

Initial Stages in the Formation of Galls Induced by *Geoica utricularia* in *Pistacia Terebinthus* Leaflets: Origin of the Two Vascular Bundles Which Characterize the Wall of the Galls

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ABSTRACT

Only a few species of aphids induce galls. Among these, *Paracletus cimiciformis*, *Forda marginata*, *Forda formicaria*, *Geoica utricularia* and *Baizongia pistaciae* induce galls on *Pistacia terebinthus* leaflets. Prior to present study the author examined microscopically *P. terebinthus* leaflets. He also studied the microscopic morphology of galls induced by the five species mentioned above. A clear microscopic difference between these galls is that in the wall of galls induced by the genera *Paracletus* and *Forda* a single vascular bundle is seen. The interpretation is that these galls are laminae of the modified leaflets. However, in the walls of the galls induced by the genera *Geoica* and *Baizongia*, two vascular bundles are observed. In the present paper a study of the early stages of development of galls produced by *G. utricularia* is described. The study was designed to explain the origin of the two vascular bundles present in the walls of these galls. The findings indicate that the aphid induces a massive development of the two vascular bundles present in the midvein of the leaflets of *P. terebinthus*: the main vascular bundle and the small supernumerary vascular bundle. Both these extremely developed vascular bundles occupy the walls of the galls induced by *G. utricularia*.

Keywords: Microscopic Study, Galls, Gallicolous Aphids, *Geoica Utricularia*, *Pistacia Terebinthus*, Vascular Bundles

1. Introduction

Aphids are insects that feed on the phloem sap that flows through the plant [1,2]. Most aphids live in populations in the open air. Only a few species (the Eriosomatinae) form galls on host plants. The structure of the galls is controlled by the aphids themselves [3]. Gall-inducing aphids do not eat the tissue of the host, but they reorganize the vascular tissues to be able to access them easily [4]. Among the species of gall-forming aphids, *G. utricularia* induces galls on leaflets of plants of the genus *Pistacia*.

P. terebinthus is found in the study area. On these shrubs at least five different galls can be identified - according to Inbar *et al.* [5]—induced by as many aphid species (all belonging to the tribe Fordini): *P. cimiciformis*, *F. marginata*, *F. formicaria*, *G. utricularia*, and *B. pistaciae*. They are prosoplasmatic galls, *i.e.* they have a defined size and shape and show a clear tissue differentiation [6]. They are also monothalamic galls, *i.e.* they

have only one chamber [7,8]. The first three types of galls are formed as a fold in the leaflet margins. The other two types are voluminous galls and are balloon-shaped or banana or goat horn-shaped, respectively [9,10]. Prior to the present study, the author microscopically examined the normal morphology of *P. terebinthus* leaflets [11]. Subsequently the same author microscopically studied galls induced by *P. cimiciformis*, *F. marginata*, and *F. formicaria* [9]. And recently, he studied the walls of the galls induced by *G. utricularia* and *B. pistaciae* [10]. The microscopic morphology of the galls induced in the leaflet margin is different from that induced by the genera *Geoica* and *Baizongia*: in the wall of the galls of the first three species there is only one vascular bundle, whereas in the wall of the galls of the other two species two vascular bundles are observed [9,10]. This structural difference supports the existence of two clades within the tribe Fordini, in agreement with several authors [5,12]. In addition, in the galls induced by the genera *Paracletus* and

Forda the xylem is oriented toward the lumen of the gall. Aphids inside the chamber of the gall must circumvent the xylem or pass through it to reach the phloem. In galls induced by the genera *Geoica* and *Baizongia*, the vascular bundle farthest removed from the lumen of the gall positions itself in the same way as the one before it. However, the second vascular bundle is positioned opposite the first (xylem facing xylem), leaving the phloem oriented toward the lumen of the galls [9,10].

In the present paper, newly formed and young galls of *G. utricularia* are analyzed microscopically, in order to learn the origin of the two vascular bundles present in the walls of these galls.

2. Material and Methods

Essentially the same protocol was followed as used in 3 previous studies by the same author [9-11].

Galls on *P. terebinthus* induced by aphids of the species *G. utricularia* were collected from the wild. Samples were taken in April and May at sites in the west of the province of León (Spain). Samples were collected from the early stages of development of the galls, from incipient galls to young galls (**Figures 1(a)-(e)**). The corresponding uninfested (control) leaflets were also collected. Samples were placed *in situ* in FAA (formaldehyde, acetic acid, and ethyl alcohol) and were fixed for 48 hours. They were subsequently dehydrated through an increasing ethyl alcohol series, passed through isoamyl acetate as an intermediate liquid, impregnated with Paraplast for 90 minutes in an oven at 64°C, and finally blocks were formed. Of these blocks, 12 µm thick serial sections were obtained using a paraffin microtome. The Safranin-Fast Green staining method was used. The preparations were mounted permanently on microscope slides with Entellan.

