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Induced Activity of Pathogenesis Related (PR) Proteins in Aphid Galls

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Abstract

The favorable abiotic conditions within aphid galls may provide an optimal microhabitat for pathogenic microorganisms such as fungi and bacteria. Pathogens, especially fungi may be one of the main sources of mortality of the aphid gall-formers. We found high levels of pathogenesis related (PR) proteins in the tissue of galls induced by the aphids *Smynthurodes betae* (West.), *Forda riccobonii* (Stephani), and in particular *Slavum wertheimae* HRL (Homoptera: Pemphigidae: Fordinae) on *Pistacia atlantica* (Anacardiaceae). Compared with adjacent ungalled leaves, activity levels of chitinase and peroxidase, but not β-1,3-glucanase, were significantly higher in the galls. These PR proteins are an important component of the plant defense mechanisms against pathogens. The local induction of PR proteins in the galls suggests that manipulation of anti-microbial activity in the host tissue by gall-forming aphids may be self-benefiting.

Keywords: Fordinae, fungi, gall, host manipulation, pathogens, *Pistacia*, PR proteins

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1. Introduction

The mechanism of gall induction by insects and other invertebrates is unknown (Shorthouse and Rohfritsch, 1992). The plant-gall formers' relationships are considered parasitic, negatively affecting the host plant. Gall-forming insects gain protection against abiotic factors and natural enemies, and obtain increased nutritional values (Price et al., 1987). Gall formers exploit the development, morphology, physiology, and chemistry of the host plant (Weis et al., 1988; Shorthouse and Rohfritsch, 1992). Thus, plant traits are modified to the benefit of the gall former (e.g., Inbar et al., 1995). Numerous extensive studies have examined the biochemical changes in gall tissues including the levels of plant hormones (Mapes and Davis, 2001), primary metabolites such as proteins (e.g., Schonrogge et al., 2000), and secondary metabolites including phenolics, tannins and terpenes (Cornell, 1983; Hartley, 1998; Monaco et al., 1973; Nyman and Julkunen-Titto, 2000).

Pathogenesis related (PR) proteins are plant proteins that are induced in pathological situations (Bowles, 1990). Usually they are produced via the salicylic-dependent pathway and are considered a part of the multiple defense systems of plants (Kombrink and Somssich, 1997). For example, chitinase and β-1,3-glucanase have the ability of degrading fungal and bacterial cell walls. Peroxidases are key enzymes in lignification and hypersensitive responses in plants, which limit disease spread (Bowles, 1990). Only recently has it been shown that sap-feeding arthropods (aphids, whiteflies, mites) induce PR proteins in plants (Bronner et al., 1991; Mayer et al., 1996; Walling, 2000). Insect galls are attractive to pathogenic microorganisms, especially fungi, that can destroy the galls and the gall-formers (Taper and Case, 1987; Wool and Bar-El, 1995). High levels of tannins and phenolics in the gall tissue are often considered as a general host-plant manipulation by the insects to reduce the risk of pathogen infection and predation by natural enemies (Cornell, 1983; Taper and Case, 1987; Taper et al., 1986). In this study we evaluated the induction of three PR proteins by three gall forming aphid species as induced plant defense mechanisms against pathogens.

2. Material and Methods

The organisms

Pistacia atlantica Desf. (Anacardiaceae) is a deciduous monoecious tree with a typical Irano-Turanian distribution from central Asia through the Middle East to north Africa (Zohary, 1952). In Israel, *P. atlantica* is distributed discontinuously from the Golan Heights, upper and lower Galilee, to the Negev

highlands (Zohary, 1952). The sampled trees grow naturally in the lower Galilee, ca. 30 km east of Haifa. The galls of three aphids (Homoptera, Pemphigidae, Fordinae) were examined in this study: *Smynthurodes betae* (West.) (SB), *Forda riccobonii* (Stephani) (FR), and *Slavum wertheimae* HRL (SW). All three aphids form typical galls only on *P. atlantica* (Inbar and Wool, 1995). The first (temporary) galls of SB and FR are formed early in the spring on the leaflet midrib by the fundatrices (F1). Within each gall, ca. a dozen second generation aphids (F2) are produced parthenogenetically. The aphids leave the temporary galls and produce the final galls on the leaflet margin (Inbar and Wool, 1995). SW has only one cauliflower shaped gall that is formed in late spring on lateral buds (Wertheim and Linder, 1961). Samples of SW galls and final galls of SB and FR were collected in mid-August. We sampled 14 trees that had SB galls, 9 of them had FR galls, and SW was found on five trees. From each tree we randomly collected 20 galls and adjacent ungalled leaves that were pooled. Only "clean" galls that had no visual signs of pathogen infections were sampled. Galls were opened with a scalpel and the aphids were removed; ungalled leaves were similarly longitudinally cut.

