Ecology Letters, (2005) **8**: 370–377 doi: 10.1111/j.1461-0248.2005.00728.x

LETTER

Differential resistance to freezing and spatial distribution in a chemically polymorphic plant *Thymus vulgaris*

Justin Amiot, Yann Salmon, Christian Collin and John D. Thompson*
UMR 5175 Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 Route de Mende, 34293 Montpellier cedex 5, France
**Correspondence: E-mail: john.thompson@cefe.cnrs.fr*

Abstract

Secondary compounds play multiple ecological roles. In this study, we present novel experimental evidence of differential tolerance to freezing temperatures among chemotypes of a chemically polymorphic plant, *Thymus vulgaris*. Non-phenolic chemotypes showed a significantly better survival and re-growth after early-winter freezing (−10° in early December) than phenolic chemotypes. Comparison of temperature data (1971–2002) at a phenolic and non-phenolic site showed that whereas early-winter freezing occurred in 6 years in the non-phenolic site they never occurred at the phenolic site. Observations of trichome morphology (where the essential oil is stocked) with and without intense freezing indicate that non-phenolic chemotypes may escape any negative effects of freezing by releasing their essential oil into the atmosphere during severe freezing. The correlation between tolerance of freezing and local temperature regimes strongly suggests that differential freezing resistance is a key ingredient of the distribution of thyme chemotypes in space.

Keywords

Chemical polymorphism, freezing resistance, Lamiaceae, secondary compounds.

Ecology Letters (2005) 8: 370–377

INTRODUCTION

Plant secondary compounds have multiple biotic and abiotic functions. Well known to act as a chemical defence against herbivores and pathogens (Levin 1976; Bryant *et al.* 1991), secondary compounds may modulate interactions with competing plants (Rice 1979; Ehlers & Thompson 2004) and pollinators (Beker *et al.* 1989; Ayasse *et al.* 2000) and provide UV protection (Close & McArthur 2002) or carbon/nutrient balance adjustment in response to sunlight (Shure & Wilson 1993).

The existence of genetically based polymorphisms for secondary compound production has been reported in various species, several of which show spatial variation in the occurrence of different chemical forms, e.g. *Pinus halepensis* (Schiller & Grunwald 1987), *Trifolium repens* (Daday 1954a,b), *Mentha citrata* (Murray & Lincoln 1970), *Origanum vulgare* (Vokou *et al.* 1993), and a range of *Thymus* spp. (Stahl-Biskup 2002; Thompson 2002). Such spatial variation provides an appropriate situation in which to evaluate the different biotic and abiotic functions of secondary compound production. However, untangling the ecological role of different factors has proven complex. In several species which show spatial variation in relation to temperature and other abiotic environmental features, chemical forms also show differential resistance to herbivory, e.g. *Trifolium repens* (Jones 1973; Dirzo & Harper 1982a,b; Hughes 1991), *Lotus corniculatus* (Ellis *et al.* 1977; Compton *et al.* 1983), and *Thymus vulgaris* (Linhart & Thompson 1995, 1999). It thus remains unknown whether abiotic factors directly cause spatial segregation of chemical morphs or whether their impact is via an effect on herbivore abundance.

Thymus vulgaris L. (Lamiaceae), a small aromatic woody shrub in the western Mediterranean, has natural populations which contain one or more of six different chemical morphs, or chemotypes (Granger & Passet 1973). Each chemotype is named after the main component of its essential oil, which is either a phenolic monoterpene, thymol (T) or carvacrol (C), or a non-phenolic monoterpene, linalool (L), thuyanol (U), α-terpineol (A) or geraniol (G). All six are genetically controlled by an epistatic series of five loci and are part of the same biosynthetic pathway (Passet 1971; Vernet *et al.* 1986). In

T. vulgaris, the essential oil is sequestered within glandular trichomes at the surfaces of leaves, calices and young shoots. Phenolic chemotypes are common close to the Mediterranean Sea, whereas non-phenolic chemotypes predominate in inland sites, particularly above 400 m elevation or in basins which experience a winter temperature inversion (Granger & Passet 1973; Gouyon *et al.* 1986; Thompson 2002). Although phenolic chemotypes are generally more toxic than non-phenolic chemotypes to potential herbivores in controlled conditions, such deterrence varies dramatically across the spectrum of different antagonists (Linhart & Thompson 1999). Spatial variation in biotic selection could thus contribute to the maintenance of the chemical polymorphism if herbivores vary in spatial abundance, perhaps because of differences in abiotic conditions. Alternatively, the different chemotypes may vary in their tolerance of environmental factors, such as soil moisture and freezing temperatures in winter.

