

Changes in Oak Gall Wasps Species Diversity (Hymenoptera: Cynipidae) in Relation to the Presence of Oak Powdery Mildew (*Erysiphe alphitoides*)

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Mohammed Reza Zargaran, Nadir Erbilgin, and Youbert Ghosta (2012) Changes in oak gall wasps species diversity (Hymenoptera: Cynipidae) in relation to the presence of oak powdery mildew (*Erysiphe alphitoides*). *Zoological Studies* 51(2): 175-184. Plant-mediated interactions usually lead to multipartite interactions in a community of organisms. To evaluate the impact of oak powdery mildew *Erysiphe alphitoides* infestation on the distributions of cynipid oak gall wasps (Hymenoptera: Cynipidae), a field survey was conducted in West Azerbaijan Province, Iran, in 2 consecutive years of 2009-2010. Multiple samples were taken from both infected and uninfected trees (*Quercus* spp.) at 4 different sites where maximum activity of *E. alphitoides* occurred and cynipid galls exhibited complete development. The species diversity and richness of gall-forming wasps were estimated and also parameters such as Simpson's index, Shannon's H', and the Sorensen similarity quotient were calculated. Data were also analyzed by independent-samples *t*-test to compare the mean numbers of galls occurring on infected and uninfected trees. Results clearly indicated that the highest richness and diversity of oak gall-forming wasps were consistently found on uninfected trees at all study sites in the 2 consecutive years. Further, the number and diversity of gall-forming wasps were negatively correlated with the extent (percentage) of pathogen infection, and trees with the heaviest *E. alphitoides* infection had the lowest numbers of gall-forming wasps. In addition, *E. alphitoides* decreased the rate of Sorensen's coefficient between regions where oak trees infected with *E. alphitoides* were sampled. This study demonstrates plant-mediated interactions between a native pathogen and a community of gall-forming insects on oak trees.
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Key words: Cynipid gall wasps, Tree-mediated interactions, Species diversity, Abundance, Oak forest.

Plant-mediated interactions were commonly reported in many studied systems (Karban and Baldwin 1997, Nakamura et al. 2003, Foss and Rieske 2004, Eyles et al. 2010, Staley et al. 2010). Such interactions usually lead to multipartite interactions in a community through indirect interactions as 1 organism may change a host's suitability for others, and hosts become more or less suitable for subsequent attackers (Karban and Baldwin 1997). Most current knowledge of plant-mediated interactions was obtained through

studies on herbaceous annuals or short-lived perennials, but much less is known about trees, either angiosperms or gymnosperms (Eyles et al. 2010).

In recent years, plant-mediated interactions in forest ecosystems were documented (see review by Eyles et al. 2010, Colgan and Erbilgin 2011), although the implications of those studies are limited, in part, due to associations of a large diversity of insects and pathogens with different growth stages of trees (Eyles et al. 2010).

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Further, it is difficult to compare different systems because their relationships usually depend on the interacting organisms, the intensity of damage, and the time since induction (Herms and Mattson 1992, Eyles et al. 2007, Colgan and Erbilgin 2011). Nevertheless, those studies clearly demonstrated indirect interactions between species based on the initial damage they caused to trees. In some cases, 1 organism lowered the host suitability to a subsequent organism (Kosaka et al. 2001, Eyles et al. 2007), whereas other researchers found increased host susceptibility to subsequent attackers (Raffa et al. 1998, Wallin and Raffa 2001). For example, defoliation by the pine looper (*Bupalus piniaria* L.) resulted in a strong decline in the resistance of Scots pine to the blue-stain fungus *Leptographium wingfieldii* (Långström et al. 2001). Trees in the lowest-defoliation classes were less susceptible to *L. wingfieldii* than those in higher-defoliation classes.

An overwhelming majority of such studies focused on interactions between a few organisms at the same or different trophic levels, and roles of plant-mediated interactions among a community of organisms have seldom been documented, although in nature, trees serve as foci for communities of insects and diseases. This study provides an example of plant-mediated interactions in naturally occurring groups of organisms in natural oak (*Quercus* spp.) forests.

We focused on interspecific interactions between a native tree disease, oak powdery mildew (*Erysiphe alphitoides*), and a community of native oak gall-forming wasps (Hymenoptera: Cynipidae). We were particularly interested in whether prior infection of oaks by *E. alphitoides* influenced the spatial abundance and richness of oak gall-forming wasps on these oaks.

