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Artificial infestations of *Tapinanthus ogowensis* (Engler) Danser (*Loranthaceae*) on three host species in the Logbessou Plateau (Douala, Cameroon)

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In Cameroon today, Loranthaceae has become a major pest against which a great "battle" must be launched if food production in the country has to be maintained at a self-sufficient level. However, an effective battle against pests can only be achieved through a better understanding of their geographical distribution and biology. Eight Loranthaceae species (Globimetula braunii, Globimetula dinklagei, Globimetula opaca, Helixanthera mannii, Phragmanthera capitata, Tapinanthus globiferus, Tapinanthus ogowensis, and Tapinanthus preussii) have been identified in the Douala area. Among these species, T. ogowensis is the only one limited to a surface area of about eight hectares in the Logbessou plateau located in a direction of North-East from Douala (Latitude 03°40 - 04°11' N, Longitude 09°16' - 09°52' E, and at an altitude of 13 m). In this zone, the hemi-parasite infests only one host tree (Dacryodes edulis) in the orchards, gardens and agricultural plantations. The hemiparasite is however common and adapted to all the different ecological regions in the southern part Cameroon where it infests several host trees. In order to study the stages involved in the germination, fixation, as well as the initial stages involved in the development of the seedlings of T. ogowensis, three of the most frequent host species (D. edulis, Mangifera indica and Persea americana) on the plateau were artificially infected. The results revealed that the host species are sensitive to the parasite, T. ogowensis, at least during their early stages of growth and development. D. edulis is the most sensitive host species with a 22% yield of young seedlings as against 5 and 4% for P. americana and M. indica, respectively. This sensitivity of the mango tree to T. ogowensis had never been demonstrated before. The percentages of seeds germinating on the different host species however remained high; 96% on both D. edulis and M. indica and 93% on P. americana. It was also revealed that the development of the young seedlings of T. ogowensis is greatly influenced by the availability of light, a plausible reason why Loranthaceae occupies mainly the uppermost branches of the oldest trees found here.

Key words: Artificial infestations, Cameroon, host species, Loranthaceae, Tapinanthus ogowensis.

INTRODUCTION

Parasitic plants are sub-divided into two categories depending on the region of attachment on the host spe-

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cies. These two sub categories are: epiphytes which are attached on the aerial parts (branches and stems); and eparchies which are attached on the root system of the host plant (Kuijt, 1969). About 64% of the parasitic plant species are eparchies (Raynal-Roques and Paré, 1998). Based on the nutrient requirements, parasitic plants are sub-divided into hemi parasites and holoparasites.

The hemiparasites contain chlorophyll and synthesise their organic needs using carbon. However, some species have a reduced photosynthetic activity compared to their hosts. For such species to develop, they are obliged to extract organic matter from their host (Tuguet and Sallé, 1996). These plants that are less adapted for the parasitic mode of life form the majority (88%) of parasites. They include the facultative parasites (Rhamphicarpa, Buchnera, Orthocarpus, Bellardia, Parentucellia, Euphasia, Rhinanthus and Odonites), which represent the first stage of the differentiation in the parasitic mode of life (Raynal-Roques and Paré, 1998). In the absence of the host plants, they can still complete their life cycle successfully even though they remain weak and less prolific (Raynal-Roques and Paré, 1998).

The holoparasites, do not contain chlorophyll, are more specialized, and depend entirely on their host for their nutritive needs since they are unable to synthesis organic nutrients using atmospheric carbon. They include the endoparasites, whose development takes place inside the host tissues and are only visible during the flowering season (Raynal-Roques and Paré, 1998). The endoparasites are found mainly in the *Rafflesiaceae* and *Balanophoraceae* (Kuijt, 1969). Their mode of life is more specialized and their parasitism is absolute or obligatory (Raynal-Roque and Paré, 1998).

The parasitic plants are either dicotyledonous angiosperms and constitute only 2% of all the phanerogams or approximately 4700 species (Raynal-Roques and Paré 1998). *Loranthaceae* is a member of Santalales. They are chlorophyll containing hemiparasitic epiphyte shrubs. Their fruits are berries consumed by birds which also serve in dispersing the seeds. In each seed, there are multiple embryo sacs (polygonum type), the suspensor being very long and arranged in multiple series, and the viscous layer is located in the vascular region of the fruit.