The lack of conclusive evidence of differential tolerance of abiotic conditions in a chemically polymorphic plant has stimulated us to quantify whether the different thyme chemotypes show variation in their tolerance to severe freezing temperatures in controlled experimental conditions. We examined three questions. First, do plants of different chemotypes show variation in survival, growth and reproduction following severe freezing temperatures? Second, is trichome morphology affected by freezing? Third, are severe freezing events more frequent or more severe in areas where chemotypes with better freezing tolerance predominate? The underlying hypotheses to this work are that plants with a non-phenolic chemotype occur in colder environments and show evidence of greater resistance to freezing than those with a phenolic chemotype, which may suffer some form of chemical poisoning as a result of freezing.

MATERIALS AND METHODS

Meteorological data

We obtained meteorological data from two sites, one near the town of St Martin-de-Londres, in a basin where non-phenolic chemotypes are the dominant component of thyme populations (hereafter non-phenolic site) and one at the CEFE-CNRS experimental gardens on the outskirts of Montpellier (phenolic site), in an area where phenolic chemotypes, in particular carvacrol, are predominant in local populations. The latter site is where we performed experimental studies of freezing tolerance in controlled conditions. Data for the non-phenolic site are from the Direction de l'Aménagement Rural et de l'Environnement de l'Hérault (1955–2002) and data for the phenolic site are unpublished data collected and archived at this site for the period from 1971 to 2002 (C. Collin, unpublished data).

Plant material

Plant material originated from bulk collections in natural populations, within and around the Saint Martin-de-Londres Basin. Each collection site contains a single chemotype at a frequency of at least 90% of the population.

For freezing experiments we used four chemotypes: two which produce a non-phenolic monoterpene (geraniol and α-terpineol) and two which produce a phenolic monoterpene (carvacrol and thymol). We did not use the linalool and thuyanol chemotypes because the former may have a phenolic phenotype during the first few months after germination, and because we know of no site where the latter occurs at a frequency above 60% of the population. Use of the latter would thus require that seedlings be analysed (i.e. have leaves removed) prior to freezing experiments. Seeds were collected in one population of each chemotype in early July 2002. After removal from the calyx, seeds were scarified (to improve germination rates) by automatic shaking (for 45 min) in Eppendorf tubes (*c.* 100 seeds per tube) containing Fontainebleau sand. Seeds were sown in trays in a glasshouse at the CEFE-CNRS experimental garden in Montpellier. Roughly 1 month after germination, seedlings were transplanted to individual pots and placed outdoors. Seeds were sown in May 2003 (experiments 1 and 2) or October 2003 (experiment 3). Seeds germinate in moist and mild conditions, i.e. in the autumn or spring. Growth is rapid in autumn, as this represents the period of re-growth and establishment after the summer drought. Hence, although the seeds for experiment 3 were sown later, they were of an equivalent size to those in experiments 1 and 2 at the time of our experiments.

We observed trichome morphology on plants of known chemotype collected from natural populations in 1997 and grown in the CEFE-CNRS experimental gardens.

Effect of freezing on survival, growth and reproduction

Based on the minimum temperature values (Fig. 1) we carried out three experimental tests of freezing resistance of seedlings. All three experiments were conducted in identical settings, only the date of freezing and the minimum temperature varied among experiments. Experiment 1 was conducted in early December and involved a minimum temperature of −10 °C to simulate an early winter frost, observed during 11 winters at the non-phenolic site between 1962 and 2002. Experiment 2 took place in mid-January and employed a minimum temperature of −15 °C, which occurred during four winters. Experiment 3 was conducted in late February with a minimum temperature of −10 °C to simulate a late-winter frost, which happened during nine winters in February and early March. These minimum

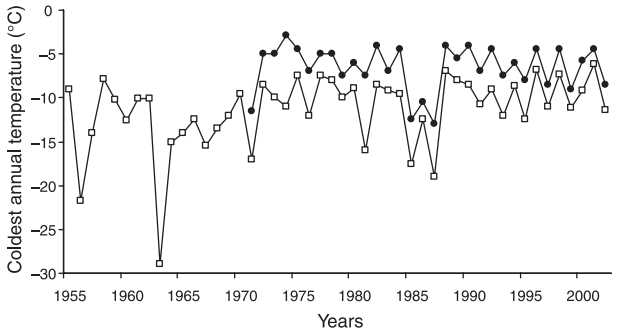

Figure 1 Coldest annual temperatures in a non-phenolic site (Saint Martin-de-Londres Basin) from 1955 to 2002 (open squares), and in a phenolic site (CEFE-CNRS experimental gardens in Montpellier) from 1971 to 2002 (filled circles).

temperature values were selected on the basis of local meteorological records, and also in relation to data on frost resistance of other aromatic plants (Sakai & Larcher 1987), and previous transplant experiments in which seedlings of *T. vulgaris*, submitted to minimum temperatures of −5, −8, −10, −12 and −15 °C, showed no damage and no mortality at temperatures between 0 and −10 °C (J. Thompson and J. Amiot, unpublished observations).