Large populations of many western Palearctic species, including oaks, are commonly found in Eastern Europe, Turkey, the Caucasus, and Iran (Hewitt 1999). In the northern, southern and western Iran, *Q. pubescens* Willd, *Q. cerris* L., *Q. infectoria* Olivier, and *Q. macranthera* Fisch are predominant; while junipers and oak forests such as *Q. infectoria*, *Q. brantii*, and *Q. pubescens* are predominant in the eastern region (Zargaran et al. 2008).

Oaks are reported to be primary hosts for a larger number of plant pathogens and insect herbivores (Stone et al. 2002). Among pathogens, powdery mildew fungi infestations, including species of *Erysiphales* are very common (Braun 1995). Detailed on world-wide distributions of

powdery mildew species were reported by Farr and Rossman (2010). *Erysiphe alphitoides* is a common fungal disease that appears on many oak species (Griffon and Maublanc 1912, Mougou-Hamdane et al. 2010).

Oaks are also commonly attacked by gall-forming insects (Ronquist and Liljeblad 2001). Cynipid wasps (Hymenoptera) are the 2nd most diverse family after cecidomyiid midges, and the majority of cynipid wasps are obligate parasites on oaks (Stone et al. 2002). There are about 1300 species of cynipid oak gall-forming wasps globally, with the majority occurring in the Nearctic (Cornell 1983, Ronquist and Liljeblad 2001, Stone et al. 2002).

Several studies documented the abundance and richness of gall wasps with respect to the richness and abundance of host plants (Chodjai 1980, Starzomski et al. 2008, Zargaran et al. 2008), plant quality (Genimar-Reboucas et al. 2003, Egan and Ott 2007), other herbivorous insects and natural enemies (Veldtman and McGeoch 2003, Cuevas-Reyes et al. 2004, Prior and Hellmann 2010), and abiotic factors, such as water stress (Stone et al. 2002). Few studies focused on community-level interactions in gall-forming insects. For example, Nakamura et al. (2003) demonstrated that gall-formers had a positive plant-mediated effect on other insect herbivores and reported that the stem gall midge *Rabdophaga rigidae*, and adults of 2 leaf beetles, *Plagiodera versicolora* and *Smaragdina semiaurantiaca*, on *Salix eriocarpa* were more abundant on lateral shoots and leaves of galled shoots than on ungalled shoots, respectively. However, roles of other organisms, particularly diseases, on cynipids are largely unstudied (Foss and Rieske 2004). Further, how gall-forming species are locally distributed and what biological factors affect their local distributions (Veldtman and McGeoch 2003) are largely unknown, given that the suitability of oviposition sites has the potential to generate indirect interspecific competition between gall-forming insects and other species (Stone et al. 2002).

In this study, we attempted to determine whether prior *E. alphitoides* infestation of oak trees affected the community of oak gall-forming wasps. Specifically, we addressed whether gall-forming wasp abundance and diversity were affected by *E. alphitoides* infestations.

MATERIALS AND METHODS

Study sites

Sampling was performed in West Azerbaijan Province, Iran in 2009-2010 (Table 1).

Studied species

Chodjai (1980) reported 36 oak gall wasp species associated with the oak *Q. infectoria* from Iran. Recent surveys were conducted on the cynipid fauna of Iran (Tavakoli et al. 2008, Zargaran et al. 2008) and according to the latest results, so far 82 species of oak gall wasps were recorded in oak forests of Iran, of which 25 species were reported for the 1st time (Sadeghi et al. 2010). Those surveys confirmed that the cynipid fauna of Iran includes widespread western Palaearctic species such as *Andricus kollari* (Hartig) and *Cynips quercusfolii* (Hartig) (Chodjai 1980, Zargaran et al. 2008). Oak powdery mildew *Erysiphe alphitoides* L. was reported on *Q. infectoria* Oliv. for the 1st time in Iran (Tavanaei 2006).

Sampling methods

At each site, oak cynipid galls were collected from *Q. infectoria* in late Sept. when the maximum activity of *E. alphitoides* occurred and the development of cynipid galls was completed. The optimal number of trees (sample unit) per site was determined according to Southwood and Henderson's (2000) formula of $N = [(t \times s) / (D \times m)]^2$, where t is Student's t -test from standard statistical tables, D is the predetermined confidence limit for estimation of the mean as a decimal, m is the sampling mean and s is the standard deviation. Based on this analysis, the

optimal number of trees was determined to be 20 per site. Twenty trees infected with *E. alphitoides* and 20 trees without infection were surveyed at each site and in each of 2 consecutive years. All cynipid galls were counted on 4 randomly selected branches per tree. Galls found on plant surfaces (branches and leaves) were identified based on their morphology.