The family of Loranthaceae is of worldwide distribution and includes 77 genera and 950 species (Polhill and Wiens, 1998). Seven of these genera subdivided into about 25 species have been identified in Cameroon (Balle, 1982). They infest many wild and cultivated woody tree species thereby limiting their yields. In Cameroon today, Loranthaceae has become a major pest against which a great "battle" must be launched if food production in the country has to be maintained at a self-sufficient level. The list of plants mentioned in the literature where the parasite infests only one host species refers to homogeneous plant groups (Boussim et al., 1993a; Engone et al., 2005). So far, we have not found any literature that has mentioned, a situation where the parasite infests only one host species among many, in a heterogeneous plant group.

In the Douala region (latitude, 03°40' - 04°11' N; longitude, 09°16' - 09°52' E; altitude, 13 m) (Din et al., 2008), eight *Loranthaceae* were identified. These include *Globimetula braunii, Globimetula dinklagei, Globimetula opaca, Helixanthera mannii, Phragmanthera capitata, Tapinanthus globiferus, Tapinanthus ogowensis*, and *Tapinanthus preussii.* Among these parasitic species, *T. ogowensis* limited to a surface area of about eight hectares on the Logbessou plateau (a quarter located north of the city) was found to infest and parasite only one host tree, *Dacryodes edulis.* This host species accounts for 2/3 of the fruit trees counted on this site. The remaining 1/3 consists of peer, guava and mango trees. To understand this absolute and specific sensitivity, artificial infestations (Armillotta, 1984; Hariri et al., 1990) on three identified host species were conducted using a large number of *T. ogowensis* seeds.

MATERIAL AND METHODS

Site of the study

The study was conducted at Logbessou (P.K. 14), a quarter located north of Douala. Douala (located between latitude, $03^{\circ}40 - 04^{\circ}11'$ N; longitude, $09^{\circ}16' - 09^{\circ}52'$ E; and at an altitude of 13 m) has a particular type of equatorial climate described as « camerounian ». It is characterised by two seasons, with a long rainy season (of at least 9 months), with abundant rainfall, (about 4000 mm per year), high and stable temperatures (26.7° C). The average minimal temperature in Douala for 30 years (1961 - 1990) is 22.6° C in July while the average maximal temperature is 32.3° C in February. The relative humidity of the air remains high through out the year and is close to 100% (Din et al., 2008).

Artificial infestations on the field

Artificial infestations were carried out in December 2007 on the three most common and abundant host species in the orchards and gardens of the Logbessou plateau. These three species are D. edulis (Burseraceae), Mangifera indica (Anacardiaceae) and Persea americana (Lauraceae). Ripe fruits of T. ogowensis were collected pressed and the epicarp removed. The seeds were then deposited on the top branches (whose diameters were less than or equal to 1 cm) where adhered to these branches through viscin (Boussim et al., 1993b). For each species, 30 individuals of Diameter at Breast Height (DBH) 30 cm were selected in the orchards and gardens on a mapped surface area of about 8 hectares. On each individual, 100 seeds of T. ogowensis were deposited on five of its top branches, that is, 20 seeds per branch. The germination of the seeds and the developmental stages of parasite were followed for nine months, that is, the follow up lasted until the end of August 2008. Two parameters were calculated:

Germination (%) = (No. of germinated seeds / No. of seeds deposited) \times 100

Fixing (%) = (No. of young seedlings / No. of germinated seeds) \times 100

Ripe fruits and the first developmental stages of the parasite on the host branches were collected, immediately fixed in FAA which is a mixture of formol, ethanol 70 °C and acetic acid (Langeron, 1949) and were used for histological studies. Macrophotographs were taken using a digital camera of the type Kodak Easy Share CX6330 (3.1 Mega pixels).

Histological study of seeds and the young seedlings of *T. ogowensis* at the laboratory

For light microscope observation, samples were firstly fixed with

solution, then dried in a gradient concentrated alcohol and then in paraffine at 56 - 58 °C. Thirteen microns thick particles were either coloured with blue toluidine or with green light/fuchsine binocular magnifying glass Zeiss Stemi 2000-C. Pictures were numerized with a camera Zeiss Axiocam MRC using Axio Vision AC Rel. 4.5 software.

RESULTS

Distribution and ecology of *Loranthaceae* on the site of the study

Two species of Loranthaceae are present on the Logbessou plateau. The least abundant and least common is Phragmanthera capitata. It is heliosciaphilous and grows mainly on the wet and ventilated zones. It develops outside as well as inside the foliage or shelter (Figure 1A), and does not seem to have preference vis a vis the light intensity. This is not the case of T. ogowensis which rather occupies heliophilous biotopes (uppermost branches). It generally develops at the periphery of the foliage (Figure 1B) in order to have maximum sunlight. This behaviour justifies the parasite's preference for trees with light foliage particularly *D. edulis* for which it is specific in the Logbessou plateau. In fact, P. capitata infests and parasites other cultivated fruit trees (P. americana and Psidium quajava) but not the plum trees.

