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One aphid species induces three gall types on a single plant: Comparative histology of one genotype and multiple extended phenotypes

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ABSTRACT

In aphids, usually only the fundatrix (F1) induces galls. In *Smynthrodes betae* (Fordini) however, three gall types may coexist on the leaflets of a single host species, *Pistacia atlantica* (Anacardiaceae). The fundatrix induces pea-shaped galls on the leaflet midvein early in spring and its genetically identical daughters (F2) disperse from these galls and induce spindle-shaped galls on leaflet margins. In some of the fundatrix' galls, the daughters (F2) continue to reproduce and complete their normal life cycle within the same gall (CLC galls). Using a comparative anatomy approach, the aim of this study is to evaluate the role of different aphid generations and gall location in controlling gall structure. Histological sections of the three gall types as well as galled and ungalled leaflets were examined. The leaflet's mesophyll is highly modified in all gall types expressing hyperplasia and hypertrophy and collateral veins occupy the inner part of the gall's wall. The epidermis lining the chamber of only the F1 and the CLC galls is covered with trichomes. The F1 galls have a rather simple structure, composed mostly of large parenchyma cells. The F2 and CLC galls have two parenchyma layers separated by a sclereid layer that creates a protective hardened structure. The structural similarity of the F2 and the CLC galls indicates that most histological modifications are controlled by the aphids and depend on either the continuous or cumulative activity of the following aphid generations in the gall.

Introduction

Galls are abnormal plant structures induced by various organisms, in particular insects (Mani, 1964). The mechanism of gall induction by insects remains unknown, but it seems that the insect controls gall development, subject to suitability and reactivity of the plant tissues (Weis et al., 1988). This hypothesis is supported by phylogenetic studies of several lineages of gall-forming insects (Cook and Gullan, 2008; Inbar et al., 2004; Nyman et al., 2000; Stern, 1995; Stone and Cook, 1998). Galls are therefore described as the extended phenotypes of the insects (Dawkins, 1982). The galls serve as an "incubator" for the insects that may gain better nourishment and protection from unfavourable abiotic conditions and natural enemies (Price et al., 1987).

Most insect galls are highly organised structures; defined as histoid prosoplasmic galls (sensu Küster, 1911). They have a definite size and shape, often symmetrical, and usually display a novel tissue pattern compared to ungalled organs (Chakrabarti, 2007; Raman, 2007). However, the complexity of gall structure varies enormously among and within insect groups (Raman, 1996; Rohfritsch, 1992), ranging from local overgrowths (e.g., Rey, 1992) to highly differentiated structures with orderly arrangement of various types of cell layers as exhibited by cecidomyiids and cynipid wasps (Dorchin et al., 2002; Rey, 1992; Rohfritsch, 1992; Sliva and Shorthouse, 2006). A nutritive tissue may develop in the inner layer of some insect induced galls (e.g., Bronner, 1992; Rohfritsch, 1977). A lignified sclerenchyma sheath, which provides physical protection may be formed external to the nutritive tissue (e.g., Dorchin et al., 2002; Rohfritsch, 1992).

Usually each insect species induces a single gall type. In some cases two gall types are induced by the same species, on different host plant or organs, or at different periods (e.g., Ananthakrishnan, 1992; Ananthakrishnan and Raman, 1989; Shorthouse and Rohfritsch, 1992). The induction of two gall types extends the ability of the insect to exploit variable plant resources (Dorchin et al., 2009; Miller, 1998; Rhomberg, 1980; Wool and Burstein, 1991). In cynipid wasps, distinct generations of the same species may induce different galls on different plant organs (e.g.,

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Rey, 1992). Different plant organs (galling sites) might restrict gall development (Wool, 1997) and thus promote gall dimorphism. Dimorphic galls can also be related to different induction signals given by the insects. For example, some scale insects (Coccidea) induce sexually-dimorphic galls, often on the same plant organ, suggesting that different signals are involved (Gullan et al., 2005). The longer feeding period and the sessile habit of the coccoid female may also influence gall size and structure (Gonçalves et al., 2005). The development of sexually dimorphic gall structures in the pteromalid wasps was related to cytokinins and auxins levels (Dorchin et al., 2009).

Aphids (Hemiptera: Aphididae) induce a variety of galls with different morphologies and structural complexities (Inbar et al., 2004). Aphids in the tribe Fordini induce galls on *Pistacia* spp. (Anacardiaceae) hosts (Wool, 1995). Their life cycle is complex and includes alternation between a primary host (*Pistacia* spp.) and roots of secondary hosts, on which they do not induce galls. Aphids are phloem-feeders and their galls may have an extensive internal vascularisation (Wool et al., 1999).

