Plant Ecol (2019) 220:777–788
https://doi.org/10.1007/s11258-019-00954-9

Spatial heterogeneity of gall formation in relation to chemotype distribution in *Thymus vulgaris*

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Received: 21 March 2019 / Accepted: 28 June 2019 / Published online: 5 July 2019

Abstract The utilization of host plants by herbivorous insects depends on plant traits and physical environment. The purpose of the present work is to test the hypothesis that spatial variation in the presence of galls of the specialist fly *Janetiellathymicola* in natural populations of its host plant *Thymus vulgaris* differ in relation to spatial variation in chemotype presence. We quantified gall infection rates in 59 populations that differ in chemotype presence across a sharp ecological gradient in the South of France. We also quantified spatial aggregation of galls and plants and made a 3-year study of infection, biomass and plant survival in three populations. The proportion of galled plants was significantly higher in populations with non-phenolic chemotypes on deeper soils in sites with cold winter temperatures than in populations of phenolic chemotypes on stony soils in sites with mild winters. In a population with two non-phenolic chemotypes, galls were significantly more present on plants of the chemotype with the highest proportion of galls in the multi-population survey. In a 3-year study, galled plants had a significantly greater probability of being infected by a subsequent generation than non-galled plants. This probability declined with distance. Galls absorbed a significant proportion of vegetative biomass on a shoot, but their presence was unrelated to survival. Host plant chemistry, habitat characteristics and dispersal limitation conjointly affect this host–parasite interaction.

Keywords Parasitism · Gall · Chemical polymorphism · Dispersal limitation · Mediterranean

Communicated by Thomas Abeli.

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Introduction

Due to an intimate biological relationship, specialist gall-making parasites have a unique ecological relationship with their host plants. Gall formers are often highly specific and the benefits of a food resource and protection may go primarily to the gall former (Redfern 2011). Gall forming is thus generally antagonistic in nature. However, in some cases, the presence of the gall former may benefit the plant, e.g. enhanced

freezing resistance (Rocha et al. 2013). The nature of the relationship may thus shift from antagonism to mutualism depending on environmental variation and plant traits (Thompson 1994).

The fact that a change of host is impossible for a given generation of larvae makes the initial choice of a host plant for oviposition a critical stage in the life cycle of gall formers. Genetic variability in host–plant traits, which make the plant more or less suitable for larval development, can have a marked influence on gall formation and larval development (McCrea and Abrahamson 1987; Akimoto 1990; Horner and Abrahamson 1992; Cronin and Abrahamson 2001; Egan and Ott 2007; Evans et al. 2012). Here, secondary compounds can play an important role in plant defence and influence the choice of laying sites (Tija and Houston 1975; Zucker 1982; Hartley 1998; Abrahamson et al. 1991). In addition, although the gall is plant tissue, the insect can modify its form and composition (Weis and Abrahamson 1986; Hartley 1998), and the concentration of defence compounds in galls can be markedly higher in the gall than elsewhere on the plant (Abrahamson et al. 1991; Hartley 1998). Hence, the secondary compounds may be used by the parasite to further enhance its own defence.

Finally, galling insects occupy a habitat defined by the presence of its resource, as in specialist Lepidoptera (Dennis et al. 2003), and which resembles a spatial mosaic of habitable patches where the host plant is embedded in a matrix of uninhabitable patches comprised of other plant species (Overton 1994). As a result, in addition to variability of the local ecological context there may be marked dispersal-related patterns of infestation. For example, McCrea and Abrahamson (1987) showed that gall presence on *Solidago altissima* may be closely linked to spatial proximity of host plants and dispersal limitation of flies of the stem galler *Eurosta solidaginis*.

Thymus vulgaris L. (Lamiaceae) populations in southern France contain one or more of seven different genetically determined chemotypes (Granger and Passet 1973; Gouyon et al. 1986; Thompson 2002; Keefover-Ring et al. 2009). Two chemotypes have an essential oil dominated by a phenolic monoterpene (thymol and carvacrol), and five have non-phenolic monoterpenes (linalool, 1,8-cineole, thuyanol, α-terpineol and geraniol). The monoterpenes originate along the same biosynthetic pathway (Passet 1971; Croteau 1987) in association with an epistatic chain of five loci (Vernet et al. 1986). The chemotypes show marked spatial segregation associated with local adaptation to climatic conditions (Amiot et al. 2005; Thompson et al. 2007). Phenolic chemotypes tend to be better defended against a range of potential generalist herbivores and grass competitors (Linhart and Thompson 1995, 1999; Tarayre et al. 1995; Ehlers and Thompson 2004; Linhart et al. 2015; Ehlers et al. 2016).

In addition, *T. vulgaris* is the host plant for a specialist Diptera, *Janetiella thymicola* (Cecidomyiideae) that lays its eggs in meristematic tissues, stimulating gall formation in which 1–8 larvae develop. Adults are thought to be short-lived, making the choice of a host plant critical. Up to three generations per year can occur depending on environmental conditions, as reported for closely related *J. brevicauda* (Clark and Dennehy 1989).