PR protein analyses

Fresh samples were weighed, lyophilyzed, and then ground in liquid N_2 using an Omni-Mixer (OCI Instruments, Waterbury, CT). The resulting powder was suspended in cold 0.1 M sodium phosphate (pH 7.4) and homogenized for 1 min. The homogenate was filtered through four layers of cheesecloth and centrifuged at 15,000 g for 15 min at 4°C. The supernatant was filtered through a layer of Miracloth (Calbiochem-Novabiochem Corp., La Jolla, CA). Filtrates were desalted by dialysis against water and lyophilyzed. Chitinase activity was measured colorimetrically using dye-labeled chitin (Loewe Biochemica, Munich, Germany) as the substrate (Wirth and Wolf, 1990). β-1,3-Glucanase activity was assayed using the method of Abeles and Forrence (1970) with laminarin (*Laminaria digitata*, Sigma, St. Louis, MO) as the substrate. Peroxidase analysis was based on the method outlined in the Worthington Enzyme Manual (Worthington Biochemical Corporation, 1993), using 4-aminoantipyrine as a hydrogen donor. Differences in PR protein activity levels were analyzed with a One Way ANOVA, and followed by mean separations post hoc tests.

3. Results

The levels of peroxidase and chitinase activities were low and hardly detectable in mature *P. atlantica* leaves (Figs. 1 and 2). Both enzymes had high

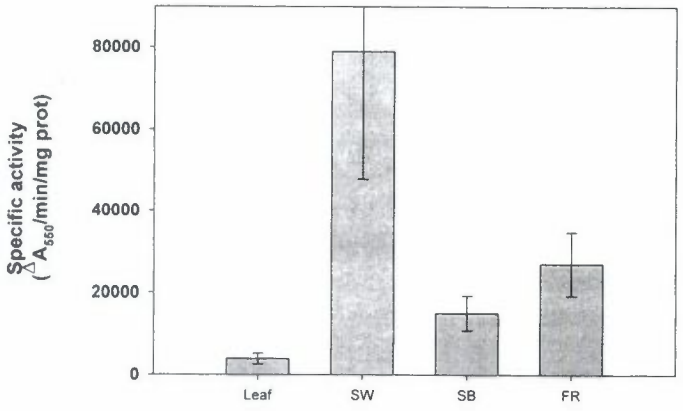

Figure 1. Peroxidase activity levels (mean ± se) in galls and ungalled leaves of *P. atlantica*.

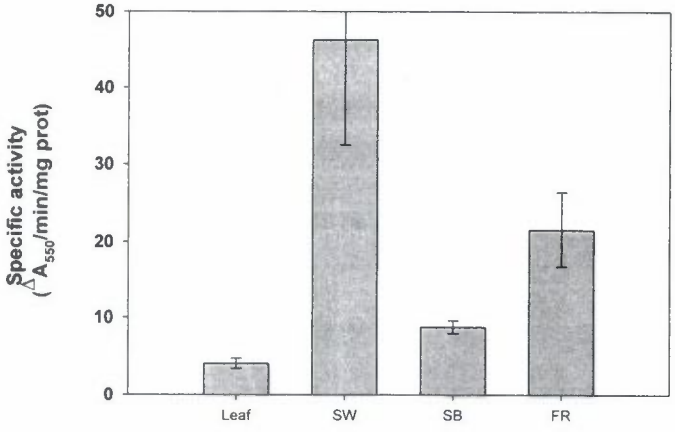

Figure 2. Chitinase activity levels (mean ± se) in galls and ungalled leaves of *P. atlantica*.

activity levels in the galls. Peroxidase activity was nearly 18-fold higher in SW galls compared with the ungalled leaves. Significant higher activity was also observed in SB and FR as well ($F_{3\text{-}39}$ = 10.1, P<0.01, Fig 1.). A similar trend was found with chitinase. Again, compared with ungalled leaves, the sharpest increase in chitinase activity was found in SW galls. A moderate (but significant) increased was also found in FR and SB galls (Fig. 2, $F_{3\text{-}39}$ = 17.1, P<0.01). β-1,3-Glucanase activity levels in the galls were not statistically different from the activity levels in the ungalled leaves, although it was approximately 2-fold higher in the SW galls (Fig. 3). Nevertheless, mean separation test reviled that the significant ANOVA results ($F_{3\text{-}39}$ = 4.4,