Preparations were studied using a Nikon E600 microscope under bright-field, epi-fluorescence, and polarized light conditions.

3. Results

In the midvein of young *P. terebinthus* leaflets, 8 areas can be distinguished (**Figure 1(f)**): 1/abaxial epidermis; 2/annular collenchyma (2 - 3 rows of cells); 3/storage parenchyma; 4/main vascular bundle: collateral bundle with conspicuous schizogenic ducts in the phloem and abundant xylem elements; 5/storage parenchyma; 6/supernumerary vascular bundle: collateral bundle with few xylem elements and no schizogenic ducts in the phloem. The 7/storage parenchyma; and 8/adaxial epidermis are facing the main vascular bundle (**Figure 1(g)**).

In the first stages of development of the galls (**Figure 1(h)**) a higher cellularity is observed than in the control

leaflets. This increased number of cells is evident in the parenchyma. In addition, the supernumerary vascular bundle presents more xylem elements than the control leaflets. Unicellular trichomes were observed in the epidermis lining the chambers of the galls (**Figures 1(c)-(d)**). Subsequently (**Figure 1(i)**) an increase is observed in the number of cells of the three parenchyma layers considered. The vascular bundles are very developed, and show an evident procambium. At a later stage, the increased cellularity is evident (**Figure 1(j)**). The development of the two vascular bundles is also evident, and a prominent procambium is observed in both. The generalized hypertrophy causes a displacement of the midvein with respect to control leaflets. It can be seen how the two vascular bundles enter the wall of the gall (**Figure 2**). Specifically, it can be observed that the main vascular bundle occupies the outermost part of the wall. The supernumerary vascular bundle occupies the innermost part of the wall, *i.e.* the part closest to the lumen of the gall. Unicellular trichomes are located exclusively in the most proximal part of the galls.

4. Discussion

Only a few species of aphids induce galls. Among them, *G. utricularia* forms spherical galls on the abaxial side of *P. terebinthus* leaflets.

Prior to the present study, the author did a histological study of the leaflets on which these galls are found. Of that study, it should be noted here that the leaflets of *P. terebinthus* have pinnate venation, *i.e.* a main midvein or midrib [11,13]. Specifically, in the midvein of the leaflets of *P. terebinthus* two vascular bundles can be observed microscopically: one primary vascular bundle and one supernumerary vascular bundle with few elements. Both are collateral vascular bundles. Xylem is seen facing xylem, separated by few parenchyma cells. The phloem of the supernumerary bundle is oriented toward the adaxial side of the leaflet, and the phloem of the main vascular bundle is oriented toward the abaxial side.

Subsequently, galls induced by *P. cimiciformis*, *F. marginata*, and *F. formicaria* in the margins of the leaflets of *P. terebinthus* were studied microscopically [9]. Such galls can be understood as laminae of the modified leaflets. A single vascular bundle, developed to a greater or lesser extent, is observed in them: the phloem is oriented toward the outside of the gall, and the xylem is oriented toward the lumen of the chamber. That is, the veins, with varying degrees of hypertrophy, of the lamina itself are observed in them. These are veins in which the small supernumerary vascular bundle that characterizes the midvein of the leaflets of *P. terebinthus* is not observed. Next, the walls of the galls induced by *G. utricularia*

