce inside the floret parenchyma tissues (Fig. 1 H) which are hyperplastic as a consequence of the secretions released by *B. psenes* during oviposition. Nematodes can also migrate inside and outside staminate florets (Fig. 1 G). *B. psenes* eggs hatch and the newborn larvae feed on the hyperplastic floret tissues (Fig. 1 C). Entomogenous nematodes appear and they penetrate and reproduce inside the hemocoel of the *B. psenes* larvae (Fig. 1 C). The larvae complete their development with nematodes inside the hemocoel (Fig. 1 E). Wingless males (Fig. 1 B.p./a), which are not associated with nematodes, appear and fertilize winged females inside the floret galls. Mated winged females (Fig. 1 B.p./b) emerge from galls and exit the syconium carrying nematodes in their hemocoel and pollen grains removed from staminate florets which adhere to their body surface. These *B. psenes* females will start a new cycle in a new generation syconium. The life cycle of *Philotrypesis caricae* starts with an ovipositing female (Fig. 1 P.c./b) having cleptoparasitic habits. *P. caricae* does not enter the syconium cavity (Fig. 1 A), but inserts its long ovipositor in to the syconium from outside. This wasp lays an egg and probably releases nematodes inside the pistillate florets which are already parasitized by a *B. psenes* larva and *S. caprifici* nematodes (Fig. 1 D). The egg hatches and the larva of *P. caricae* outcompetes the *B. psenes* larva for food, causing it to die (Fig. 1 F). The larva of *P. caricae* completes its development. Wingless males (Fig. 1 P.c./a), which are not associated with nematodes, are produced and fertilize female wasps inside the floret galls. Mated winged females (Fig. 1 P.c./b), which have acquired entomogenous *S. caprifici* nematodes in the hemocoel, exit the syconium as do *B. psenes* females in search of other syconia infected by *B. psenes* to start another cleptoparasitic cycle.

In winter (February) and at the beginning of spring (March), a low number of *S. caprifici* females were found in the hemocoel and ovipositor of *P. caricae* and *B. psenes* from winter syconia (Fig. 2). No preadult nematode stages were found at this sampling date. These findings suggest that *S. caprifici* is able to use the cleptoparasite *P. caricae* as a transport host as it does with *B. psenes.* Association of the nematode with the ovipositor of both wasps may favour nematode transmission during oviposition. In June, nematode reproduction was