The spatial segregation of *T. vulgaris* chemotypes offers a novel opportunity to test the hypothesis that a specialist gall former shows variation in its use of different genetically distinct chemotypes as a host plant. First, we test for differences in the presence and abundance of galled plants in relation to chemotype presence. Second, we quantify the spatial and temporal dynamics of gall abundance within populations. Third, we assess whether the presence of galls influences biomass allocation and seed output?

Materials and methods

Spatial variation in gall abundance

To study the spatial distribution of infection rates, we sampled populations in a regularly spaced grid in and around the St. Martin-de-Londres Basin, ~ 20 km north of Montpellier in southern France, where chemotypes of *T. vulgaris* have previously been mapped (Vernet et al. 1977a, b; Gouyon et al. 1986). The sampled area was delimited by a 6 km by 6 km grid which allowed us to sample one population/km^2 and which covered part of the area containing phenolic and non-phenolic chemotypes (Fig. 1a). Within a fairly central portion of the grid (which also contained populations of different chemotypes), where preliminary work indicated large numbers of plants with galls (Amiot 2001), we sampled one population in each of 36 squares each 0.5 km by 0.5 km (0.25 km^2).

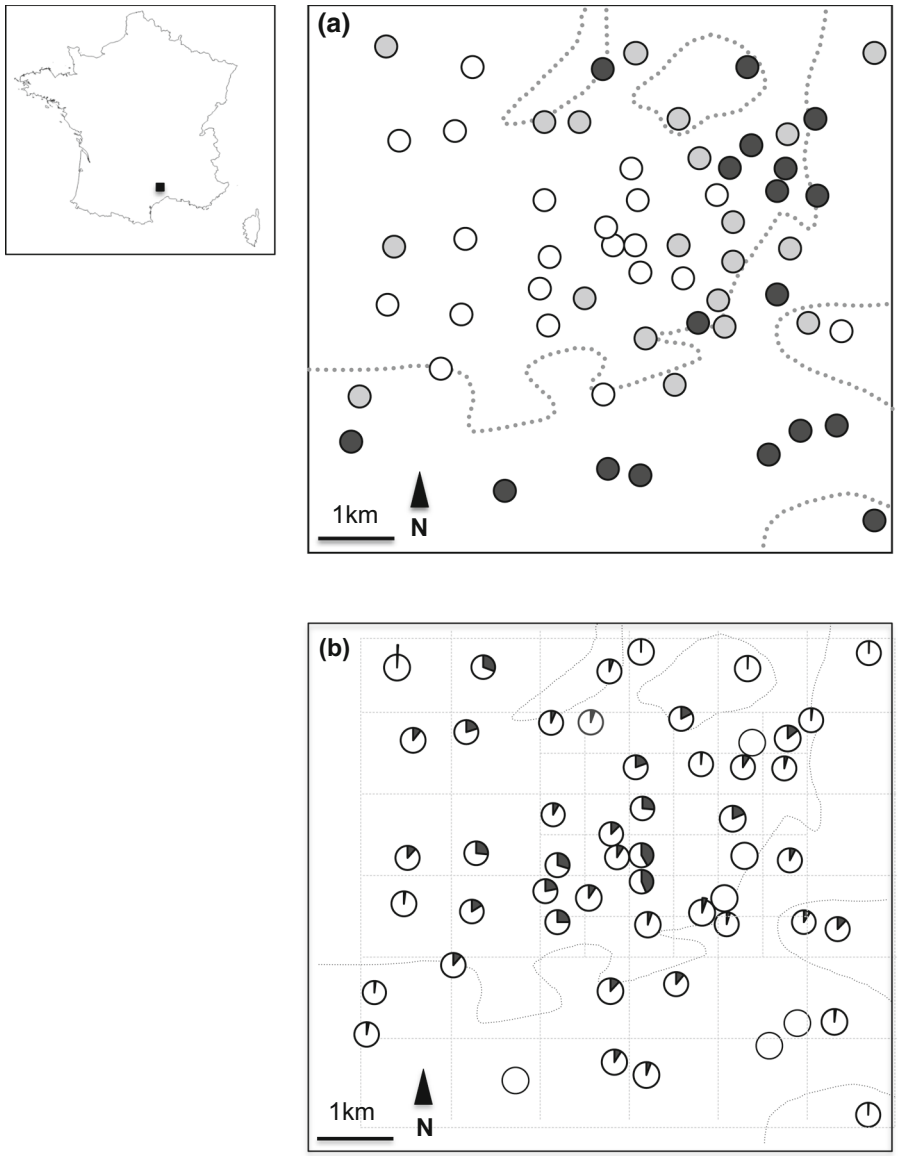

Fig. 1 The spatial distribution of sampled *Thymus vulgaris* populations in and around the Saint-Martin-de-Londres Basin. The dashed line is the 220 m contour line (mean elevation of all sampled populations). **a** The distribution of the three types of populations (phenolic—black circles, non-phenolic—open circles, and mixed phenolic and non-phenolic—grey circles). **b** The proportion of plants (black part of the circle) with at least one gall induced by *Janetiella thymicola*

This grid-sampling scheme produced a total of 63 squares for population sampling: 36 squares (0.25 km^2) in the central zone and 27 squares (1 km^2) around this central zone (Fig. 1a). In four central squares, we were unable to locate a previously sampled (or even a new) *T. vulgaris* population, hence a total of 59 populations were studied.