For each experiment, 360 seedlings, 90 per chemotype, were kept outdoors prior to treatment to allow natural frost hardening processes to occur. On the day of treatment, 30 pots of two chemotypes were randomly placed into two isolated trays which were designed to protect roots from freezing. The trays were then placed in a programmed upright freezer overnight and taken out at dawn to avoid any photoperiod bias. The freezer's temperature was programmed (Campbell CR23X micrologger, programmed with Campbell PC208W version 3.3 software; Campbell Scientific Inc., Logan, Utah, USA) as follows. Starting at room temperature, air temperature decreased 1 °C every 30 min, until it reached the programmed minimum temperature (−10 or −15 °C) where it was kept stable for 5 h. The temperature was then allowed to rise gradually. The temperature within the freezer was automatically checked every 30 s in order to avoid significant fluctuations.

After the freezing treatment, plants in each experiment were fully randomized in a single block and kept outdoors throughout the 2004 growing season. For each plant we measured initial plant size at the time of freezing treatment and recorded four parameters. Resistance to freezing was estimated by recording plants with green leaves 3 weeks after treatment. Survival was estimated at the peak of flowering (28 April 2004). A plant was recorded as flowering if it produced at least one flower in which reproductive organs were identifiable. Finally, growth rate was estimated as the difference between size at peak flowering and size at the time of freezing. As plants were young and not yet branched, size was measured as the height of the plant above the soil. In young seedlings, height and width show a very strong positive correlation with each other and with plant biomass (J. Thompson and Y. Linhart unpublished data). We also recorded fruit set, however the number of individuals actually setting fruits was too low to conduct statistical analysis.

Trichome observations

Leaves of *T. vulgaris* bear trichomes on their surface, i.e. glandular cells containing the essential oil. When they are full, trichomes are round and shiny under a binocular microscope. They appear flat, wrinkled and dull when empty. We thus quantified the ratio of intact to damaged trichomes on treated and untreated plants to estimate any impact of frost damage on trichomes and their content. Leaves observed were collected on adult plants of all six chemotypes originally collected from a natural population and cultivated in an open field at the phenolic site. The trichomes were sampled as follows. We collected one shoot on each of 20 plants per chemotype. Five leaves, carefully removed from each shoot in order not to damage the trichomes, were placed under a binocular microscope and 20 randomly sampled trichomes were observed on the surface of each leaf. Every observed trichome was quantified as either 'intact' or 'damaged'.

Three sets of observations, or treatments, were obtained. For the first set of observations shoots were collected in February 2004 and put in a freezer overnight prior to leaf removal. These shoots were subject to a minimum temperature of −15 °C (as in experiment 2 above). For the second set of observation, shoots were also collected in February 2004 but received no particular freezing treatment before observation. For the third set of observation, shoots were collected in June 2004 and observed without freezing treatment in order to compare the state of trichomes during winter and summer. Prior to observations made in February 2004 (with or without the freezing treatment), the adult plants in the experimental field experienced 17 days of frost during the winter, with a minimum of −5.5 °C in January. A total of 12 000 trichomes (2000 per chemotype) were observed.

Statistical analysis

Ninety seedlings per chemotype were assessed for resistance and survival in relation to each of the three experimental freezing treatments. Because of mortality, flowering and growth rate were assessed on a variable number of < 90 plants per chemotype and per trial. For resistance, survival and flowering, data were analysed using PROC GENMOD in

SAS Institute (1999–2000). In these analyses, chemotype was treated as a fixed effect. For growth rate, data were analysed with ANOVA using PROC GLM in SAS Institute (1999–2000), with chemotype as a fixed factor. The ratio of intact/damaged trichomes was analysed on a per plant basis using PROC GENMOD SAS Institute (1999–2000) with chemotype, treatment and interaction effects. In each analysis, *a priori* contrasts were employed to test for differences between phenolic and non-phenolic plants. Variation among chemotypes, and contrasts between phenolic and non-phenolic plants, were also assessed in separate analyses for each treatment.