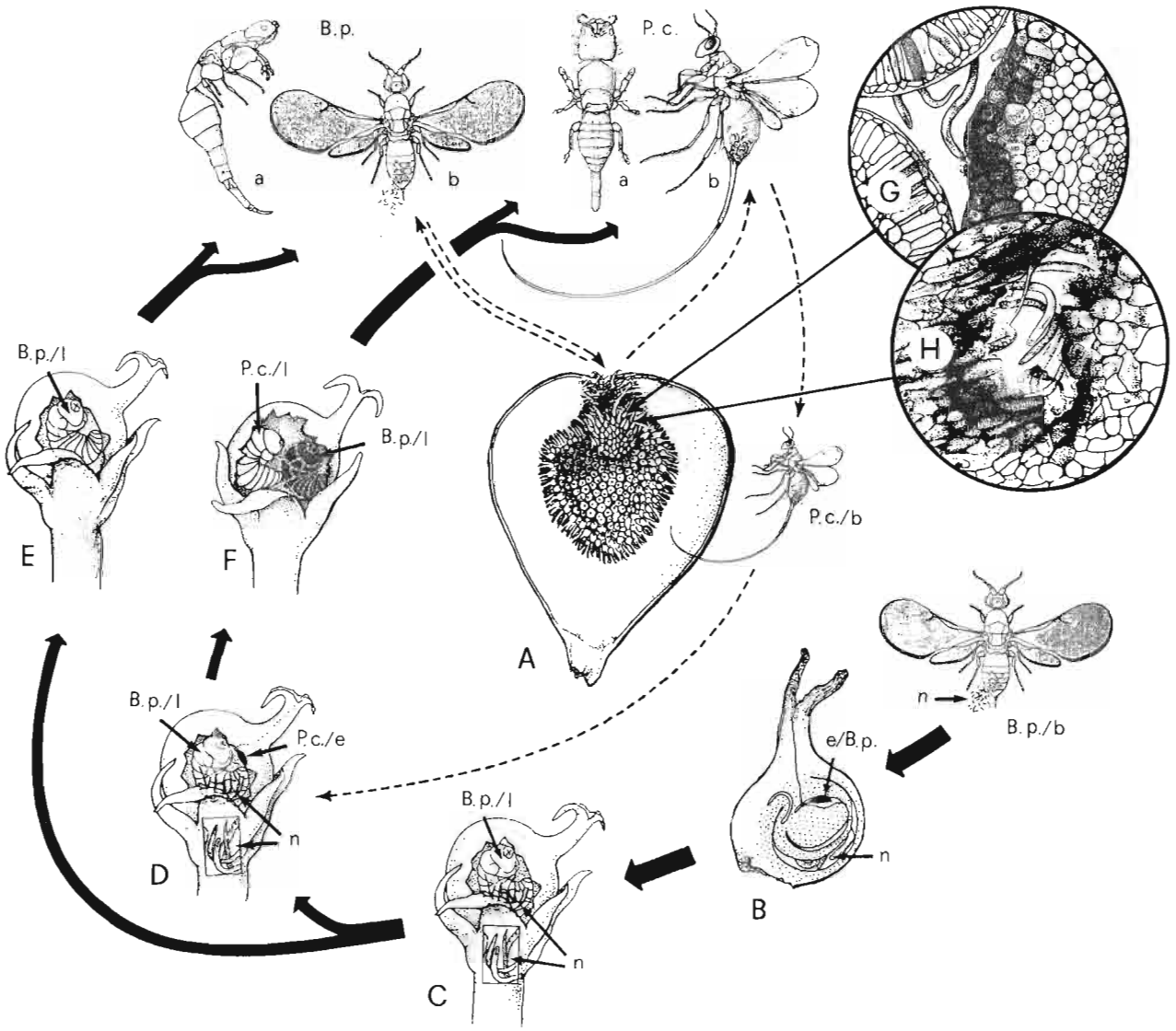

Fig. 1. *Anatomical parts of* Ficus carica *var.* sylvestris *with associated life cycles of the pollinator wasp* Blastophaga psenes (B.p.), *its cleptoparasite wasp* Philotrypesis caricae (P.c.), *and the associated phytoparasitic and/or entomogenous nematode species* Schistonchus caprifici. *A : Syconium; B : Egg and nematodes released by the vector* B. psenes; *C :* B. psenes *newborn larva feeding on the hyperplastic florets tissues; D :* P. caricae *egg and nematodes inside the pistillate florets which are already parasitized by a* B. psenes *larva; E :* B. psenes *larva developing with nematodes inside the hemocoel; F :* P. caricae *larva suppressing* B. psenes *larva; G : Phytophagous* Schistonchus caprifici *migrating inside and outside staminate florets; H : Nematodes invading and feeding on the floret parenchyma tissues.*
(Abbreviations and symbols used : B.p. = Blastophaga psenes; *P.c.* = Philotrypesis caricae; *n = nematode; l = larva; P.c./l* = Philotrypesis caricae *larva; B.p./l* = Blastophaga psenes *larva; e = egg.).*

observed in the hemocoel of both wasps. All the entomogenous life stages of the nematode, eggs, juveniles, females, and males, were found at this sampling date in both wasps from spring and summer syconia. The nematode burden per female in both species was greater in June than in March and ranged from 1-23 and 1-3 in *P. caricae* and from 1-116 and 1-4 in *B. psenes* in June and March, respectively. No nematodes were found in the hemocoel of the wingless males of *P. caricae* of *P. caricae* or *B. psenes*. The percentage of female wasps carrying nematodes inside the hemocoel increased from 12 and 25 % in March, to 15 and 80 % in June for