Most sites had been sampled previously for chemotype determination either by Vernet et al. (1977a, b) or Thompson et al. (2003). In all sites, we sampled ten plants at random to check the chemotype based on smell (by two people). In a small number of cases (of doubt), chemotype designation was verified by analytical gas chromatography (following Keefover-Ring et al. 2009). We classified three types of population depending on their majority chemotype, i.e. "phenolic", "non-phenolic" and "mixed" populations. Chemical analyses that we have done on these

populations show no evidence of changes in non-volatile compounds or other terpenoid compounds in relation to chemotype variation (Thompson et al. 2003) that could eventually have had a relationship with gall infection.

To quantify the presence and abundance of galls in each population, in April and May 2002, we mapped all *T. vulgaris* plants in fifty 0.25 m^2 quadrats regularly spaced in a grid pattern. Ten quadrats were placed in each of five transects 10 m long with each quadrat and line 0.5 m distant from one another. This design allowed us to move around quadrats and not trample plants within quadrats where counts were made. The number of plants per quadrat and the number of galls present on each plant was recorded. We thus obtained data on the density of *T. vulgaris* plants, the number of plants with at least one gall ("infected" plants) and the mean number of galls per "infected" plant for each population. Based on these data, we obtained information on the spatial distribution and aggregation of both galls and *T. vulgaris* plants. In the Pâtus-2 population (within the central zone), where infection rates were high and in which two easily distinguishable non-phenolic chemotypes were present, we recorded the precise chemotype identity (geraniol or linalool), for all counted plants.

Temporal variation in gall abundance

Three *T. vulgaris* populations within the central portion of the sampling grid were selected for the assessment of temporal variation in gall abundance over three consecutive years. These populations all had a fairly high proportion of galled plants and they had only non-phenolic plants (geraniol and linalool in population 2, geraniol in the population 5 and thuyanol and linalool in population 16). In 2001, 100 plants were chosen at random in each population, labelled with a plastic tag and their spatial location and sex (female or hermaphrodite) recorded. The number of galls per plant was recorded as plants were labelled. On each of three occasions per year, before, during and after the summer drought (i.e. early May, July and late September, respectively), all plants were observed and the number of galls per plant quantified. This was repeated each year until July 2004. Plant mortality was also recorded.

Impact of gall presence on plant performance

For nine populations, we measured and removed two shoots of comparable length, one with a single gall and the other without a gall on 12 galled plants. These shoots were sampled on different portions of the plant. Both shoots were oven-dried and weighed. On the infected shoot, the biomass of the gall and the remaining part of the shoot (once the gall had been removed) were determined.

In each of 20 populations within the central zone, 24 plants were sampled at random and labelled. The sex (female or hermaphrodite) and the size of each plant (height and maximum width) were recorded.

Finally, during the study, we discovered six plants with a carvacrol chemotype that were infected with galls and growing in a common garden at the CEFE in Montpellier where we cultivate thyme for experimental work. To provide a preliminary observation of whether galling may modify chemical composition and yield in uniform conditions we sampled two branch tips (about 0.5 cm) on each plant, one with a gall (G) and one lacking a gall (L). These samples were accurately weighed and placed in 0.5 mL microcentrifuge tubes. Terpenes were extracted with 0.4 mL of an internal standard solution and the biomass of terpenes per gram of fresh weight in the galls (G) and leaves (L) measured and gas chromatography was done to assess the proportion of carvacrol in the oil, following Keefover-Ring et al. (2009). We thus compared terpene production and oil composition for galled and un-galled stems.

Statistical analysis

The number of galled plants was analysed as a binomial response (with or without galls) using PROC GENMOD in SAS (1999–2000). The mean number of galls for plants with at least one gall was analysed in ANOVA using PROC GLM in SAS (1999–2000). In all analyses, chemotype was treated as a fixed effect and population as a random effect. A priori contrasts were used to assess differences among phenolic, non-phenolic and mixed populations. For each chemotype, we compared infection rates among populations where the chemotype was either present or absent. Spatial aggregation of thyme plants in populations containing at least five plants with galls (38 populations) was

tested using the index of clumping (ICS) proposed by David and Moore (1954) and by chi-squared analyses.