Statistical analysis

The Shannon-Weiner diversity index uses the following formula:

$$\text{Shannon's } H' = - \sum_{i=1}^{N_0} [p_i \cdot \log p_i]$$

where p_i is the proportion of the total number of individuals belonging to a morphotype, and N_0 is the total number of morphotypes seen in that sample. Simpson's diversity index is calculated using the following formula:

$$\text{Simpson's } D = 1 - \sum_{i=1}^N \frac{n_i(n_i - 1)}{N(N - 1)}$$

where n_i is the number of individuals of a particular morphotype and N is the total number seen in the sample (Magurran 2004).

Diversity indices like the Shannon's entropy ("Shannon-Wiener index") and the Gini-Simpson index are not in themselves diversities. The number of equally-common species required to impact a particular value to an index is called the "effective number of species". This is the true diversity of the community. Converting indices to true diversities gives them a set of common behaviors and properties. After conversion, diversity is always measured in units of the number

Table 1. Characteristics at 4 study sites selected to investigate the effect of powdery mildew infestation on oak gall wasp species diversity and richness on oaks in West Azerbaijan Province, Iran in 2009-2010

Characteristic	Site			
	Ghabre-hossein	Mirabad	Rabat	Dare-ghabr
Quercus species	<i>Q. infectori</i> <i>Q. brantii</i> <i>Q. libani</i>	<i>Q. infectoria</i> <i>Q. brantii</i>	<i>Q. infectoria</i> <i>Q. brantii</i>	<i>Q. infectori</i> <i>Q. brantii</i> <i>Q. libani</i>
Latitude	36°28'N	36°15'N	36°14'N	36°11'N
Longitude	45°18'W	45°22'W	45°33'W	45°24'W
Weather	Very humid and cold	Very humid and cold	Humid, mildly cold	Humid, mildly cold

of species (Jost 2006). Conversion of common indices to true diversities can be achieved as described in table 2.

Evenness, the other information-statistical index, is affected by both the number of species and their equitability or evenness compared to a community's actual diversity, and the value of E is constrained to 0-1.0. Shannon's evenness is calculated by the formula: $H'/H_{\max}$.

Beta diversity is generally thought of as the change in diversity among various alpha diversities (variation in species composition among sites in a geographic region) (Koleff et al. 2003, Magurran 2004). The classical Sorensen index is based on both the number of species present in the total sample and numbers only seen in each individual sample (Koleff et al. 2003). Sorensen's measure is regarded as one of the most effective presence/absence similarity measures. The Sorensen similarity index is calculated by $C_s = 2a/(2a + b + c)$, where a is the number of species common to both sites, b is the number of species at site B but not at A, and c is the number of species at site A but not in B (Magurran 2004). It is used when research is conducted on more than 1 site and begins with a table or matrix giving the similarity between each pair of sites (using any similarity coefficient). The 2 most similar sites are combined to form a single cluster. The analysis then proceeds by successively combining similar sites until all are combined into a single cluster (a dendrogram). Cluster analysis measured using the hierarchical cluster and cluster method are based on Ward's method. Sorensen's similarity index value was used in a cluster analysis to illustrate similarity patterns at the 4 sites. Also, data were analyzed with an independent-samples t -test to compare mean numbers of galls occurring on infected and uninfected trees. The surface of the infected leaves was measured by a leaf area meter and Pearson's correlation coefficient was used to test the relationship between percent leaf infection and number of leaf galls.

RESULTS

At 4 sites, 25 species of oak gall wasps (asexual generation) were collected and identified as the following species groups: *Andricus* (20 species), *Cynips* (3 species), and *Neuroterus* (2 species) (Table 3). Overall, stem gall wasps were more abundant (20) than leaf gall wasps (5). All stem gall wasps belonged to a single genus, *Andricus*. Leaf-causing gall wasps were members of *Cynips* and *Neuroterus*. The *Andricus* species group had the highest abundance among species groups collected from oaks.

