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ONGOING ADAPTATION TO MEDITERRANEAN CLIMATE EXTREMES IN A CHEMICALLY POLYMORPHIC PLANT

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Abstract. Although extreme climatic events may have profound effects on ecological systems, there is a marked lack of information on adaptation to such events. In this study, we employed reciprocal transplantation on both a geographic scale (experimental sites 200 km apart in different parts of the range of the study species) and a local landscape scale (reciprocal populations separated by 2–8 km) to study the performance of different chemical forms of *Thymus vulgaris* which naturally occur in different climatic environments. Survival and growth were analyzed in relation to long-term and contemporary climate data in natural populations and our experimental sites. The reciprocal transplants involved a period of six years for clones transplanted in experimental field sites on a geographic scale and three years for seedlings transplanted among natural populations at the local landscape level. Cloned transplants on a geographic scale produced evidence for local adaptation to either summer drought, primarily following the extreme summer drought of 2003, or severe early-winter freezing. Chemotypes that show high survival after intense summer drought showed poor survival after intense early-winter freezing and vice versa, results which directly accord with climate data for their original sites. On the local landscape scale, we found further evidence for local adaptation to summer drought but not to winter freezing (probably due to the absence of extreme freezing during the three years of this study). Future modifications to the occurrence and frequency of extreme climate events may have a profound influence on the spatial distribution of thyme chemotypes.

Key words: *adaptation; chemical polymorphism; drought; freezing tolerance; Mediterranean climate.*

Introduction

Across the world, plants in different regions experience dramatic differences in environment caused by the local climatic regime. An understanding of how climatic variation shapes species distributions, interactions among species, and population abundance and adaptation is an integral feature of models aimed at predicting the ecological effect of predicted future climate change (Easterling et al. 2000). By analyzing the impact of climatic selection regimes on transplanted populations of the annual herb *Chamaecrista fasciculata*, Etterson (2004*a*, *b*) reported that native populations show adaptive variation to local climate. As a result of low heritability, genetic correlations and poor demographic performance in alien habitats, northern populations may however face a "severe evolutionary challenge" since predicted rates of evolutionary response are slower than predicted rates of climate change (Etterson and Shaw 2001). Studies of a range of plant species which show latitudinal variation in various traits (Reinartz 1984, Lacey 1988, Weber and Schmid 1998, Rehfeldt et al. 2002) also provide information on adaptive variation in relation to climate. However, we still have little information concerning within-species variation in response to infrequent but extreme climate events which may create episodes of strong natural selection.

In five areas of the world, the occurrence of a summer drought defines the occurrence of a Mediterranean climate region (Quézel and Médail 2003) and imposes a major constraint on plant growth, reproduction and survival (Thompson 2005). In the Mediterranean Basin, plants show two main functional strategies that provide a response to summer drought (Thompson 2005); species either avoid drought stress by shedding of leaves and summer dormancy or show tolerance of drought stress by virtue of their low specific leaf area and/or high stomatal regulation. Drought tolerance may vary greatly among species (Damesin et al. 1997, Joffre et al. 1999, Martinez-Vilalta et al. 2002, Ogaya and Peñuelas 2003, Gratani and Varone 2004, Serrano and Peñuelas 2005, Valladares and Sánchez-Gómez 2006) and drought stress can limit the southern-most distribution of species (Arrieta and Suárez 2006) and contribute to population decline (e.g., see Gulías et al. 2002). However, despite early recognition by Stebbins (1952) of the potential role

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of aridity stress in plant evolution, there is but a scanty literature documenting population differentiation and adaptive variation of traits in relation to such stress in the Mediterranean flora (see Volis et al. 2002*a*, *b*, Atzmon et al. 2004, Peleg et al. 2005, Thompson 2005).

The Mediterranean Sea is almost completely surrounded by hills and mountains where severe winter freezing temperatures occur at high elevation and within basins and enclosed valleys as a result of dramatic temperature inversions. Since summer drought often occurs for more than two to three months at a time when temperatures would otherwise facilitate growth, a period of intense winter cold may represent a serious additional constraint on plant growth in this region. Indeed, several studies point to the potential importance of frost resistance for species' distributions in the Mediterranean Basin (Larcher 1981, Mitrakos 1982, Gianoli et al. 2004, Cavender-Bares et al. 2005, Hekneby et al. 2006).