Size measurements of the plants in the field survey were used to construct an index of plant biomass, $B = (h + w)/2$ (where h is height of the plant and w its maximum width), which is correlated with plant biomass and has been used in other work on the study plant species to assess performance variation (Thompson et al. 2004). The biomass of calices and seeds of each plant was divided by the value of B for a given plant to standardize reproductive biomass. The values obtained were then plotted against numbers of galls rate in a linear regression test using PROC REG in SAS (1999–2000). The same method was applied to plot sex ratio against number of galled plants.

In the monitoring of galled and un-galled plants and their survival, the probabilities of bearing a gall and of mortality were treated as multinomial data because each plant could have one of three states at a given time: with or without a gall or mortality. We used PROC GENMOD in SAS (1999–2000) using population, previous state of infection and interaction between them as fixed factors. For 2 by 2 tables of frequency we performed chi-squared analyses of goodness of fit. The spatial pattern of variation in infection rates over time was analysed with ArcView GIS (ESRI 1999–2002). For a given generation, the distance between each plant and the closest infected plant in the previous generation, i.e. the closest source of infection, was calculated. These data were analysed in ANOVA using PROC GLM in SAS (1999–2000) with population, state of infection, and interaction between them, as fixed effects. Data from each population were then treated independently, with state of infection treated as a fixed effect.

To estimate any effect of gall development on vegetative biomass allocation, we standardized shoot and gall biomass values by dividing them by the length of the shoot. Standardized biomass values were analysed in ANOVA using PROC GLM in SAS (1999–2000) with shoot status (infected including gall versus un-infected) as a fixed effect. A Scheffé means comparison was employed to assess differences in the biomass of the gall, an infected shoot with the gall removed and an un-infected shoot.

Results

Spatial distribution gall formation

The three different types of populations we sampled occur at significantly different ($df = 2$, $F = 13.04$, $p < 0.001$) elevations due to the higher elevation of phenolic populations (Scheffé means tests) (Fig. 2a). In these sites they incur milder winters (due to a temperature inversion) and greater drought stress in summer (due to a more stony and rocky substrate). Due to the spatial distribution of the different population types (Figs. 1a, 2a), the proportion of galled

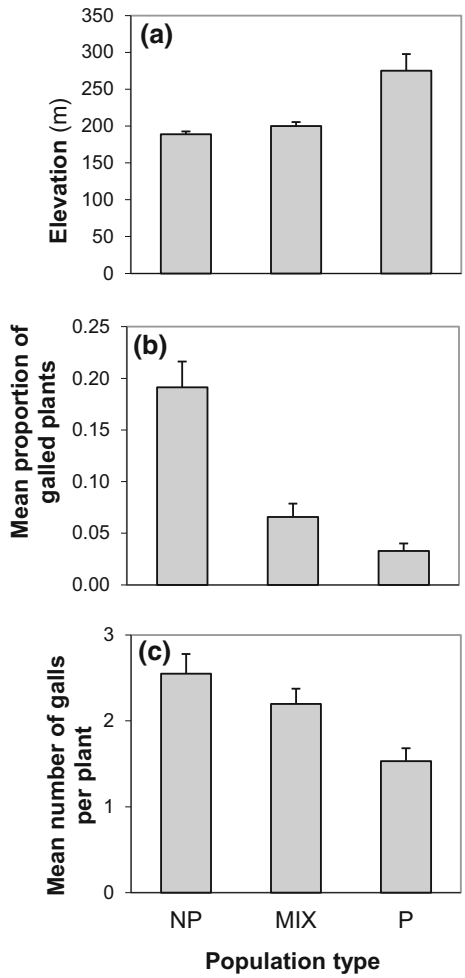

Fig. 2 Variation among non-phenolic (NP), mixed (MIX) and phenolic (P) populations of *T. vulgaris* in terms of: **a** mean site elevation (± SE), **b** mean proportion (± SE) of galled plants and **c** mean number (± SE) of galls per plant with at least one gall

plants was higher within the basin of St-Martin-de-Londres than at higher elevations around its outskirts (Fig. 1b).

The proportion of galled plants in non-phenolic populations was in fact significantly higher ($df = 1$, $\chi^2 = 312.43$, $p < 0.001$) than in mixed populations, whose proportion of galled plants was in turn significantly higher ($df = 1$, $\chi^2 = 18.86$, $p < 0.001$) than in phenolic populations (Fig. 2b). Plants in non-phenolic populations (with at least one gall) had a significantly higher mean number of galls per plant ($df = 1$, $F = 7.91$, $p < 0.01$) than phenolic plants (Fig. 2c). Mixed populations had intermediate values that were not significantly different from phenolic ($df = 1$, $F = 3.62$, $p > 0.05$) and non-phenolic ($df = 1$, $F = 1.72$, $p > 0.05$) populations.