RESULTS

Meteorological data

A plot of the minimum temperature of the coldest day each winter over the 31 years from 1971 to 2002 (Fig. 1) shows that minimum temperatures are regularly 4–5 °C (and often more) colder at the non-phenolic site. The lower the minimum temperature at the non-phenolic site the greater the difference in minimum temperature between the two sites. Over the 31 years, a minimum temperature of −10 °C was reached in 14 years at the non-phenolic site and four years at the phenolic site. At the latter site, three of the four occasions occurred in consecutive years. A minimum temperature of −15 °C occurred in 4 years at the non-phenolic site, but never at the phenolic site during the observation period. Severe freezing temperatures are thus more regular, and markedly more severe, at the non-phenolic site. In addition, since 1971, the occurrence of a severe freezing event (−10 °C) in early winter, i.e. in December (as simulated in experiment 1), was detected in 6 years at the non-phenolic site but in none of the years at the phenolic site. At the phenolic site, severe freezing temperatures only thus occurred later in winter, nearly always in January.

Effect of freezing on survival, growth and reproduction

In experiment 1, i.e. freezing to −10 °C in early December, non-phenolic plants showed significantly better resistance to freezing, better survival and growth, and higher rates of flowering than phenolic chemotypes (Fig. 2a–c; Table 1). The contrast between phenolic and non-phenolic plants was highly significant for each of these parameters (Table 1). Growth rates showed negative values (Fig. 2d) because of the fact that the upper portion of a shoot often frequently dried and fell, hence plants became smaller after freezing. In some cases growth rates were not sufficient to bring plants back to a height greater than prior to freezing.

In experiment 2, i.e. freezing to −15 °C in mid-January, chemotype effects were only detected for resistance and survival (Fig. 2e,f; Table 1). For these parameters, plants with the α-terpineol and carvacrol chemotypes performed better than the geraniol and thymol chemotypes. However the slight reversal of these patterns for flowering and growth rate suggests that such effects may be minimized by trade-offs with other performance estimates (Fig. 2g,h). In this experiment, the contrast between phenolic and non-phenolic chemotypes was not significant.

In experiment 3, i.e. freezing to −10 °C in late-February, a significant chemotype effect on resistance, flowering and growth rate was observed because of enhanced performance of the geraniol and carvacrol chemotypes after freezing (Fig. 2i,k,l; Table 1) As in experiment 2 the contrast among phenolic and non-phenolic chemotypes was not significant.

Trichome observations

The ratio of intact to damaged trichomes on plants of each chemotype in three trials (summer, winter and freezing treatment) showed highly significant differences among the three treatments and the six chemotypes and a significant interaction between chemotype and treatment (Fig. 3; Table 2). A comparison of chi-squared values for the different analyses reveals a much larger difference between winter freezing treatment and winter control than between winter and summer controls (Table 2). Contrasts among phenolic and non-phenolic plants in each treatment showed significant differences in the ratio of intact to damaged trichomes in each treatment, particularly the freezing treatment (Table 2). Together, these results illustrate two important differences between phenolic and non-phenolic chemotypes. First, although in all chemotypes the ratio of intact to damaged trichomes decreased significantly in the freezing treatment, a much greater effect was observed in non-phenolic plants compared with phenolic plants. Second, in non-phenolic plants the ratio of intact to damaged trichomes was lower in winter (after plants experienced cold temperatures of *c.* 0 °C) than in summer, whereas no such difference was observed in phenolic plants. A preliminary experiment conducted in 2003 using a smaller sample size gave identical results (J. Amiot, unpublished data).

DISCUSSION

In this study, we have shown that seedlings of non-phenolic chemotypes of *T. vulgaris* have a markedly higher ability to tolerate and re-grow after early-winter freezing temperatures of −10 °C than seedlings from phenolic chemotypes. Meteorological data for a non-phenolic and a phenolic site for the period 1971–2002 show that the former more frequently experiences winter freezing temperatures, which