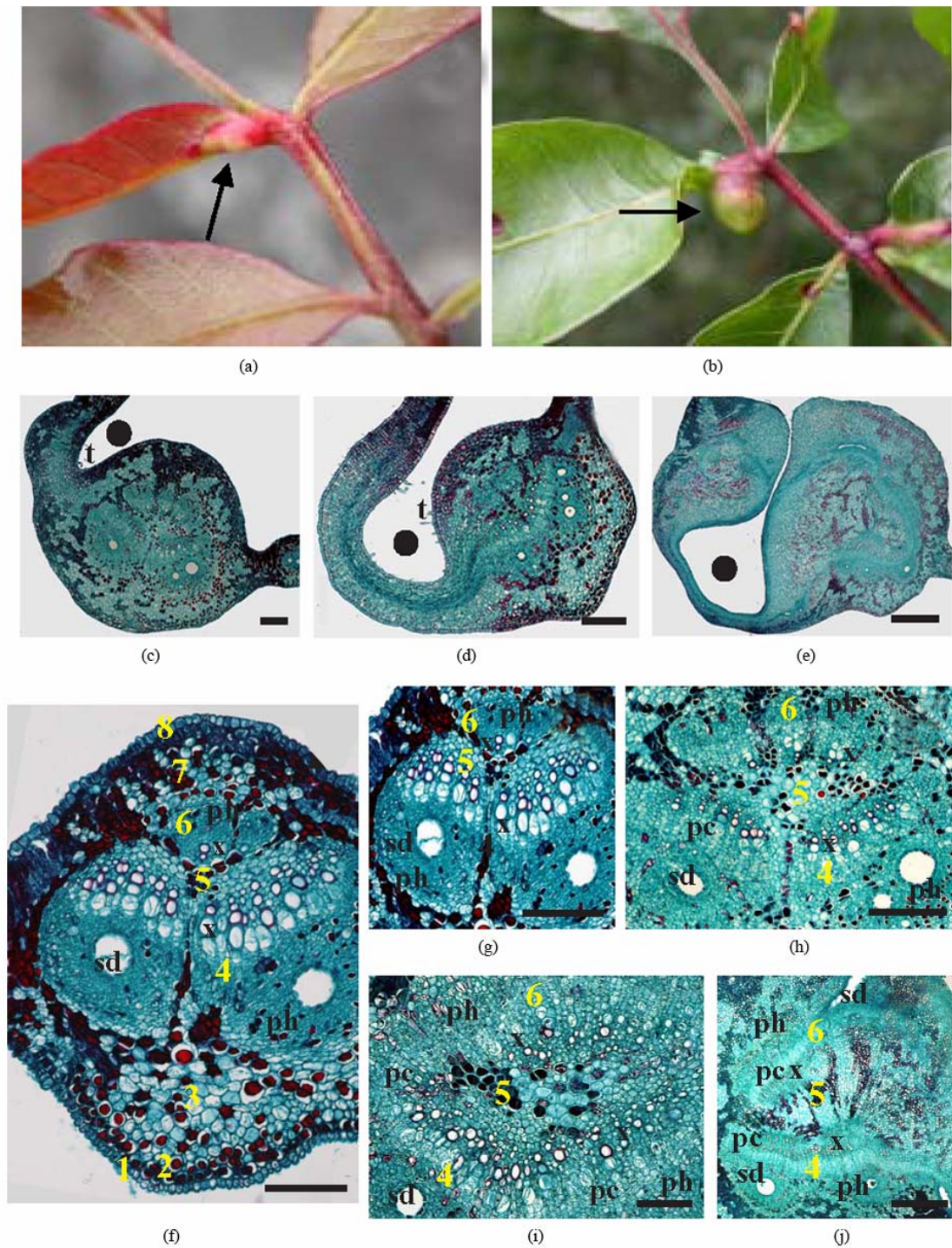


Figure 1. *Pistacia terebinthus* leaflets bearing galls (arrows) induced by *Geoica utricularia* (a, b). An incipient gall is shown in (a), and a young gall is shown in (b). Microscopic sections of galls induced by *Geoica utricularia* (c-e). The dot indicates the evolution of the chamber of the gall. Transverse section of the midvein of the leaflet of *Pistacia terebinthus* (f). Detail of the midvein shown in (f, g). Evolution of the midvein of leaflets bearing galls (h-j), from initial stages (h) to young galls (j). (c-j) Safranin-Fast Green. Bright-field light microscopy. Numbers and abbreviations: 1, abaxial epidermis; 2, collenchyma; 3, abaxial parenchyma; 4, main vascular bundle; 5, parenchyma between bundles; 6, supernumerary vascular bundle; 7, abaxial parenchyma; 8, adaxial epidermis; pc, procambium; ph, phloem; sd, schizogenic duct; t, trichomes; x, xylem. Scale bars (c, f, g) = 100 μ m; (d, h, i) = 200 μ m; (j) = 500 μ m; (e) = 1000 μ m.