Distributions of oak gall wasps among sites differed (Table 3). Ghabre-hosseini had the highest number of species among sites, with 21 species in 2009 and 17 species in 2010. Mirabad had the lowest species abundance, with 6 in 2009 and 5 in 2010. Naturally some species overlapped between sites. There was a slight decline in species abundances from 2009 to 2010.

The highest and lowest number of *Andricus* species were observed at Ghabre-hosseini (16) and Mirabad (4), respectively, in 2009 (Table 3), whereas in 2010, the highest number of *Andricus* species was found at Dare-ghabr (13) and the lowest number was collected at Mirabad (3) (Table 3). All species belonging to the genera *Cynips* and *Neuroterus* were only found at Ghabre-hosseini.

Table 3 also shows the distributions of species between infected and uninfected oak trees at 4 sites. Two *Cynips* species (*C. quercus* and *C. quercusfolii*) and 2 *Neuroterus* species (*N. numismalis* and *N. quercus-baccarum*) were commonly found on uninfected trees, occasionally found on slightly infected trees, and virtually absent from highly infected trees. Other species, exclusively stem galls, were found on both infected and uninfected trees; however, they were more commonly found on infected trees. It was interesting to note that between *C. quercus* and *C. quercusfolii*, the latter was overall more abundant. Likewise between *N. numismalis* and *N. quercus-*

Table 2. Conversion of common indices to true diversities

Index x	Diversity in terms of x	Diversity in terms of p_i
Shannon entropy $x \equiv - \sum_{i=1}^N p_i \ln p_i$	$\exp(x)$	$\exp(- \sum_{i=1}^N p_i \ln p_i)$
Gini-Simpson index $x \equiv 1 - \sum_{i=1}^S p_i^2$	$1/(1-x)$	$1 / \sum_{i=1}^S p_i^2$

baccarum, the later was more abundant. Several stem gall wasps and 1 leaf gall wasp, *C. cornifex*, were found equally on infected and uninfected trees. None of the leaf gall wasps were found together on the same leaf in the current study.

Overall, oak trees with oak powdery mildew infection had reduced richness and diversity of oak gall wasps (Table 4). The highest species richness was found on uninfected trees in all study sites in the 2 consecutive years. Gini-Simpson indices were 1.75 in 2009 and 1.71 in 2010 for infected and 2.33 in 2009 and 2.13 in 2010 for

uninfected trees. Gini-Simpson indices were lower in 2010 compared to values in 2009. Among sites, uninfected trees at Ghabre-hosseini had the highest Gini-Simpson indices in both years, followed by Dare-ghabr and Rabat. Mirabad had the lowest Gini-Simpson indices. Likewise, Shannon's H index indicated that uninfected trees had the highest oak gall wasp diversity compared to infected trees at all sites (Table 4). Differences among sites were similar to the Gini-Simpson index, with Ghabre-hosseini having the highest Shannon's indices, followed by Dare-ghabr and

Table 3. Gall wasps species associated with infected and uninfected oak trees in 2009 and 2010. We collected 25 gall wasps species in this research from 4 sites. The presence and absence of any gall wasps are shown by (+) and (-), respectively. The 1st sign indicates that the specimen was either present on (+) or absent from (-) uninfected trees, while the 2nd sign indicates that the specimen was either present on (+) or absent from (-) infected trees. For example, *Andricus aries* was present on uninfected trees (+), but absent from infected trees at Ghabre-hosseini in 2009