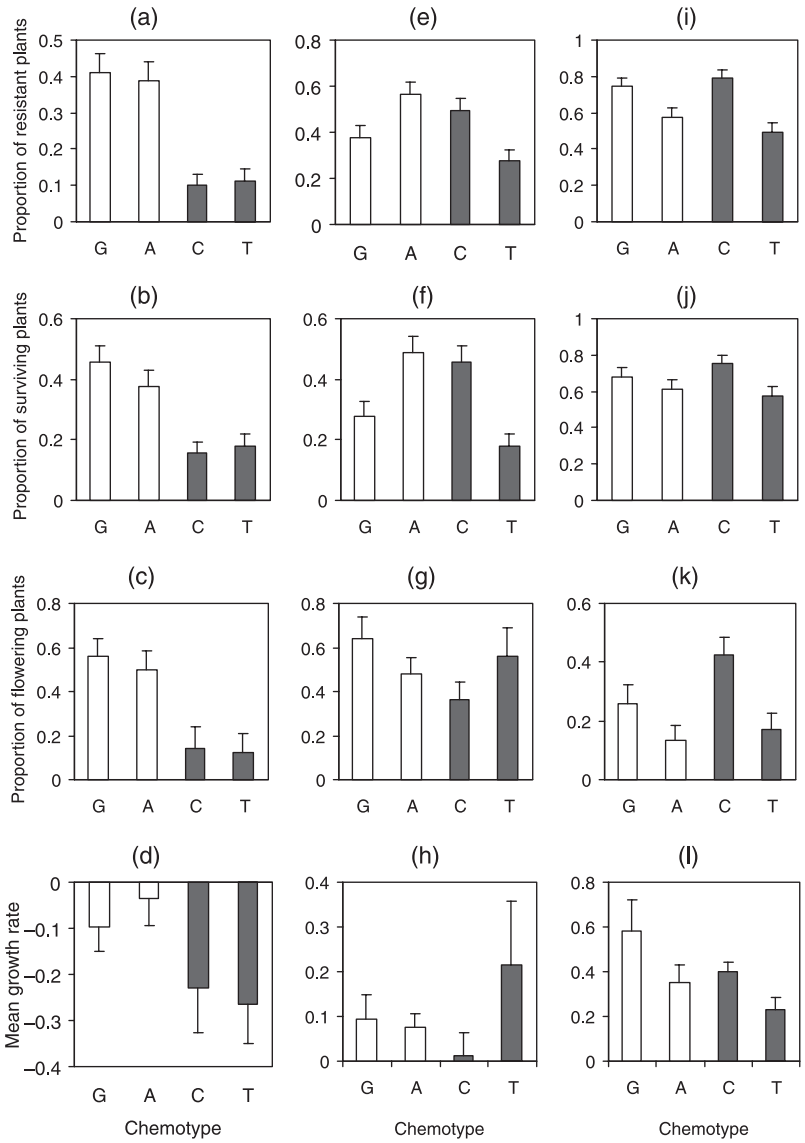

Figure 2 Mean performance values (±SE) of seedlings of four chemotypes of *Thymus vulgaris* after a minimum temperature of −10 °C in early December (experiment 1; a, b, c and d), −15 °C in mid-January (experiment 2; e, f, g, and h) and −10 °C in late-February (experiment 3; I, j, k, and l). Open bars are the two non-phenolic chemotypes, geraniol (G) and α-terpineol (A); filled bars are the two phenolic chemotypes, carvacrol (C) and thymol (T).

are more severe than those at the phenolic site. Indeed, early-winter freezing temperatures have frequently occurred in December at the non-phenolic site, roughly every 5 years over a period of 30 years, but have not been observed at the phenolic site during the same period.

The results of freezing in January and February showed no consistent differences between phenolic and non-phenolic chemotypes, primarily because of enhanced performance of carvacrol plants. Hence we detected seasonal variability in freezing tolerance, which could be explained by a number of factors. First, frost hardening may reduce differences in freezing tolerance among chemotypes as winter progresses (Sakai & Larcher 1987). Second, the experiments were conducted at the CEFE-CNRS experimental garden in Montpellier, a site which occurs within the distribution of carvacrol plants. At this site carvacrol plants have higher survival and larger size relative to the other chemotypes, primarily as a result of greater survival and regrowth after the summer drought (Thompson *et al.* 2004). These inherent differences in performance at the phenolic site may influence the results of our study. Third, temporal variation in monoterpene composition of the essential oil

Table 1 Results of the statistical analyses of comparison among chemotypes (d.f. = 3) and contrasts (d.f. = 1) among phenolic (P) and non-phenolic (NP) chemotypes of *Thymus vulgaris* in three freezing treatment experiments

Experimental conditions	Parameters	Chemotype	Contrast P vs. NP
Experiment 1: early December (−10 °C)	Resistance	$\chi^2 = 43.57$***	$\chi^2 = 43.42$***
	Survival	$\chi^2 = 29.19$***	$\chi^2 = 27.76$***
	Flowering	$\chi^2 = 15.90$**	$\chi^2 = 15.28$***
	Growth rate	$F = 2.13$ n.s.	$F = 5.83$*
Experiment 2: mid-January (−15 °C)	Resistance	$\chi^2 = 17.96$***	$\chi^2 = 3.09$ n.s.
	Survival	$\chi^2 = 29.79$***	$\chi^2 = 2.30$ n.s.
	Flowering	$\chi^2 = 5.19$ n.s.	$\chi^2 = 0.98$ n.s.
	Growth rate	$F = 1.50$ n.s.	$F = 0.24$ n.s.
Experiment 3: late-February (−10 °C)	Resistance	$\chi^2 = 22.97$***	$\chi^2 = 0.04$ n.s.
	Survival	$\chi^2 = 7.40$ n.s.	$\chi^2 = 0.30$ n.s.
	Flowering	$\chi^2 = 15.92$**	$\chi^2 = 2.39$ n.s.
	Growth rate	$F = 3.09$*	$F = 3.52$ n.s.