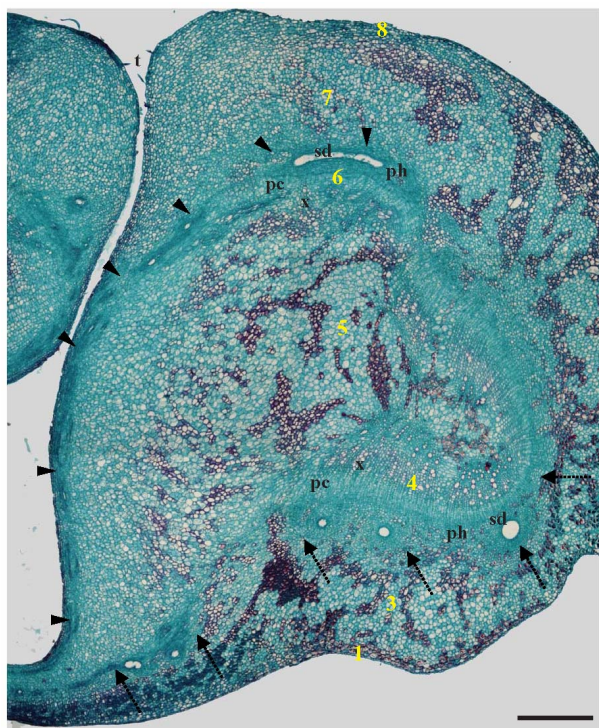


Figure 2. Young gall induced by *Geoica utricularia*. Note the development of the primary vascular bundle (arrows) and the supernumerary vascular bundle (arrowheads). Safranin-Fast Green. Bright-field light microscopy. Numbers and abbreviations: 1, abaxial epidermis; 3, abaxial parenchyma; 4, main vascular bundle; 5, parenchyma between bundles; 6, supernumerary vascular bundle; 7, abaxial parenchyma; 8, adaxial epidermis; pc, procambium; ph, phloem; sd, schizogenic duct; t, trichomes; x, xylem. Scale bar 500 μ m.

and *B. pistaciae* were studied [10]. These walls have two vascular bundles, and not just one as seen in the galls studied before. The question that gave rise to the present study related to the origin of the two vascular bundles seen in the walls of the galls induced by *G. utricularia*. To this end, newly formed galls and young galls were analyzed microscopically and compared with the leaflets of *P. terebinthus*.

The two -hypertrophied- vascular bundles present in the midvein of the leaflets of *P. terebinthus* occupy the walls of the galls induced by *G. utricularia*. This is demonstrated not only by the detailed study of the serial sections, but also by the study showing that the two vascular bundles in the wall of the gall are facing each other. Just like the supernumerary vascular bundle and the main vascular bundle in the midvein of the leaflets of *P. terebinthus* are facing each other [11]. The fact that a prominent procambium is observed in the two vascular bundles provides further support.

Several authors have made comparisons between the lamina of the leaflets of the genus *Pistacia* and the walls of the galls induced by species of the genus *Geoica* [4,14]. In view of the results of the present study, such a comparison may not be correct. However, it does make sense to make such a comparison regarding galls induced by aphids in the genera *Paracletus* and *Forda* [9]. Therefore, the structure of galls induced by *G. utricularia* should be compared with the midveins of the leaflets of *P. terebinthus*. Or, to say it differently, the galls induced by *G. utricularia* are structures depending on the midvein of the leaflets. This observation is in agreement with the suggestion of Inbar *et al.* [5] that the subtribe Baizongiini (which includes the genus *Geoica*) evolutionarily acquired the ability to develop large galls on the midvein. In the absence of microscopic studies, these findings do not contradict the suggestion of Remaudière *et al.* [15]. These authors state that *G. swirskii* forms a gall on *P. atlantica* in the leaf rachis but not on the midvein of these leaflets, and not in the lamina of the leaflets either.

Somehow the aphid causes hypertrophy of the vascular bundles and of the accompanying parenchyma. Based on 1/the similarity between the microscopic composition of the walls of galls induced by *G. utricularia* and *B. pistaciae* [10]; and 2/the field observation that the galls induced by *B. pistaciae* concern the leaflet as a whole and not a part of it, the following hypothesis can be formulated: galls induced by *B. pistaciae* may follow a process similar to that of galls induced by *G. utricularia*. But in this process, *B. pistaciae* would act on the meristematic masses of the bud primordia of *P. terebinthus*. This means that *B. pistaciae* would settle on *P. terebinthus* during earlier stages of development of the leaflets than those in which *G. utricularia* acts. Microscopic studies and field studies are required to reject or accept this hypothesis.

Moreover, Al-Saghir *et al.* [16] state that species of the genus *Pistacia* do not have trichomes. However, in the first of the studies cited above [11] the existence of glandular trichomes on young *P. terebinthus* leaves was established. The same author also noted unicellular nonglandular trichomes at the “entrance” of the galls induced by *F. formicaria* [9]. In the present study trichomes similar to those induced by *F. formicaria* are observed. In both cases these should be interpreted as elements that are part of the protection system of the galls against attack by intruders on their interior [17-19].

To summarize the above, it should be underscored that the origin of the two vascular bundles seen in the wall of the galls induced by *G. utricularia* is found in the interference of the aphid with the normal development of the

midvein of the leaflets, especially of the two vascular bundles and the surrounding parenchyma cells.

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