To determine the possible influence of the presence or absence of a specific chemotype within a population on gall numbers, we compared the mean proportion of total galled plants in populations where a specific chemotype was present compared to those from which that chemotype was absent. For all chemotypes (Fig. 3), we observed a significant difference in the mean proportion of galled plants in populations where that chemotype was present compared to those from which it was absent (G: $df = 1$, $\chi^2 = 135.34$, $p < 0.001$. A: $df = 1$, $\chi^2 = 177.68$, $p < 0.001$. U: $df = 1$, $\chi^2 = 94.72$, $p < 0.001$. L: $df = 1$, $\chi^2 = 79.29$, $p < 0.001$. C: $df = 1$, $\chi^2 = 138.82$, $p < 0.001$. T: $df = 1$, $\chi^2 = 113.10$, $p < 0.001$). What is important here is that the pattern of variation was very different among chemotypes and generally reversed for phenolic and non-phenolic chemotypes (other than L). For non-phenolic chemotypes, the proportion of galled plants was greater in populations where they are present whereas for phenolic chemotypes the pattern is reversed; the proportion of galled plants in a population is greater when the chemotype is absent. Despite a similar pattern of variation, these differences were not significant for the mean number of galls per infected plant (Fig. 3).

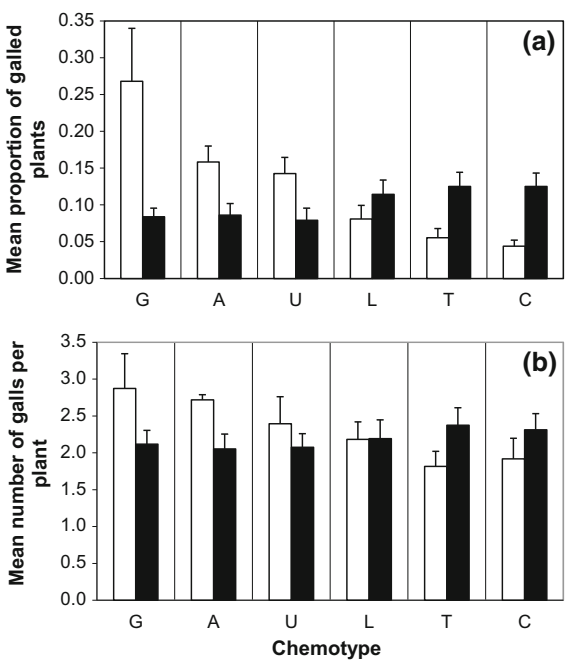

Fig. 3 Mean proportion (± SE) of galled plants and the mean number (± SE) of galls for plants with at least one gall in populations where a given chemotype of *T. vulgaris* is present (empty bars) or absent (closed bars). Chemotypes are geraniol (G), α-terpineol (A), thuyanol (U), linalool (L), carvacrol (C) and thymol (T)

For non-phenolic populations, the highest proportion of galled plants was observed in populations where the geraniol chemotype was present, followed by populations containing the α-terpineol and thuyanol chemotypes. Populations where the linalool chemotype was present had proportions of galled plants more similar to populations containing the phenolic chemotypes than those containing the other non-phenolic chemotypes. Finally, geraniol plants were significantly more often infected than linalool plants where the two chemotypes coexist at similar frequencies in population 2 ($df = 1$, $\chi^2 = 4.89$, $p < 0.05$) but there was no significant difference in the number of galls on plants with at least one gall for the two chemotypes in this population ($df = 1$, $\chi^2 = 0.18$, $p > 0.05$).

Analysis of the spatial pattern of gall occurrence in the 38 populations (18 non-phenolic, 12 mixed, 8 phenolic) that contained at least five galled plants showed that only six populations (three non-phenolic, two mixed and one phenolic) presented a significantly aggregated pattern of galled plants (Table 1); three of which also showed significant aggregation of thyme plants with or without galls. 24 of the 38 populations showed significant aggregation of all thyme plants.

Temporal variation

The previous presence of galls (time t) had a significant effect ($df = 1$, $\chi^2 = 24.7$, $p < 0.001$) on

Table 1 Numbers and percentages of galled plants and test for spatial aggregation of galled plants and all plants in 38 populations of *Thymus vulgaris* containing at least five galled plants