Flowering-fructification

T. ogowensis is monoecious plant whose flowering period occurs from December to August with a 50% activity on May. The violet flowers (Figure 1D), generally occur in groups of three at the base of triangular bracts in the form of a cross with the leaves of the node bearing them. The male flower is made up of four petals (Christmann, 1960) each bearing on its face, an anther with many pollen sacs. The mush more smaller female flower, is made up of a perianth with four sepals inserted on the floral receptacle below the ovary. Fruit production begins immediately after the flowering period and lasts for two months. The ovoid fruits are either white or green during the juvenile stage but are red when mature (Figure 1F). The observation of longitudinal sections of the ripe T. ogowensis fruits enables us to distinguish its different regions. From the outside towards the centre these regions are the red epicarp, the yellow mesocarp (Figure 1F) whose internal part represents viscin, the white endocarp and the seed. Viscin is a tissue made up of elongated cells coated with polysaccharide mucilage (Armillotta, 1984).

The production of seeds is limited compared to the great number of flowers produced. Seed production by *T. ogowensis* is, however, abundant compared with that of *P. capitata* where it is limited (Figure 1).

Germination and fixing

T. ogowensis seeds are more or less regular and bear an embryo containing chlorophyll. This embryo completely enclosed by an endosperm containing no chlorophyll (Figure 2A). The embryo itself (Figures 2B and C) consists of two zones; a hypocotyl zone which ends in a rounded apical part, pointing towards the style. The cotyledonous zone is found on the same side as the flower stalk. These two areas are separated by a depression, near to the hypocotylous zone (Figure 2A to C). In the depression is the caulinear meristem (Figure 2D). The hypocotylous region is covered with outgrowths (Figure 2B and E) which play an important role during the binding of the seedling to the host branch. The cotyledonous zone is much more elongated and is made up of two cotyledons separated by a narrow furrow (Figure 2D). The meristem appears to be acute in the plane of the cotyledon (Figure 2C).

Germination of the seed takes place when the chlorophyllian hypocotyls cross the endocarp and become visible (Boussim et al., 1993b). Irrespective of the host species considered, the percentage germination of *T*. *ogowensis* seeds was high and constant, \geq 93%. Germination of mature seeds (having a red epicarp and a yellow mesocarp) occurs within 48 h. Trial artificial infestations with a mixture of mature and immature seeds (with a green epicarp) resulted in percentage germination lower than 50%; this low percentage may probably be due to the fact the white viscin of the immature seeds is not sticky, hence most of the seeds fell off after they were deposited.

Fixing or attachment takes place through the free end of the hypocotyls, a meristematic zone at the origin of the elongation of this part of the embryo (Thoday, 1951; Boussim, 2002). When this end comes into contact with the surface of the host branch, it flattens and forms an attachment cone. At the centre of this cone, the cells actively multiply to form a young sucker which penetrates the host's tissues. The percentage of seedling attachment on the branches of each retained *T. ogowensis* was always < 25%; 22% for *D. edulis*, 5% for *P. americana* and 4% for *M. indica*.

These stages took place during the first 14 days following the deposition seeds on the surface of the host branch. On day 14, the first leaves appeared above the attachment cone in the furrow between the cotyledons. The appearance of the second leaf took one more week; and for all the three host species, it took place either at shelter or in the light (Figure 2).

Growth and development of the seedling

The development of the aerial part of the seedling occurs only on *D. edulis* and depends on the intensity of the light received and more especially on the amount of nutrients supplied by the parasitized organ of the host. Under