In most gall-forming aphids, only the fundatrix (F1) is capable of inducing a gall despite the genetic identity with her offspring (Moran, 1988; Wool, 2004). This paper deals with one of the most common Fordini species, *Smynthurodes betae* Westw., which forms galls on leaflets of *P. atlantica* Desf. The fundatrix induces small, pea-shaped ("temporary") galls on leaflet midveins early in spring (Fig. 1A). About three weeks later, her offspring (F2) induce different ("final") galls on leaflet margins of adjacent young leaves (Fig. 1B). Usually, the fundatrix dies within a few weeks but the empty temporary galls may remain on the tree until leaf-fall in autumn (Wertheim, 1954). Each final gall is induced by a single F2 aphid in which two more parthenogenetic generations are produced. In autumn, winged migrants (F4) disperse from the F2 gall to various secondary hosts (see Fig. 1 in Wool and Burstein, 1991). The F2 galls contain about 30 winged aphids. In *S. betae* there is a further complication, since in some of the fundatrix galls, the daughters (F2) continue to reproduce and complete their life cycle within this temporary gall. The "CLC" (complete life cycle) galls contain about twelve aphids and they are quite rare, comprising between 3 and 24% of the fundatrix galls (Wool and Burstein, 1991). Morphologically they are not easily distinguished from F1 galls. Usually they appear to be darker and larger (Fig. 1C) and become harder than normal fundatrix galls during the summer (Inbar personal observations; Wool and Burstein, 1991). This is a rare example of induction of three gall types by the same species on a given host plant (see also Gonçalves et al., 2009).

Pistacia atlantica is a dioecious and deciduous tree with an Irano-Turanian distribution (Zohary, 1952). Inter-population variability in some leaf morpho-anatomical characters has been reported (Ait Said et al., 2011; Belhadj et al., 2007; Inbar and Kark, 2007). For example, the thickness of the epidermis, cuticle, palisade parenchyma and total lamina as well as the density of epidermal trichomes increased with the degree of aridity (Ait Said et al., 2011).

Histological studies in the Fordini are limited to galls induced on *P. palaestina* and *P. terebinthus* (Álvarez et al., 2009; Álvarez, 2011a, 2011b; Wool and Bar-El, 1995; Wool et al., 1999). These galls induced on the same plant organ and even at the same galling site (e.g., leaflet margins) exhibit different histological modification of the ungalled leaflet tissues (e.g., Álvarez et al., 2009).

Here we present a comparative histological study of three gall types induced by a single aphid species (*S. betae*). Each gall is induced and/or inhabited by different generations on the leaflets (leaflet midvein vs. leaflet margin). This system provides an opportunity to evaluate the role of different aphid generations and location in the modifications that occur in the plant tissues during gall formation.

Material and methods

Plant material

Galls induced on leaflets of *P. atlantica* by the aphid *S. betae* and ungalled control leaves were collected from trees growing in several sites in northern Israel: Kiryat Tiv'on; Beit Lehem HaGlilit; Gamla and Bar'am. Seven fundatrix (hereafter, F1) and 11 final (hereafter, F2) galls were sampled during August-September from four and five *P. atlantica* trees, respectively. Six CLC galls were collected in late June from two trees. All galls were collected with the galled leaf (control 1). In addition, another ungalled leaf from the same branch was sampled as a second control (control 2). Samples were kept cool from the time of collection until their arrival at the laboratory (for a few hours at the most) and then fixed in 96% ethanol and glacial acetic acid (ratio 3:1). All fixed samples were kept in the fixative solution for at least several months.

Histology

The histological procedures were performed according to Ruzin (1999) with few modifications. The fixed samples were cut transversely with a sharp razor blade into smaller segments (~1 cm long). Three types of samples were prepared: galls only, galls with their attached galled leaflets and ungalled control leaflets. These samples were placed in 20 ml vials with 50% ethanol overnight and then dehydrated in a series of ethanol and *tert*-butanol solutions (% ethanol/% *tert*-butanol: 40/10; 50/20; 50/35; 45/55; 25/75; 0/100; 0/100; 0/100) for at least 8 h in each stage. After dehydration, the samples were infiltrated with paraffin for over one week in an oven at 60 °C. The paraffin was replaced every two days and then the samples were embedded in paraffin until complete solidification. These paraffin blocks were used to prepare 8–10 μm thick serial sections of the whole gall (data not shown) on a rotary microtome (Leica RM 2135). Histological sections were stained with Safranin and Fast-Green and mounted permanently on microscope slides with Entellan New (MERCK).

Microscopy and measurements

The sections were examined under an Olympus BX61 microscope equipped with an Olympus DP70 digital camera under bright-field, fluorescence and polarised light illumination and also under a bright-field Olympus CX21 microscope. Measurements were done using the AnalySIS® software.

The following parts were studied (Fig. 1D–F): (1) The external epidermis of the gall, derived from the abaxial leaflet epidermis; (2) the internal epidermis of the gall, derived from the adaxial leaflet epidermis; (3) the modified mesophyll between both epidermal layers; (4) the zone where the gall's lumen is closed and a narrow opening (ostiole) is formed. Henceforth, the term "ostiolar zone" refers to the gall's walls adjacent to the ostiole; (5) the midvein; (6) the part of the leaflet lamina adjacent to the ostiolar zone, which separate between the galled tissue and a normal leaflet tissue (transition zone). In addition, we examined different parts of the galled leaflets, located at increasing distance from the transition zone (galled leaflet 1, 2, 3, Fig. 1A–C). Not all zones were found in all sections.

The thickness (in μm) of the gall's wall and the leaflet lamina were measured in the different zones (i.e., the main part of the gall (excluding the ostiolar zone), ostiolar zone, transition zone, galled leaflet, ungalled leaflet) using the AnalySIS® software. The number of cell layers that composed each zone was counted and the quotient between the thickness (in μm) and the number of cell layers was used to evaluate cell size (height). Similar measurements