Population		Galled plants		Statistical analyses (χ^2)			
Code	Type	Number	Percentage	Galled plants	*df*	All plants	*df*
1	NP	31	43.7	29.1 ns	37	ns	37
2	NP	20	41.7	28.0 ns	31	ns	31
3	NP	82	30.7	70.2*	47	***	47
4	NP	39	26.5	38.3 ns	44	***	44
5	NP	49	26.5	44.0 ns	48	ns	48
6	NP	24	20.3	40.8 ns	36	***	36
7	NP	20	20.0	27.3 ns	42	ns	42
8	NP	21	19.4	29.1 ns	38	**	38
9	NP	22	16.1	56.5 ns	47	ns	47
10	NP	14	12.5	58.0*	41	ns	41
11	NP	28	12.2	42.3 ns	40	***	40
12	NP	28	11.9	63.0 ns	48	**	48
13	NP	14	11.3	46.0 ns	41	***	41
14	NP	18	10.7	35.8 ns	43	***	43
15	NP	15	9.1	67.8*	45	***	45
16	NP	8	7.4	33.0 ns	40	ns	40
17	NP	5	2.1	62.2 ns	47	***	47
18	NP	10	1.9	40.0 ns	49	***	49
19	MIX	35	18.6	46.0 ns	44	***	44
20	MIX	27	17.4	33.9 ns	46	ns	46
21	MIX	13	14.3	25.1 ns	32	***	32
22	MIX	11	11.7	59.8*	40	ns	40
23	MIX	23	11.2	50.0 ns	47	*	47
24	MIX	22	9.1	37.1 ns	49	***	49
25	MIX	10	7.6	39.0 ns	48	ns	48
26	MIX	5	5.5	58.0 ns	34	***	34
27	MIX	6	5.4	50.0 ns	41	ns	41
28	MIX	46	5.2	71.4*	49	***	49
29	MIX	12	4.7	37.0 ns	48	***	48
30	MIX	5	1.3	45.0 ns	49	***	49
31	P	7	9.3	54.3*	38	ns	38
32	P	13	9.1	45.8 ns	44	ns	44
33	P	8	7.5	35.0 ns	42	ns	42
34	P	7	5.6	34.0 ns	40	**	40
35	P	9	5.3	57.4 ns	45	***	45
36	P	8	4.5	39.0 ns	46	***	46
37	P	6	3.1	55.3 ns	45	***	45
38	P	5	1.9	42.0 ns	46	***	46

NP non-phenolic, *MIX* mixed, *P* phenolic populations

$^{ns}p > 0.05$; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$

the probability of a plant having a gall (time $t + 1$) due to the fact that previously galled plants had a relatively higher probability of being infected at the next parasite generation (proportion at time $t + 1 = 0.35$) than a non-galled plants (proportion at time $t + 1 = \ = 0.10$) (Fig. 4). All three populations showed a very similar pattern so we only present pooled results here. Un-galled plants had a probability of mortality in a given transition stage twice that of infected plants

Fig. 4 Probability of a plant being galled, un-galled or dead at generation $t + 1$, according to whether the plant was either **a** galled or **b** un-galled during the previous (t) parasite generation in three populations of *Thymus vulgaris*

(probabilities of 0.10 and 0.05 respectively, $df = 1$, $\chi^2 = 7.2$, $p < 0.01$).

At time $t + 1$, galled plants were spatially closer to previously galled plants than were un-galled plants (Fig. 5). Gall presence in a previous generation ($df = 1$, $F = 3.04$, $p < 0.001$) and population ($df = 2$, $F = 7.15$, $p < 0.001$), but not the interaction between population and gall presence ($df = 2$, $F = 1.27$, $p > 0.05$), had significant effects on spatial distance to previously galled plants. Galled ($t + 1$) plants were closer to sources of previously galled (t) plants than un-galled ($t + 1$) plants in all three populations (population 2: $df = 1$, $F = 5.34$, $p < 0.05$; population 5: $df = 1$, $F = 25.69$, $p < 0.001$; population 16: $df = 1$, $F = 8.18$, $p < 0.01$).

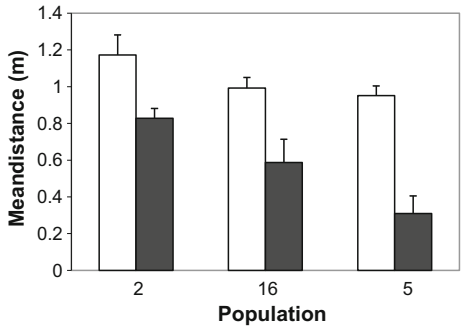

Fig. 5 Mean distance (± SE) of galled (closed bars) and un-galled (open bars) plants in a given parasite generation ($t + 1$) to the closest galled plant in the previous generation (t) in three populations of *Thymus vulgaris*

Influence of galls on biomass and chemical composition

The biomass index of galled shoots (including the gall) was significantly higher ($df = 1$, $\chi^2 = 64.2$, $p < 0.001$) than that of un-galled shoots on the same plant. However, the biomass index of galled shoots after removal of the gall was significantly lower ($df = 1$, $\chi^2 = 57.8$, $p < 0.001$) than un-galled shoots. The greater biomass allocated to galled shoots compared to un-infected shoots is thus primarily due to the biomass of the gall. There was no significant correlation between plant size and number of galls. Females and hermaphrodites showed no differences in the proportion of galled plants.