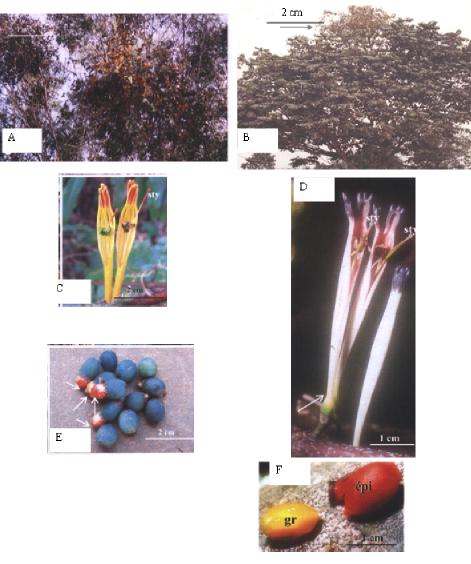


Figure 1. A. General aspect of the *P. capitata* tufts on *P. americana*. B. Arrow indicates *T. ogowensis* located at the periphery of *D. edulis*. C. Details of two opened flowers of *P. capitata*, cheesecloths rolled up on themselves towards the interior and recovering the bicolour style (sty). D. Details of two opened flowers of *T. ogowensis*, highly strangled at the base, arrow showing a style rounded at its end. E. Adult berries, each bearing a seed covered by an orange or red viscin (arrow). F. Seed surround-ded by yellow viscin and removed from its epicarp.

these conditions not more than one seedling of each infested individual host succeeded to develop through the 9 months after germination. Attachment on pear and on mango trees failed and all the *T. ogowensis* seedlings degenerated to a stage where they had only two leaves. During the first five months the rate of development is relatively slow. At the end of the second month, 2 other leaves appear while the first 2 leaves disappeared during the next month. During the 4th month, 2 other leaves appeared making a total of 4 leaves. No morphological change was observed during the 5th month. During the 6th month, the oldest leave degenerated and two other leaves appeared on the stem which now measures 0.4

cm. The 7th month is characterized by a significant increase in the length of the stem (6.5 cm) though the number of leaves remained the same. From the 8th month, growth obeyed the law of increase in number of leaves and length of the stem with seven leaves developing for a 9.7 cm increase in length. The diameter of sucker also thickens and reaches 2 cm. During the 9th month, two new leaves as the stem length increased to 23.9 cm. During the 10th month, the seedling measures 38.3 cm, and has thirteen leaves seven of which developed vigorously but with progressively decreasing sizes from the bottom to the top of the stem (Table 1).

During the first five months, the increase in the length

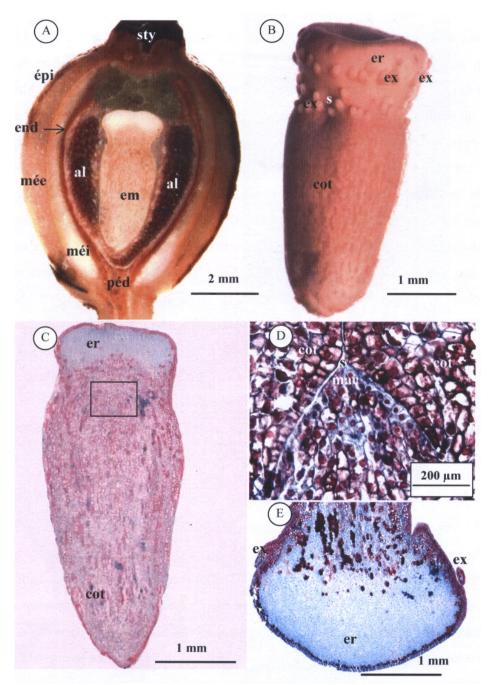


Figure 2. A. Longitudinal section of a berry, shown without colour. The section shows the epicarp, the external mesocarp, the internal mesocarp or viscin surrounding the endocarp, endosperm, embryo, floral stalk and style base. B. Macrophotograph of mature embryo extracted from seed; hypocotyled area covered with outgrowths, reinflated end, area of cotyledon, beginning of the inter cotyledon furrow. C. Longitudinal section of an embryo showing the reinflated end, location of the apical meristem, insertion and the area of cotyledon as seen under a light microscope. D. Details of the insertion shown in "C". E. Details of the reinflated end showing the outgrowths.

of the leaves is more remarkable than their widening and the first four leaves with weopon form an alternate on the stem. The host forms a blister on the contact zone while the remaining part of the seed consisting of hypocotylous and cotyledon start to decrease. After this period, widening becomes more marked so that the five new appearing leaves are bigger and larger than the first ones, subovals and always alternate on the stem (Table 1).