n.s., non-significant
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

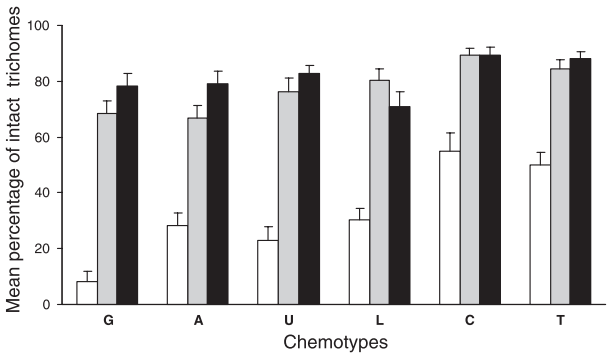

Figure 3 Mean (±SE) percentage of intact trichomes on leaves of each chemotype of *Thymus vulgaris* in three environmental conditions: freezing treatment (open bars), winter control (grey bars) and summer control (black bars). Chemotypes are: geraniol (G), α-terpineol (A), thuyanol (U), linalool (L), carvacrol (C) and thymol (T).

occurs in several chemotypes (Passet 1971) and could contribute to variation in freezing resistance. For example, in the carvacrol chemotype the relative amount of the precursor γ-terpinene in the oil increases relative to the amount of carvacrol from autumn to late-winter.

Due to their toxicity, monoterpenes of aromatic plants such as *T. vulgaris* could have a detrimental effect on the plant if trichomes release their content within the plant. Such self-poisoning, first proposed to explain the low frequency of cyanogenic forms of *Trifolium repens* in areas of Europe with cold winters (Daday 1954a,b), could apply to *T. vulgaris*. Our experimental trials indicate that trichomes on non-phenolic adult plants are more fragile with respect to

Table 2 Results of the overall statistical analyses and contrasts among treatments (frost, winter control and summer control) or among phenolic (P) and non-phenolic (NP) chemotypes of *Thymus vulgaris* in each treatment for the ratio intact/damaged trichomes

Analyses	Effect	d.f.	χ^2
Main effects	Chemotype	5	1556.2***
	Treatment	2	8029.4***
	Interaction	10	517.5***
Contrasts	Frost/Winter	1	5460.8***
	Winter/Summer	1	47.8***
Frost treatment	Chemotype	5	1483.1***
	Contrast P–NP	1	1176.9***
Winter control	Chemotype	5	462.2***
	Contrast P–NP	1	301.7***
Summer control	Chemotype	5	306.3***
	Contrast P–NP	1	211.1***

***$P < 0.001$.

freezing temperatures than those on phenolic plants. It is possible that this trichome fragility may allow plants to liberate monoterpenes outside the plant rather than into the leaf upon freezing, perhaps as a result of modifications to the trichome wall or basal cells. A microphysiological study of trichome morphology after freezing would provide valuable information here.

We interpret our results with a certain degree of caution. Although our results show a correlation between chemotype distributions, the occurrence of freezing events and freezing resistance, indicative of adaptive frost resistance of non-phenolic chemotypes, the observed differences may not be the cause of the spatial segregation of chemotypes. Other factors such as differences in herbivore abundance and their preference for the different chemotypes (Linhart & Thompson 1995, 1999) may also have contributed to the spatial segregation of thyme chemotypes. Despite this caveat, it is probable that differential freezing resistance currently contributes to the maintenance of the sharp cline in chemotype frequency in our study region, where the presence of phenolic (P) and non-phenolic (NP) plants in a single population is rare and limited to spatial zone of transition from phenolic to non-phenolic types (Thompson 2002). The absence of a sharp cline in neutral gene frequency across this transition (Tarayre & Thompson 1997), further points to adaptive differentiation associated with chemotype variation.