Gall wasp species	Site							
	Ghabre-hosseini		Mirabad		Rabat		Dare-ghabr	
	2009	2010	2009	2010	2009	2010	2009	2010
Stem gall								
<i>Andricus aries</i> ²	+ (-)	+ (-)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)
<i>A. askewi</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	+ (-)	+ (-)
<i>A. caputmedusae</i> ²	+ (+)	+ (+)	- (-)	- (-)	+ (+)	+ (+)	- (-)	- (-)
<i>A. conglomerates</i> ²	+ (-)	+ (-)	- (-)	- (-)	- (-)	- (-)	+ (-)	+ (+)
<i>A. coriarius</i> ²	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)
<i>A. galeatus</i> ²	+ (+)	+ (-)	- (-)	- (-)	- (-)	- (-)	+ (-)	+ (-)
<i>A. hystrix</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>A. kollari</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (-)
<i>A. lucidus</i> ²	- (-)	- (-)	+ (+)	+ (+)	- (-)	- (-)	+ (+)	+ (+)
<i>A. mediterraneae</i> ²	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)
<i>A. megalucidus</i> ²	+ (+)	- (-)	+ (+)	+ (+)	- (-)	- (-)	+ (+)	+ (+)
<i>A. panteli</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (-)
<i>A. polycerus</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	+ (+)	- (-)
<i>A. quercuscalicis</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)
<i>A. quercustozae</i> ²	+ (+)	- (-)	+ (+)	- (-)	+ (+)	+ (+)	+ (-)	- (-)
<i>A. seckendorffi</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>A. sternlichtii</i> ²	+ (-)	+ (-)	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)
<i>A. theophrastea</i> ²	+ (+)	- (+)	- (-)	- (-)	+ (+)	- (+)	- (-)	- (-)
<i>A. tomentosus</i> ²	- (-)	- (-)	- (-)	- (-)	+ (+)	+ (+)	+ (+)	+ (+)
<i>A. megatruncicolus</i> ²	+ (+)	- (-)	+ (+)	+ (+)	+ (+)	+ (+)	- (-)	- (-)
Leaf gall								
<i>Cynipis cornifex</i> ²	+ (+)	+ (+)	- (-)	- (-)	- (-)	- (-)	- (-)	- (-)
<i>C. quercus</i> ¹	+ (-)	+ (-)	+ (-)	+ (+)	+ (-)	+ (-)	- (-)	- (-)
<i>C. quercusfolii</i> ¹	+ (-)	+ (-)	+ (-)	+ (-)	+ (-)	+ (-)	+ (-)	+ (-)
<i>Neuroterus numismalis</i> ¹	+ (-)	+ (-)	- (-)	- (-)	+ (+)	+ (-)	+ (-)	+ (-)
<i>N. quercus-baccarum</i> ¹	+ (-)	+ (-)	- (-)	- (-)	+ (-)	+ (-)	+ (-)	+ (-)

¹Species commonly found on uninfected trees, rarely found on lightly infected trees, and virtually absent from highly infected trees.

²Species commonly found on both infected and uninfected oak trees.

Rabat. Mirabad had the lowest Shannon's indices. An increase in either the Gini-Simpson index or Shannon's H index reduced the evenness of gall wasps (Table 4). The highest and lowest species evenness values were found at Mirabad and Ghabre-hosseini, respectively.

Cluster analysis dendrogram are shown in figures 1 and 2. Dendrograms cluster sites according to how strongly correlated the sites are, and if sites are highly correlated, they will have a correlation value of 1 or close to 1. In the current study, the highest value of the Sorensen similarity between Dare-ghabr and Ghabre-hosseini was 0.72 for uninfected trees in 2009, while the similarity between these 2 sites measured from infected trees was 0.41. The lowest index of similarity was recorded between Rabat and Dare-ghabr on infected trees in 2009. In 2010, Sorensen similarity indices of infected and uninfected trees at Ghabre-hosseini and Rabat were 0.27 and 0.40

respectively. The mean number of oak galls was generally higher on uninfected trees than infected trees at all sites in 2 the consecutive years (Table 5). Trees infected with powdery mildews showed the lowest mean number of cynipid galls. Among uninfected trees, the mean number of galls on uninfected trees ranged from 14.8 at Rabat in 2010 to 61.2 at Ghabre-hosseini in 2009, while the same means for infected trees ranged from 6.2 at Rabat to 25.4 at Ghabre-hosseini in 2009. The maximum and minimum uninfected: infected ratios were 2.8 and 2.1 in Dare-ghabr in 2009 and 2010, respectively.

Pearson's correlation coefficients between the number of galls and percent infection showed significant negative correlations in 2009 ($r = -0.714$, $n = 20$, $p < 0.01$) and 2010 ($r = -0.581$, $n = 20$, $p < 0.01$) (Fig. 3). On infected oak trees, leaf gall abundances declined with increasing levels of powdery mildew.