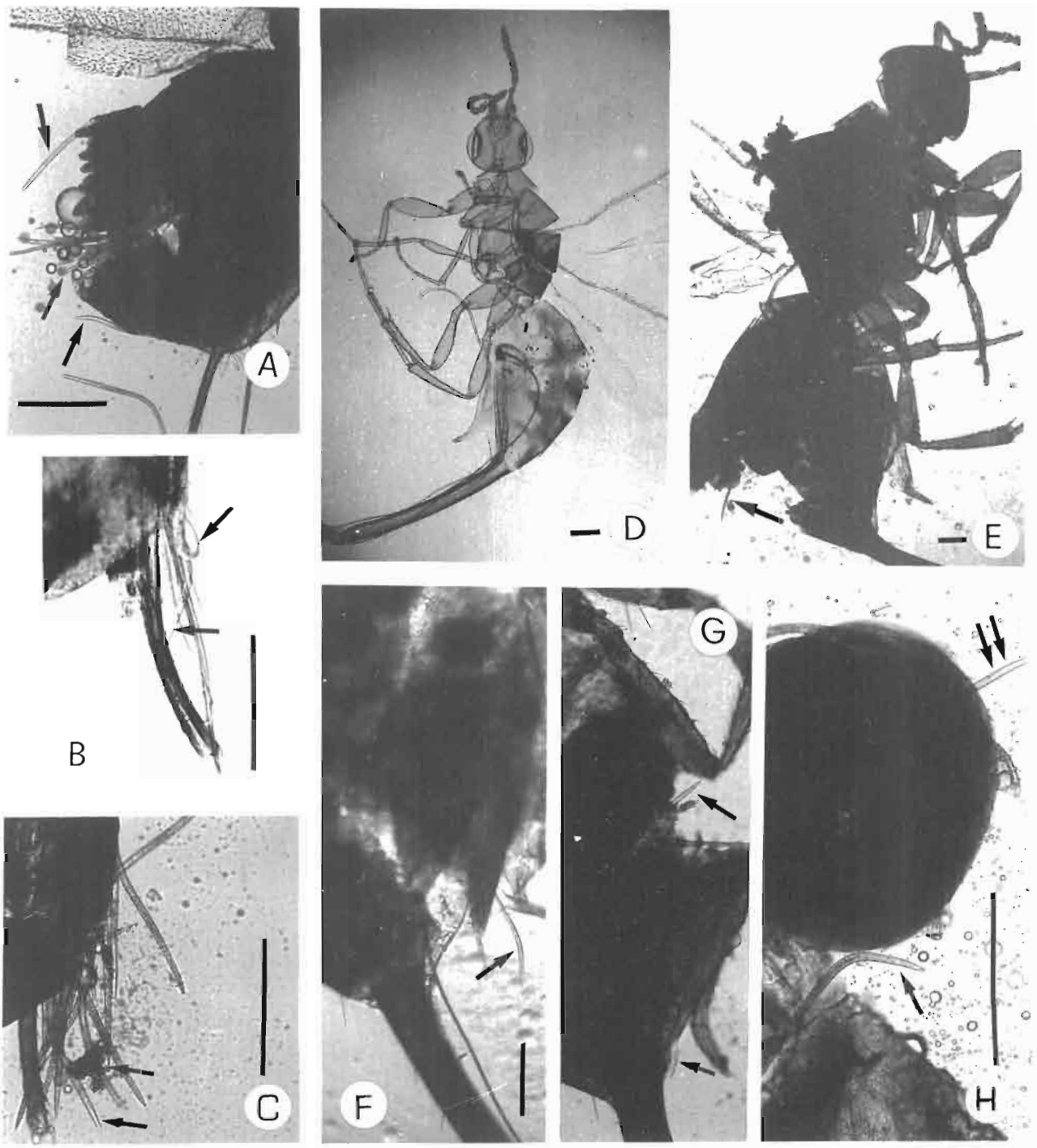

Fig. 2. *Association of* Schistonchus caprifici *with winged wasps of* Blastophaga psenes *(A-C) and with winged wasps of its cleptoparasite* Philotrypesis caricae *(D-H). A-C : Specimens of* S. caprifici *(arrow) protruding from partially dissected wasps; B : Specimens of* S. caprifici *associated with ovipositor* (Bar equivalents : A-C : 100 μm; D-H = 200 μm).

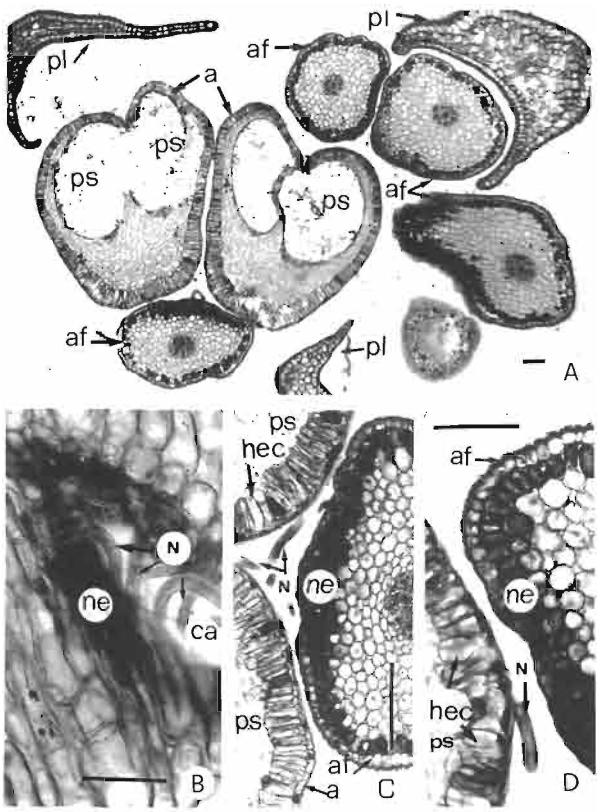

Fig. 3. *Anatomical alterations induced by phytophagous specimens of* Schistonchus caprifici *in caprifig florets. A : Cross section through healthy staminate florets (a = anther; af = anther filament; pl = perianth lobe; ps = pollen sac); B : Peduncle cross section of a pistillate floret showing necrosis (ne) and cavity (ca) in the cortical parenchyma caused by the nematode (N) infection; C, D : Hypertrophied epidermal cells (hec) with dark-stained cortical parenchymal cell of anther (a) and necrosis (ne) of epidermal and cortical parenchymal cells of anther filaments (af). Note nematode specimens (N) among anther filaments and anthers ps = pollen sac* (Bar = 100 µm).

P. caricae and *B. psenes,* respectively. The increase was greater in *B. psenes* than in *P. caricae*. These results suggest that *B. psenes* is a more efficient host of *S. caprifici* than *P. caricae*.

Phytophagous nematode specimens of *S. caprifici* were detected in pistillate and staminate florets of winter, spring, and summer syconia of caprifig as reported in previous studies (Vovlas *et al.,* 1992). Morphometrics of *S. caprifici* phytophagous females did not differ from those of the entomogenous females extracted from the hemocoel of both *B. psenes* and *P. caricae*. However, mean values of body length of entomogenous females were sligthly shorter than those of the phytophagous females : 453 ± 34 (392-489) µm from *B. psenes* and 431 ± 29 (400-476) µm from *P. caricae vs* 476 ± 31 (421-500) µm from syconia florets (Table 1).

Table 1. *Morphometric comparison of* Schistonchus caprifici *females from the haemocoel of* Blastophaga psenes, Philotrypesis caricae, *and from caprifig florets (June population)* (All measurements in µm).