Various hypotheses such as those related to trade-offs between growth and differentiation (Herms & Mattson 1992; Stamp 2003) or the costs of resistance traits (Bergelson & Purrington 1996) provide a framework for understanding why non-phenolic chemotypes may be absent from sites which do not incur early-winter freezing temperatures. For example, in *Trifolium repens*, although the abundance of cyanogenic plants increases in micro-sites with high mollusc

density (Dirzo & Harper 1982a), such plants are more susceptible to pathogenic rust infection and their leaflets more sensitive to frosting than acayanogenic plants (Dirzo & Harper 1982b). In *T. vulgaris*, reciprocal transplants of phenolic and non-phenolic chemotypes show a pattern of home site advantage (J.D. Thompson & J. Amiot, unpublished data) which indicates that differential frost resistance favours non-phenolic chemotypes in sites with cold winters and that enhanced tolerance of summer drought stress favours phenolic chemotypes elsewhere. In addition, phenolic chemotypes are more deterrent of mollusc herbivory (Linhart & Thompson 1995) and infection by a specialist Diptera (J. Amiot and J.D. Thompson, unpublished data) and may have a greater effect on germination and growth of associated grasses which are potential competitors (Tarayre *et al.* 1995; Ehlers & Thompson 2004). Hence, a range of factors may favour phenolic chemotypes in the absence of intense early-winter freezing.

In conclusion, our results shed new light on the natural selection pressures acting on a chemical polymorphism in the wild. Although we cannot rule out the possibility that the spatial pattern of chemotype abundance may have originally developed as an indirect result of other selection pressures, differential resistance of climatic conditions may contribute to current spatial segregation of chemotypes. Given the probability of future climate change (note the reduced frequency of severe frosts in the non-phenolic site over the last 50 years in Fig. 1), we would predict the colonization of non-phenolic sites by phenolic chemotypes.

ACKNOWLEDGEMENTS

We thank Isabelle Chuine, Yan Linhart, and Ken Keefover-Ring for helpful discussion and advice, Alain Rocheteau for technical help, and the CNRS for financial support via the Associated European Laboratory (LEA) 'Mediterranean ecosystems in a changing world'.

REFERENCES

Ayasse, M., Schiestel, F., Paulus, H.F., Lofstedt, C., Hansson, B., Ibarra, F. *et al.* (2000). Evolution of reproductive strategies in the sexually deceptive orchid species *Ophrys sphegodes*: how does flower-specific variation of odour signals influence reproductive success. *Evolution*, 54, 1995–2006.

Beker, B., Dafni, A., Eisikowitch, D. & Ravid, U. (1989). Volatiles of two chemotypes of *Majorana syriaca* L. (Labiatae) as olfactory cues for honeybee. *Oecologia*, 79, 446–451.

Bergelson, J. & Purrington, C.B. (1996). Surveying patterns in the cost of resistance in plants. *Am. Nat.*, 148, 536–558.

Bryant, J.P., Provenza, F.D., Pastor, J., Reichardt, P.B., Clausen, T.P. & du Toit, J.T. (1991). Interactions between woody plants and browsing mammals mediated by secondary metabolites. *Ann. Rev. Ecol. Syst.*, 22, 431–446.

Close, D.C. & McArthur, C. (2002). Rethinking the role of many plant phenolics – protection from photodamage not herbivores? *Oikos*, 99, 166–172.

Compton, S.G., Newsome, D. & Jones, D.A. (1983). Selection for cyanogenesis in the leaves and petals of *Lotus corniculatus* L. at high latitudes. *Oecologia*, 60, 353–358.

Daday, H. (1954a). Gene frequencies in wild populations of *Trifolium repens*. 1. Distribution by latitude. *Heredity*, 8, 61–78.

Daday, H. (1954b). Gene frequencies in wild populations of *Trifolium repens*. 2. Distribution by altitude. *Heredity*, 8, 377–384.

Direction de l'Aménagement Rural et de l'Environnement de l'Hérault (1955–2002). *Bulletin Annuel de la Commission Météorologique de l'Hérault*. Conseil Général de l'Hérault, Montpellier, France.

Dirzo, R. & Harper, J.L. (1982a). Experimental studies on slug-plant interactions. III. Differences in the acceptability of individual plants of *Trifolium repens* to slugs and snails. *J. Ecol.*, 70, 101–118.

Dirzo, R. & Harper, J.L. (1982b). Experimental studies on slug-plant interactions. III. The performance of cyanogenic and acyanogenic plants of *Trifolium repens* in the field. *J. Ecol.*, 70, 119–138.

Ehlers, B. & Thompson, J.D. (2004). Do co-occuring plant species adapt to one another? The response of *Bromus erectus* to the presence of different *Thymus vulgaris* chemotypes. *Oecologia*, 141, 511–518.