N° of the leaf	Date	04/12	04/01	04/02	04/03	04/04	04/05	04/06	04/07	04/08	04/09
	Number of leaves	2	4	2	4	4	5	5	7	9	13
1	Length		4.4	0							
	Width		0.4	0							
2	Length		3.5	0							
	Width		0.6	0							
3	Length		5	6	6.7	6.8	0				
	Width		1.1	1.3	1.3	1.3	0				
4	Length		1.3	1.4	6.7	6.7	6.7	6.7	6.9	6.9	6.9
	Width		0.3	0.4	1.1	1.1	1.1	2	2	2	2
5	Length				2	2	4.2	4.5	5	5.1	5.1
	Width				1	1.2	1.8	2	2	2	2
6	Length				0.4	0.4	7	8	8.2	8.5	8.7
	Width				0.1	0.1	2.5	3	3	3.9	3.9
7	Length						2	7.4	8.3	8.5	8.6
	Width						0.8	3.4	4	4.9	4.9
8	Length						0.8	3.5	8	8.8	9
	Width						0.2	1.7	4	4.5	5
9	Length								7.2	10.2	11
	Width								3.6	6.4	6.5
10	Length								3.9	10.2	11.4
	Width								1.7	5.9	6.3
11	Length									4.5	6.5
	Width									2.1	9.1
12	Length									1.3	7
	Width									0.8	4.4
13	Length										4.2
	Width										2
14	Length										15
	Width										3.1

Table 1. Parameters of growth and development of *T. ogowensis* young seedling during the first ten months 2007 - 2008, after germination on *D. edulis* branch.

DISCUSSION

The results of artificial infestations carried out on the *Loranthaceae* revealed that optimum germination and attachment of the seedlings occurs when the seeds are deposited on the day the fruits were harvested. On the contrary, a perturbation of seed germination occurs when the fruits are first preserved in a freezer for one or two days, resulting in a very high proportion of seedling (> 80 %) reflecting a reduction in the duration for which the seeds of these parasitic plants are viable. After harvesting the fruits, the seeds have to be deposited on the host branches within the shortest possible time to achieve optimum germination and attachment of seeds.

The viscin does not only play an important role in the dispersal of seeds by birds that feed on the sweet compounds (Gedalovich et al., 1988); but also in the

attachment of seeds on the surfaces of the hosts branches due to its viscous consistency (Edouard and Raynal Roques, 1989). This viscin forms the mesocarp, the major component of the pericarp of all *Loranthaceae*. During germination, the end of hypocotyls elongates and comes into contact with the surface of the host branch. It then flattens and forms a fixation cone which penetrates in the host tissue by negative phototropism to form the sucker. For the three host species, the development of the two leaf stage depends only on the nutritive reserves of the embryo, without any relationship with the substrate and can take place in shelter and in light. This is a characteristic of all *Loranthaceae* (Onefeghara, 1971; Boussim et al., 1993b; Engone et al., 2005).

The events that follow the two leaf stage depend on the parasitized species. For *M. indica* and *P. americana* all the seedlings of *T. ogowensis* degenerated without

attaining the four leaf stage whereas this stage was reached on *D. edulis* after two months. It is at the four leaf stage that the growth rate of *Loranthaceae* on its host decreases but without any visible morphological changes (not even the lost of the two leaves which are regenerated later). The end of this period of slow growth is marked by two important morphological and physiological events: the host reacts by forming a blister at the contact zone; and the establishment of vascular connections between the xylem elements in the absorbing system of the parasite and those of the host.

These connections which result from a stimulation of the cambial activity in the host following the attack by the parasitic were not established between the parasite and the mango tree which is refractory to Tapinanthus (Boussim et al., 1993b) and to other Loranthaceae. Somewhere else, away from the investigated area, pear trees that are sensitive to Loranthaceae also lost their T. ogowensis seedlings. In Cameroon, this Loranthaceae is found primary and secondary forests, tree groups in the savanna and the recurred forest (Balle, 1982). Its dissemination (through seed dispersal) on the indigenous and exotically cultivated fruit trees is almost exclusively by Pogoniulus chrysonocus, "the small bird with yellow face" (Boussim, 2002; personal observation). This dissemination takes place between February and August. According to Serle and Morel (1979), this frugivorous bird (Pogoniulus chrysonocus) is also found in Senegal, Chad, the Central African Republic and Congo. Pollination is carried out by Soui-manga (*Nectariniideae*) which is attracted to the flowers by the nectar located at the summit of the ovary. Soui-manga (Nectariniideae) is a small bird with beaks that are curved and morphologically adapted for sucking nectar from the tubular Loranthaceae flower. Among these birds, Nectarina senegalensis and Nectarina pulchella are the most active, spending long hours on the fruit trees visited in the investigated area. As counted, the fruit trees infested by Phragmanthera capitata are citrus trees, annon trees, pear trees, coffee trees, cacao trees, kolanut trees, hevea trees and guava trees. Two thirds of the old trees in the Logbessou plateau are D. edulis, while the remaining 1/3 consists of pear, mango and guava trees. The high colonisation of D. edulis by T. ogowensis is favoured by the adaptations that result from interactions with this host species that is also very common (Norton and Carpenter, 1998). In this less specific heterogeneous plant community in which D. edulis is relatively more abundant (≥ 70%), the degree of specificity of the parasite is reinforced (Norton and Carpenter, 1998). Under these conditions, the parasite can successfully grow on any potential host it meets as is the case of Phragmanthera capitata parasitism observed in the plantations of Hevea brasiliensis in Cameroon Development Corporation (CDC) in the Douala area.