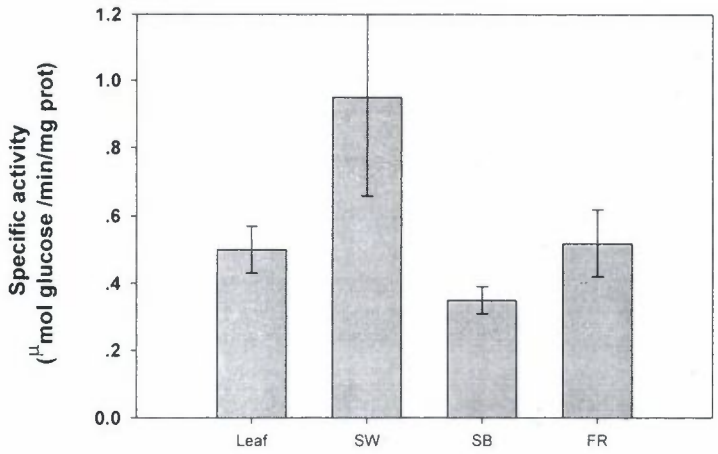

Figure 3. β-1,3-Glucanase activity levels (mean ± se) of in galls and ungalled leaves of *P. atlantica*. Mean separation revealed that the only significant differences are between SW and SB galls.

P<0.01) were due to the differences in β-1,3-glucanase activity between SW and SB galls (Fig. 3).

4. Discussion

Chitinase and peroxidase activities were highly induced in all three aphid galls especially in SW. Walling (2000) suggests that responses by plants to pathogens are similar to their responses to sap feeding arthropods such as aphids. Aphid stylet penetration causes limited tissue damage and resembles the injury pattern associated with fungal and bacterial infection that often results in induction of PR proteins. Recently it has been shown that the green peach aphid *Myzus percicae* induces several PR proteins in Arabidopsis (Moran and Thompson, 2001). The elicitors of PR proteins in the galls could be contained in the aphid's saliva and produced either by the aphid itself or by endosymbiotic bacteria (Walling, 2000 and references therein). Aphid saliva is known to contain polygalacturonases that can produce oligosaccharide fragments that are elicitors (Ma et al., 1990). Indeed, plant breeders have targeted polygalacturonases and developed resistant plant varieties that produce complex polygalacturonides (Dreyer and Campbell, 1987). Further, plant polygalacturonase inhibitor proteins (PGIP) have been reported to aid in the generation of appropriately sized oligogalacturonides that act as elicitors for a heightened defense responses (Cervone et al., 1989). Plant PGIP are know to inhibit inset polygalacturonases (Doostdar et al., 1997).

The peroxidase and chitinase activity levels in SW galls were not only

higher than ungalled leaves, but also than galls of SB and FR (Figs. 1 and 2). These results can be explained by species-specific differences in aphid abilities to induce PR proteins. However, there is another major difference between the galls of SW and those of SB and FR. SB and FR final galls develop early in the spring. By mid-August (the sampling date) SB and FR final galls are fully developed (mature) while SW galls, at the same date, are still in their early development phase, but contain larger numbers of aphids (Inbar and Wool, 1995; Wertheim and Linder, 1961). It seems likely that the young differentiating tissue of SW is more inducible than in mature galls (galls of FR and SB at that of sampling date) (see also Alarcon and Malone, 1995).

Unlike their clear role in antipathogenesis (e.g., Heil et al., 2000), the role of PR proteins in plant defense against sap feeding herbivores is unknown (Walling, 2000, but see van der Westhuizen et al., 1998). Furthermore, it appears that plant chitinases have limited effects on insect fitness (Kramer and Muthukrishnan, 1997). Peroxidases on the other hand, may have an important role in multiple plant defenses against pathogens and insects. Peroxidases are key enzymes in various metabolic cascades leading to a hypersensitive response, and production of secondary metabolites (Duffey and Stout, 1996; van der Westhuizen et al., 1998). Fungal infection may be one of the main sources of gall-former mortality (Taper and Case, 1987; Wilson 1995; Wilson and Carroll, 1997). In *Forda formicaria* Von Heyden, a gall forming aphid (Fordinae) on *P. palaestina*, rust fungus can become a major source of mortality (Wool and Bar-El, 1995). It is widely accepted that high concentrations of tannins and phenolics, especially in the outer layer of the galls, protect the gall-former from external enemies such as parasitoids, predators and fungi (Cornell, 1983; Taper and Case, 1987; Hartley, 1999).

Little is known about the chemistry of the gall forming aphids (Fordinae) on *Pistacia*, except the fact that a few species induce high level of triterpenes (Caputo et al., 1975; Monaco et al., 1973). Gall formers manipulate the host plant organs for their own needs. Extensive developmental, morphological physiological and chimerical modifications have been reported (Weis et al., 1988; Shorthouse and Rohfritsch, 1992). We suggest that the high level of PR proteins in the Fordinae galls reflects an additional aspect of host plant manipulation by the aphids. PR proteins induced locally only in the gall tissue by the Fordinae in *P. atlantica* trees, may protect the galls and aphids from pathogenic infections. Finally, since antifungal activities have been detected in (ungalled) *Pistacia* leaves (Kordali et al., 2002), it will be worthwhile for commercial use to evaluate such activity in gall extracts.

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