Ellis, W.M., Keymer, R.J. & Jones, D.A. (1977). The defensive function of cyanogenesis in natural populations. *Experientia*, 33, 309.

Gouyon, P.-H., Vernet, P., Guillerm, J.L. & Valdeyron, G. (1986). Polymorphisms and environment: the adaptive value of the oil polymorphisms in *Thymus vulgaris* L. *Heredity*, 57, 59–66.

Granger, R. & Passet, J. (1973). *Thymus vulgaris* spontané de France: races chimiques et chémotaxonomie. *Phytochemistry*, 12, 1683–1691.

Herms, D.A. & Mattson, W.J. (1992). The dilemma of plants: to grow or defend. *Quat. Rev. Biol.*, 67, 283–335.

Hughes, M.A. (1991). The cyanogenic polymorphism in *Trifolium repens* L. (white clover). *Heredity*, 66, 105–115.

Jones, D.A. (1973). Co-evolution and cyanogenesis. In: *Taxonomy and Ecology* (ed. Heywood, V.H.). Academic Press, London, pp. 213–242.

Levin, D.A. (1976). The chemical defenses of plants to pathogens and herbivores. *Ann. Rev. Ecol. Syst.*, 7, 121–159.

Linhart, Y.B. & Thompson, J.D. (1995). Terpene-based selective herbivory by *Helix aspera* on *Thymus vulgaris*. *Oecologia*, 102, 126–132.

Linhart, Y.B. & Thompson, J.D. (1999). Thyme is of the essence: biochemical polymorphism and multi-species deterrence. *Evol. Ecol. Res.*, 1, 151–171.

Murray, M.J. & Lincoln, D.E. (1970). The genetic basis of acyclic oil constituents in *Mentha citrata* Ehrh. *Genetics*, 65, 457–471.

Passet, J. (1971). *Thymus vulgaris L.* Chémotaxonomie et biogénèse monoterpénique. Faculté de Pharmacie, Montpellier, 153 pp.

Rice, E.L. (1979). Allelopathy – an update. *Bot. Rev.*, 45, 15–109.

Sakai, A. & Larcher, W. (1987). *Frost survival of plants*. Springer-Verlag, Berlin, Heidelberg, 321 pp.

SAS Institute (1999–2000). *SAS/STAT Users Guide*, Version 6. SAS Institute Inc., Cary, NC.

Schiller, G. & Grunwald, C. (1987). Resin monoterpenes in range-wide provenanced trials of *Pinus halepensis* Mill. in Israel. *Silvae Genet.*, 36, 109–114.

Shure, D.J. & Wilson, L.A. (1993). Patch-size effects on plant phenolics in successional openings of the southern appalachians. *Ecology*, 74, 55–67.

Stahl-Biskup, E. (2002). Essential oil chemistry of the genus Thymus – a global view. In: *Thyme: the Genus Thymus* (eds Stahl-Biskup, E. & Sáez, F.). Taylor & Francis, London, pp. 75–124.

Stamp, N. (2003). Out of the quagmire of plant defence hypotheses. *Quat. Rev. Biol.*, 78, 23–55.

Tarayre, M. & Thompson, J.D. (1997). Population genetic structure of the gynodioecious *Thymus vulgaris* L. (Labiatae) in southern France. *J. Evol. Biol.*, 10, 157–174.

Tarayre, M., Thompson, J.D., Escarré, J. & Linhart, Y.B. (1995). Intra-specific variation in the inhibitory effects of *Thymus vulgaris* (Labiatae) monoterpenes on seed germination. *Oecologia*, 101, 110–118.

Thompson, J.D. (2002). Population structure and the spatial dynamics of genetic polymorphism in thyme. In: *Thyme: the Genus Thymus* (eds Stahl-Biskup, E. & Saez, F.). Taylor & Francis, London, pp. 44–74.

Thompson, J.D., Tarayre, M., Gauthier, P., Litrico, I. & Linhart, Y.B. (2004). Multiple genetic contributions to plant performance in *Thymus vulgaris*. *J. Ecol.*, 92, 45–56.

Vernet, P., Gouyon, P.H. & Valdeyron, G. (1986). Genetic control of the oil content in *Thymus vulgaris* L.: a case of polymorphism in a biosynthetic chain. *Genetica*, 69, 227–231.

Vokou, D., Kokkini, S. & Bessiere, J.M. (1993). Geographic variation of Greek Oregano (*Origanum vulgare* ssp. *hirtum*) essential oils. *Biochem. Syst. Ecol.*, 21, 287–295.

Editor, Don Waller
Manuscript received 28 October 2004
First decision made 1 December 2004
Manuscript accepted 6 January 2005