	Populations		
	Blastophaga psenes	*Philotrypesis caricae*	*caprifig florets*
n	10	10	10
Body length	453.4 ± 33.9 (392.1-489.5)	431.3 ± 28.8 (400.0-476.3)	476.3 ± 31.0 (421.0-500.0)
Body width	13.5 ± 0.3 (13.0-13.6)	12.5 ± 0.5 (11.7-13.0)	13.5 ± 0.8 (12.3-14.3)
Stylet length	22.3 ± 1.0 (20.1-23.4)	22.1 ± 2.8 (15.6-24.7)	22.8 ± 0.5 (22.1-23.4)
Median bulb anterior body distance	51.3 ± 3.7 (44.2-56.5)	52.9 ± 9.3 (48.1-79.2)	49.8 ± 1.0 (48.1-51.3)
Posterior uterine sac length	10.2 ± 11.2 (9.1-12.3)	9.5 ± 1.6 (7.8-12.3)	11.7 ± 1.4 (10.4-14.9)
Tail length	34.7 ± 2.8 (30.5-39.0)	31.3 ± 3.4 (26.6-36.4)	38.1 ± 5.0 (31.8-44.8)
V	70.6 ± 1.3 (68.7-72.6)	70.9 ± 1.0 (69.1-72.6)	69.6 ± 1.4 (67.9-71.9)
a	33.7 ± 2.4 (30.2-36.9)	34.5 ± 2.7 (30.8-39.4)	35.4 ± 3.1 (29.4-40.2)
b	6.0 ± 0.4 (5.4-6.4)	5.5 ± 0.7 (4.0-6.2)	6.4 ± 0.6 (5.7-7.1)
b′	4.2 ± 0.5 (3.5-4.9)	4.1 ± 0.5 (3.4-4.6)	4.2 ± 0.4 (3.7-5.0)
c	13.1 ± 1.3 (11.4-15.1)	13.9 ± 0.9 (12.5-15.0)	12.6 ± 1.3 (10.5-14.8)

The mode of entry of *S. caprifici* inside the hemocoel of *P. caricae* wasps remains unknown. No preadult stages of *P. caricae* were examined for nematodes in the hemocoel. Therefore, it remains unclear whether *S. caprifici* infects and is transferred through preadult stages (larvae and pupae) to adult female wasps inside the floret galls as has been shown for *B. psenes* (Volvlas *et al.,* 1992), or if it enters into the wasp's body during the exit of the female wasp from the floret gall in the syconium cavity. We do not know if the nematode is retained in the hemocoel by *P. caricae* because we do not have proof that *S. caprifici* is released with the egg through the long ovipositor during oviposition. It is possible that *P. caricae* is a dead end host for *S. caprifici*.

Histopathology

Pistillate florets infected by *S. caprifici* showed necrosis and cavities in the cortical parenchyma (Fig. 3 B) as reported previously (Vovlas *et al.,* 1992). Examination of infected staminate florets revealed nematode colonies

among the anthers, anther filaments, and perianth lobes (Fig. 3 C, D). Nematode feeding, penetration, and movement induced necrosis of epidermis and three layers of cortical parenchyma of anther filaments (Fig. 3 C, D). Formation of hypertrophied cells was observed in the epidermis of the anthers exposed to nematode feeding compared to the uninfected florets (Fig. 3 C, A). These hypertrophied cells have dense and dark stained cytoplasm of anthers similar to these reported in staminate florets of *F. laevigata* infected by *Schistonchus* sp. (Giblin-Davis *et al.*, 1995). A specialized function in providing nematode nutrients by these hypertrophied epidermal cells for the nematode has been suggested by Giblin-Davis *et al.* (1995). Induction of specialized cells in the epidermal tissues of the anther seems to be a peculiar characteristic of epigeal phytophagous populations of *Schistonchus* sp.

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