For all six carvacrol plants growing in a common garden, un-galled stem tips had more total terpenes than galls, whereas the latter had a higher proportion of the dominant monoterpene (carvacrol) and less precursors (para-cymene and ϒ-terpinene) or the non-phenolic monoterpenes that dominate the oil of other chemotypes (Fig. 6).

Discussion

Infection rates of a specialist fly *Janetiella thymicola* on plants of *Thymus vulgaris* provide a clear demonstration of spatial heterogeneity in a host–parasite interaction that is correlated with a genetically determined plant trait in a heterogeneous ecological landscape. The dispersal of the galling fly appears to be spatially limited and may elicit a response in terms of chemical deterrence.

Chemotype-associated variation in gall formation

Oviposition is a critical stage in the life cycle of gall formers. Indeed, gall-forming insects are often highly specific in their choice of individual hosts (Redfern 2011). Genetic variability in host plant traits, which

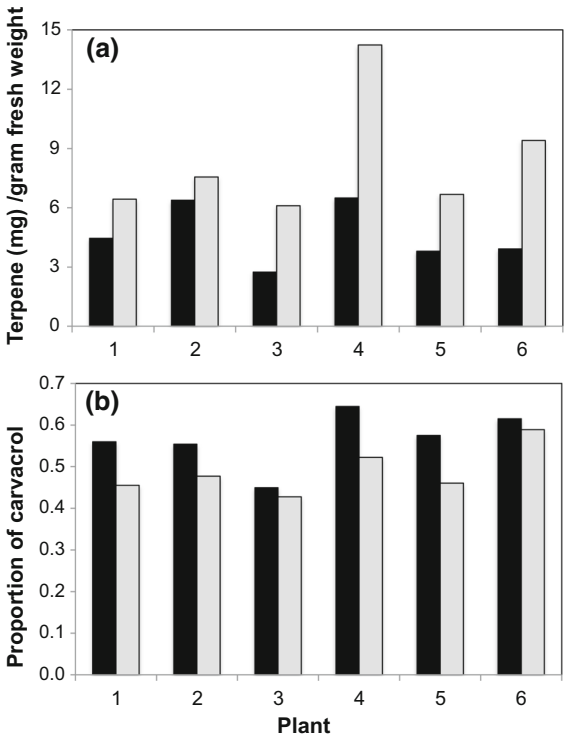

Fig. 6 Variation in **a** terpene biomass per gram of total biomass and **b** the proportion of the oil made up of carvacrol on galled (black bars) and un-galled (grey bars) stems of six carvacrol plants of *Thymus vulgaris* in a common garden

make the plant more or less suitable for larval development, can have a marked influence on the spatial distribution and dynamics of gall formation and larval development, as reported in *Solidago altissima* for resistance to gall formation by *Eurosta solidaginis* (McCrea and Abrahamson 1987; Horner and Abrahamson 1992; Cronin and Abrahamson 2001), for host tree genotype contribution to variation in the distribution of the specialist gall former *Belonocnema treatae* (Hymenoptera: Cynipidae) in populations of *Quercus fusiformis* (Egan and Ott 2007), differences among elm tree species in susceptibility to gall formation by the aphid *Tetraneura yezoensis* (Akimoto 1990), and variation in host tree susceptibility to the gall-former *Aceria parapopuli* in *Populus* species (Evans et al. 2012).

Our study of 59 *Thymus vulgaris* populations has shown that the three zones of chemotype distribution (Thompson 2002) are correlated with marked variation in gall formation by *Janetiella thymicola*. Within the basin at < 200 m elevation, non-phenolic plants are dominant in *T. vulgaris* populations and infection rates are significantly more abundant than in populations above 250 m around the basin, where the two phenolic chemotypes are predominant. In the intermediate zone where phenolic and non-phenolic plants exist in mixed populations gall formation rates are intermediate, with slightly more variation among populations than among phenolic populations. Among the four non-phenolic chemotypes, populations containing the geraniol chemotype show the most important rates of gall formation and where geraniol coexists with the linalool chemotype, individual plants of the former are significantly more susceptible to gall formation.

As a result, variation in the proportion of galled individuals follows, in a strikingly parallel fashion, the order of the epistatic chain of loci associated with the expression of secondary compound variation in this species, i.e. G, A, U, L, C, T (Vernet et al. 1986). The fact that gall formation is more abundant on plants of the geraniol chemotype than on plants of the linalool chemotype in a population where the two chemotypes co-occur, strongly implicates a role of chemotype in host plant choice by the gall former related to the chemical polymorphism.

The defence function of secondary compound production by plants is well known (Fraenkel 1959; Ehrlich and Raven 1964; Crawley 1983; Bryant et al. 1991) and in the case of gall-making insects, phenolics often play an important role in plant defence (Zucker 1982; Hartley 1998; Abrahamson et al. 1991; Horner and Abrahamson 1992). Variation associated with plant chemotype in our study could result from active choice by the insect (to avoid toxic chemotypes) or be a result of greater deterrence of gall formation by phenolic chemotypes. Thyme phenolic molecules may be generally more toxic than non-phenolic molecules to antagonists (Linhart and Thompson 1999) and may thus have a more negative effect on larvae development. Our results thus raise an interesting series of experimental perspectives for experiments in controlled conditions to test among deterrence and host choice alternatives.