Table 4. Species diversity indices and the true diversity of oak gall wasps in 2009 and 2010

Sites	Diversity indices						
	Simpson D	Species richness	Gini-Simpson	True diversity	Shannon's H'	True diversity	Evenness
Infected oak trees (2009)							
Ghabre-hosseini	0.9012	11.95	2.48	13.21	0.91	14	0.08
Dare-ghabr	0.9104	7.38	1.99	9.95	0.89	11	0.10
Rabat	0.9188	3.91	1.36	5.33	0.81	6	0.19
Mirabad	0.9342	3.21	1.16	3.49	0.71	4	0.29
All sites pooled		6.61	1.75	8.00	0.84	8.7	0.16
Uninfected oak trees (2009)							
Ghabre-hosseini	0.8958	18.36	2.91	19.65	0.95	21	0.05
Dare-ghabr	0.9197	16.96	2.83	17.21	0.94	18	0.06
Rabat	0.9176	7.32	1.99	7.79	0.87	9	0.13
Mirabad	0.9419	4.86	1.58	5.27	0.81	6	0.19
All sites pooled		11.875	2.33	12.48	0.89	13.5	0.11
Infected oak trees (2010)							
Ghabre-hosseini	0.8311	8.86	2.18	8.12	0.89	10	0.11
Dare-ghabr	0.8422	8.29	2.12	8.42	0.88	9	0.12
Rabat	0.9140	3.98	1.38	4.39	0.77	5	0.23
Mirabad	0.9205	3.25	1.18	3.29	0.69	4	0.30
All sites pooled		6.095	1.71	6.06	0.81	7	0.19
Uninfected oak trees (2010)							
Ghabre-hosseini	0.8957	12.72	2.54	16.27	0.94	17	0.06
Dare-ghabr	0.9114	11.46	2.44	15.48	0.94	16	0.06
Rabat	0.9272	8.79	2.17	7.04	0.85	8	0.14
Mirabad	0.9380	3.86	1.35	4.31	0.77	5	0.24
All sites pooled		9.21	2.13	10.78	0.87	11.5	0.13

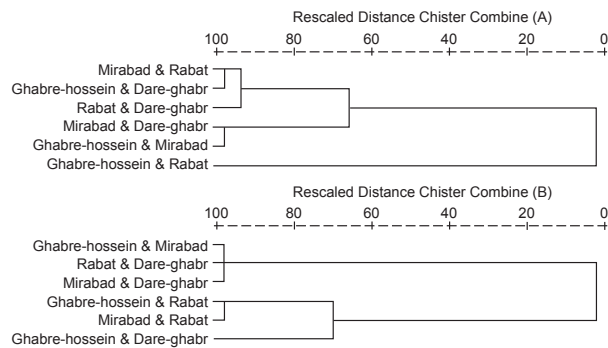


Fig. 1. Sorensen cluster analysis dendrogram of similarity coefficients for oak gall wasps occurring on infected (A, above) and uninfected (B, below) trees in 2009.

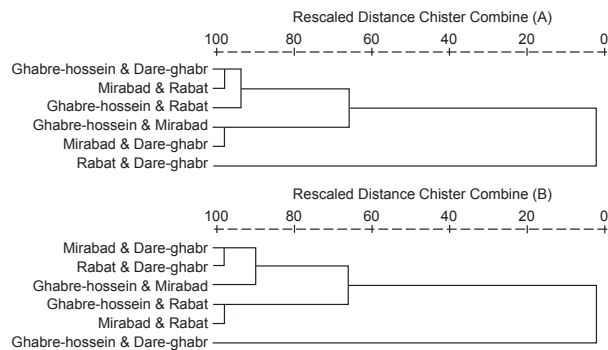


Fig. 2. Sorensen cluster analysis dendrogram of similarity coefficients for oak gall wasps occurring on infected (A, above) and uninfected (B, below) trees in 2010.

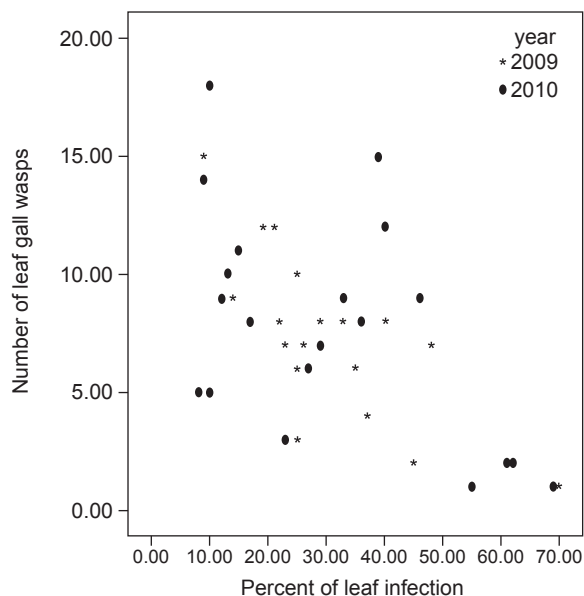


Fig. 3. Correlation between the number of leaf gall wasps and percent of leaf infection by oak powdery mildew on oaks in 2009 and 2010. An increased percent of disease infection led to a decrease in leaf oak gall wasp numbers.