The degree of specialization of *T. ogowensis* for *D. edulis* is also influenced by the duration of association between the two. In fact, *D. edulis* is one of the oldest

tree species in the study area, their diameters being at least 70 cm and as affirmed by the owners, these trees are about 20 years old. According to the second rule of Manter, a long term association between the host and the parasite creates a high specificity (Brooks and Mc Lennan, 1993). Also, a given parasite has a very close relationship with its host when it is the first among others to infest the host and if the host occurs abundantly (Shaw, 1994). The evolution of the host-parasite interface as from the two leaf stage depends on the competition between the metabolism polyphenols by the host and the growth rate of the young parasite suckers (Hariri, 1989; Hariri et al., 1990 and Sallé et al., 1993):

- If the growth rate of the sucker is higher, then the individual is sensitive;

- If the host reaction is so intense, then the progression of the sucker is blocked.

This is the case of the two woody species *M. indica* and *P. americana* which are refractory to *T. ogowensis* on the Logbessou plateau. The first species is very resistant; the two leaves stage is never exceeded no matter the medium. The second species is sensitive, but not on the Logbessou plateau. In these two cases, the host rapidly synthesizes polyphenolic compounds in large amounts. These compounds are rich in tannins and flavonoïdes. It then becomes impossible for the parasite to cross this barrier; hence it dies due to its inability to obtain nutrients from the host tissues.

The time interval (latent period) between the establishment of this phenolic barrier or connections and the death or progress in the development of *T. ogowensis* seedlings is variable; about two weeks for *M. indica* and two months for *P. americana*. The amount and the nature of the polyphenols present in the host tissues before the parasitic aggression may determine the degree of the host resistance. In the case of D. edulis, the latent period lasts for five months during which there are no apparent morphological changes. After the latent period, developmental rate of the young seedling increases. Favoured by the availability of nutritive substances drawn from the host, more leaves develop successively and connexions become established between conducting tissues of the host and parasite. Other successive stages of leaves are set up at the connections of the two partners are established. The new leaves that develop are larger and longer than the previous ones.

Conclusion

The availability of light and the relative abundance of the host species may have an important influence on the specificity of *T. ogowensis* for *D. edulis* (Hawkins et al., 1992). The relative abundance of the host found in a given area favours the parasitic specificity for that host

(Norton and Carpenter, 1998). The same factors account for the monospecificity of P. capitata for Hevea brasiliensis in the plantations of the Cameroon Development Corporation (CDC) in the Douala area. According to Rhode (1993), the relatively lower specificity of parasites for hosts in tropical areas compared to the temperate areas is a consequence of a relatively lower frequency of contact between the parasite and the individuals of host species (due probably to the presence of a large number of different tree species). Non specific parasites with a wide variety of host species prefer certain host species to others (Bernus and Chapman, 1984; Shaw, 1994). In fact, T. ogowensis have also been observed in other areas. In Penja for example, it attacks mostly Cola nitida, the most abundant host among the other woody species. D. edulis that is less abundant is not parasitized by T. ogowensis. Like P. capitata, T. ogowensis has also been observed in the forests found in south-Cameroon forest, and sometimes extending beyond (Balle, 1982). This ubiguitous species has a relatively high abundance and parasitizes mainly citrus and pear trees. In Africa, T. ogowensis is commonly found in the forest zones of the Central African Republic, Congo, Gabon and the Democratic Republic of Congo. P. capitata does not parasitize D. edulis on the Logbessou plateau as well as in the other parts of Cameroon despite the fact that this host species is relatively more abundant than the other woody species. Moreover, P. capitata is ubiquitous and is adapted to all the ecological variations in Cameroon. The duration of contact between this parasite and its host may also be a major determinant in the specialisation of the parasite for its host. Future studies on the artificial infestations of *P. capitata* seeds on *D. edulis* will clarify this issue.

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