The question remains as to whether gall distribution is the result of adult insect preference or to host toxicity to the larvae after infection. Given that tiny galls that would result from the second of these alternatives are not observed one would suspect that adult preference is involved. However, this question

requires controlled investigation via choice experiments and study of larvae development. The observation of correlated patterns of gall formation and chemotype frequency is also not completely conclusive of a direct link between plant phenotype and gall formation. Non-phenolic and phenolic chemotypes occur and are adapted to different climatic environments on different soil types (Gouyon et al. 1986; Thompson 2002; Amiot et al. 2005; Thompson et al. 2007). Differences in winter temperatures or soils that create enhanced drought conditions may thus also shape the distribution of galling if they impact on the biology of the fly, as observed for gall formation elsewhere (Traveset 1994; Evans et al. 2012; Rocha et al. 2013). Other gall-forming insects have indeed been reported to prefer colder winter environments (Irwin and Lee 2003).

Dispersal limitation of the parasite

Gall-forming insects are often sessile in terms of adult movement for oviposition (Redfern 2011) and can show a degree of territorial behaviour (Whitham 1979; Zucker 1982). Indeed, colonisation of new sites or species (more likely on widespread than rare species) is a slow process in gall formers (Strong et al. 1984). In our study, we found no evidence of spatial aggregation of gall formation. However, the temporal monitoring of gall formation in three populations over three years has shown that distance among plants affects the probability of a plant being galled, as reported for anther smut infection on *Silene alba* (Alexander and Antonovics 1988; Alexander 1990).

In addition, a key factor determining gall formation concerns whether the plant was already galled during the previous generation. The mean distance between infected plants and the closest previously galled plant is much shorter than the distance for un-galled plants, indicative that adults tend to move to one of the closest plants. In the Pâtus population, which has only the geraniol chemotype, the distance of a galled plant to a previously galled plant is smaller than in other populations that are either mixed populations with linalool and thuyanol (Aéroport population) or geraniol and linalool (Pâtus2) chemotypes. In the later two populations, adult flies could potentially choose between two chemotypes, hence distance may increase as they search for a more preferable plant. It would thus be worth investigating whether, in populations containing two or more chemotypes, a greater difference in palatability of chemotypes causes the parasite to search for a suitable host over greater distances.

Gall-induced changes on the plant

Gall forming is generally antagonistic in nature, with significant effects on growth and performance of the host in several cases (Hartnett and Abrahamson 1979; Redfern 2011) especially plant species that inhabit competitive conditions (Fay et al. 1996). Although the gall is a plant tissue, the insect can modify its form and composition (Weis and Abrahamson 1986; Hartley 1998). For example, the activity of the gall insect *Clusiamyia nitida* induces several changes in the foliar anatomy and the distribution of metabolic compounds in new tissues of *Clusia lanceolata* during gall development (Guimarães et al. 2013). Rocha et al. (2013) have reported enhanced frost resistance and higher values of a range of physiological parameters on trees of *Eucalyptus camaldulensis* that are galled by *Leptocybe invasa* compared to un-galled trees.

In our study, a greater biomass was allocated to infected shoots compared to un-infected shoots, primarily due to the biomass of the gall that absorbs the excess biomass allocated to infected shoots and furthermore causes reduced biomass on the remainder of the shoot. However, gall presence did not cause a decline in either reproductive biomass or 3-year survival. Hence, we did not detect a major cost to the plant; the perennial nature of the plant may buffer any such resource costs. In addition, we only measured biomass costs by comparing infected and un-infected shoots on the same plant, and did not assess costs associated with the presence of many galls. The fact that un-galled plants had a higher probability of mortality than galled plants in the three monitored populations may mean that gall flies avoid less vigorous plants.

Finally, the concentration of defence compounds can also be markedly higher as a result of infection by parasites, egg laying by butterflies or galling insects (Abrahamson et al. 1991; Hartley 1998; Peñuelas et al. 2006; Achotegui-Castells et al. 2015; Rand et al. 2017). We have very preliminary evidence that this is the case for volatile monoterpense in thyme, where the higher proportions of the dominant carvacrol monoterpene in galled stems compared to un-galled stems

makes for an interesting perspective for study of the plant–gall interaction, particularly across the range of chemotypes present in the study species.

Acknowledgements We thank Yannis Michalakis for advice, Laure Andrieu, Christian Collin, Marie Maistre, Annabelle Dos Santos, Pierre Reignaud, Muriel Busson and Jérôme Ghesquière for assistance with fieldwork, and the C.N.R.S. for financial support.

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