DISCUSSION

Our results clearly demonstrated an indirect plant-mediated interaction between *Erysiphe alphitoides* and a community of cynipid oak gall-forming wasps, and we found that pathogen infection significantly reduced the abundance and species richness of the native oak gall wasps. Although there were differences among sites, the highest and lowest abundance and richness values were always respectively associated with healthy and diseased oak trees, at any given site.

This is the 1st study to demonstrate plant-mediated interactions between a leaf pathogen and a community of gall-forming wasps. It was commonly reported that pathogen or insect attacks can affect the composition of insect and pathogen communities associated with plants and mediate the incidences and abundances of subsequent attackers (Stout et al. 2006, Eyles et al. 2010). In the current study, the mechanism of the plant-mediated interaction between *E. alphitoides* and gall-forming wasps is not known although, based on earlier publications on cynipid gall wasps, we

Table 5. *t*-test comparison of the mean number ($\pm$ S.E.) of oak galls per trees between infected and uninfected oak trees in 2009 and 2010. A significant difference was accepted at < 0.05

Site	Year	Mean number ($\pm$ S.E.) of oak galls per tree	
		Uninfected	Infected
Ghabre-hosseini	2009	61.2 (± 5.3)	25.4 (± 6.1)
	2010	32.4 (± 7.1)	17.3 (± 3.2)
Dare-ghabr	2009	54.2 (± 8.2)	19.4 (± 11.1)
	2010	38.4 (± 1.8)	18.3 (± 4.3)
Mirabad	2009	22.5 (± 9.4)	10.4 (± 5.8)
	2010	16.9 (± 2.7)	7.1 (± 4.6)
Rabat	2009	20.3 (± 1.4)	8.7 (± 3.3)
	2010	14.8 (± 6.3)	6.2 (± 2.4)

Site	Year	Statistics	
		<i>t</i> -value	<i>p</i> -value
Ghabre-hosseini	2009	20.16	0.002
	2010	12.23	0.036
Dare-ghabr	2009	19.05	0.001
	2010	15.64	0.026
Mirabad	2009	14.52	0.031
	2010	9.37	0.048
Rabat	2009	7.78	0.043
	2010	8.44	0.029

suspect that *E. alphitoides* can influence gall-formers in 2 possible ways. First, *E. alphitoides*-infection of oak trees most likely systemically changes the host plant suitability, particularly host nutrients and host secondary compounds, as chemical interactions between gall-formers and their host plants are important for both the success and avoidance of gall formation. For example, nutrients of plant tissues play critical roles in the selection of oviposition sites and subsequent gall development (Hartley 1998, Stone and Schönrogge 2003). Female cynipid gall wasps prefer host tissues with high nutritional quality (Stone et al. 2002), and it is likely that an *E. alphitoides* infection may diminish nutritional substances in oak tissues (Stone and Schönrogge 2003). Decreasing nitrogen or increasing carbon, due to increased metabolism of carbon-based metabolites such as tannins and lignin (Scriber and Slansky 1981, Wold and Marquis 1997) or increases in photosynthesis (Bagatto et al. 1996) may alter carbon: nitrogen (C:N) ratio of plant tissues. Scriber and Slansky (1981) suggested that tissues with high C: N ratios provide low-quality food for developing immature wasps inside galls. Additional investigations in this system should focus on changes in plant nutritional quality due to *E. alphitoides* infestations to fully understand interactions between the disease and cynipid oak gall wasps.

Further, our study cannot rule out the role of secondary metabolites, particularly tannin levels, in reducing the abundance and richness of cynipid gall wasps in diseased oak trees. Tannin is a phenolic compound used for defense against a variety of organisms and is also induced by pathogen infestation (Stone et al. 2002). Tannins in cynipid galls are known to be concentrated in the outer layers, where they may protect the gall from endophytic fungi (Taper et al. 1986, Taper and Case 1987, Wilson and Carroll 1997). For example, the endophytic fungus *Discula quercina* (Coelomycetes) was shown to cause almost 100% cynipid gall wasp mortality in artificial infection experiments (Wilson and Carroll 1997). Tannins may also protect gall-formers against parasitoids (Cornell 1983, Taper and Case 1987). This close association of cynipid gall wasp and tannin levels could explain the observed positive relationships of oak tannin levels with cynipid diversity and abundance (Taper and Case 1987, Wold and Marquis 1997, Stone et al. 2002).

Although we do not know how different severities of *Erysiphe alphitoides* infestation affect tannin contents, we suspect that pathogen

infection either increases tannin contents in all tissues such that high tannin contents in the inner layers of galls might not be suitable for the developing larvae, or significantly reduces tannin contents such that developing larvae are not protected from endophytic fungi, or a combination of both, as tannin content are very likely to vary with the severity of pathogen infection (Bonello et al. 2006).

A 2nd possible alternative to explain the plant-mediated interaction between *E. alphitoides* and cynipid gall wasps is that the presence of a fungus may prohibit oviposition by female wasps, as we observed that hyphae of *E. alphitoides* covered the surface of host leaves such that females could not lay eggs. An *E. alphitoides* infestation on leaves initially appears as light-green to yellow spots. As the disease severity progresses, spiderly or threadlike white patches typically develop with scattered small, black fruiting bodies. The presence of an infestation of plant tissues by *E. alphitoides* could also indirectly increase competition for suitable oviposition sites among leaf gall wasps (Gilbert et al. 1994). However, this avoidance mechanism might only explain the reduction in leaf gall wasp diversity and species richness, not stem galls, as *E. alphitoides* is only present on oak leaves.

Plant-mediated interactions between pathogens and herbivorous insects were commonly reported in other systems (Krause and Raffa 1992, Felton and Korth 2000, Eyles et al. 2007). For example, Krause and Raffa (1992) found that infection of larch *Larix decidua* with the fungal pathogen *Mycosphaerella laricina* induced a systemic reduction in host quality for the larch sawfly *Pristiphora erichsonii*. Likewise, Eyles et al. (2007) reported that an infection by *Diplodia pinea* elicited resistance against the defoliating European pine sawfly *Neodiprion sertifer* in Austrian pine *Pinus nigra*. In our system, plant defensive responses apparently seem to be operating against only the gall wasp community and not *E. alphitoides* because we observed continuous colonization of the same oak trees by *E. alphitoides* after an initial infection. This suggests a possible “cross-talk” between defensive pathways against *E. alphitoides* (associated with salicylic acid) and cynipid gall wasps (associated with jasmonic acid) in oaks (Bostock 2005, Heil and Ton 2008). Even though we do not have a complete understanding of the defensive pathways induced in oaks by either oak gall wasps or *E. alphitoides*, further studies of oak systems should

identify these pathways and determine whether induction of 1 pathway prevents synthesis of the other, thereby leading to an impaired capacity of a plant to respond to either pathogen infection or insect damage (Bostock 2005).

We currently do not know why the abundance and species diversity of gall wasps were higher at the Ghabre-hosseini and Dare-ghabr sites compared to the others. Despite differences in climate, both sites have similar vegetative cover and similar species abundance and richness levels of cynipid gall wasps. Despite their similarities in climate, Ghabre-hosseini and Mirabad had different vegetative cover, and the former had a larger species complex. This suggests that the distribution of host plant species may be highly critical for determining patterns of herbivore abundances (Starzomski et al. 2008) along with factors like climate and phenological synchrony of herbivores with host plants. This is not surprising considering the fact that the abundance and richness of gall wasps are related to the richness and abundance of host plant species (Starzomski et al. 2008). Likewise, the species richness of oak gall wasps in Mexico was highly correlated to their host plants (Cuevas-Reyes et al. 2004). Further, Stone et al. (2002) suggested that geographical differences in the oak gall wasp fauna were related to oak distribution patterns in different regions. The current study also added further complexity to host plant-gall wasp interactions; i.e., the role of plant pathogens in the spatial distribution of herbivorous insects. Specifically, as indicated by the cluster analysis dendrogram, although the Sorensen similarity value for uninfected trees was fairly high between Dare-ghabr and Ghabre-hosseini (0.72), *E. alphitoides* infestations dramatically reduced the similarity index between these 2 sites to 0.41.

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