In *Thymus vulgaris* L. (Lamiaceae), a short-lived aromatic woody shrub common in open garrigues from sea level to ~800 m elevation in the western Mediterranean, populations contain one or more of six different chemotypes whose distribution correlates with variation in climate (Granger and Passet 1973, Vernet et al. 1977*a*, *b*, Gouyon et al. 1986, Thompson 2002). In southern France, the two chemotypes with an essential oil dominated by a monoterpene with a phenolic structure (thymol and carvacrol chemotypes), are abundant on shallow stony soils in areas where winter temperatures tend to be mild, i.e., at elevations less than 400 m and close to the Mediterranean Sea. Within the phenolic chemotypes, populations dominated by the carvacrol chemotype tend to occur in zones of milder winter and less rainfall (to the south and closer to the Mediterranean Sea) than populations dominated by the thymol chemotype (Passet 1971, Gouyon et al. 1986, Thompson 2002). In contrast, non-phenolic chemotypes (geraniol, α-terpineol, thuyanol, and linalool) dominate areas where soils are often deeper and moister, and where freezing temperatures frequently fall below −10°C, i.e., at elevations above 400 m or in localized depressions with a temperature inversion during winter. The monoterpenes that distinguish these chemotypes originate along the same biosynthetic pathway (Passet 1971, Croteau 1987), and the genetic control of their expression is relatively well known (Vernet et al. 1986, Thompson et al. 2003). Estimates of gene flow based on neutral markers are indicative of strong selection on the chemotype genes in different environments (Tarayre and Thompson 1997, Thompson 2005). Although no study has yet fully examined the relative performance of the different chemotypes in different environments, there is evidence that in controlled conditions phenolic chemotypes may better tolerate drought (Pomente 1987), grow faster in sites with a mild winter (Thompson et al. 2004), but have low resistance to early-winter freezing (Amiot et al. 2005) compared to non-phenolic chemotypes. The spatial segregation of *T. vulgaris* chemotypes (rarely do populations contain roughly equal frequencies of both phenolic and non-phenolic chemotypes) thus offers a novel opportunity to test two questions: one is whether we can detect adaptation of a chemically polymorphic plant to local climate and the second involves the potential role of any such adaptation in the maintenance of a cline in chemotype gene frequencies on both a localized and geographic scale.

To analyze whether phenolic and non-phenolic chemotypes of thyme show local adaptation to climate variation, we performed reciprocal transplant experiments on two spatial scales. First, at each geographic extreme of the range of climatic environments in which *T. vulgaris* chemotypes occur in southern France, we monitored the growth and survival of cloned genotypes of all six chemotypes in a six-year study in experimental field sites (one at each extreme). Second, at the local landscape scale, where chemotype frequencies also show marked spatial differentiation, we monitored three-year survival and flowering of reciprocally transplanted seedlings in natural populations that differ in long-term climatic regime. The questions we addressed are as follows: (1) Do phenolic chemotypes, which naturally occur in areas with mild winter climates, show higher mortality rates than non-phenolic chemotypes during winter in sites where non-phenolic chemotypes predominate and which experience intense winter freezing? (2) At the other end of the climatic extreme, do non-phenolic chemotypes show higher mortality rates than phenolic chemotypes following summer drought in areas where phenolic chemotypes occur naturally? (3) Do the carvacrol and thymol chemotypes, which also show differences in their natural distributions, differ in their tolerance of severe summer drought and/or freezing?

The combination of a long term (six-year) geographic study in experimental field settings (i.e., over a large part of the life cycle of this short-lived shrub, for which the average lifetime of a plant which becomes a seedling in controlled conditions is less than nine years [J. D. Thompson, *unpublished data*]) with a shorter (three-year) study in natural populations in a local landscape provides a relatively complete study of adaptation to spatial and temporal climate variation, and provides key information concerning the maintenance and dynamics of a genetic polymorphism in relation to climate variation.

Materials and Methods

Geographic scale: transplants of cloned material in experimental field sites

Two experimental fields were used to compare the performance of cloned material of each chemotype in climatic and soil environments characteristic of either phenolic or non-phenolic chemotypes. The experimental field site within the phenolic zone or "phenolic site" was at the CEFE-CNRS experimental field site on the outskirts of Montpellier at ~60 m elevation and thus

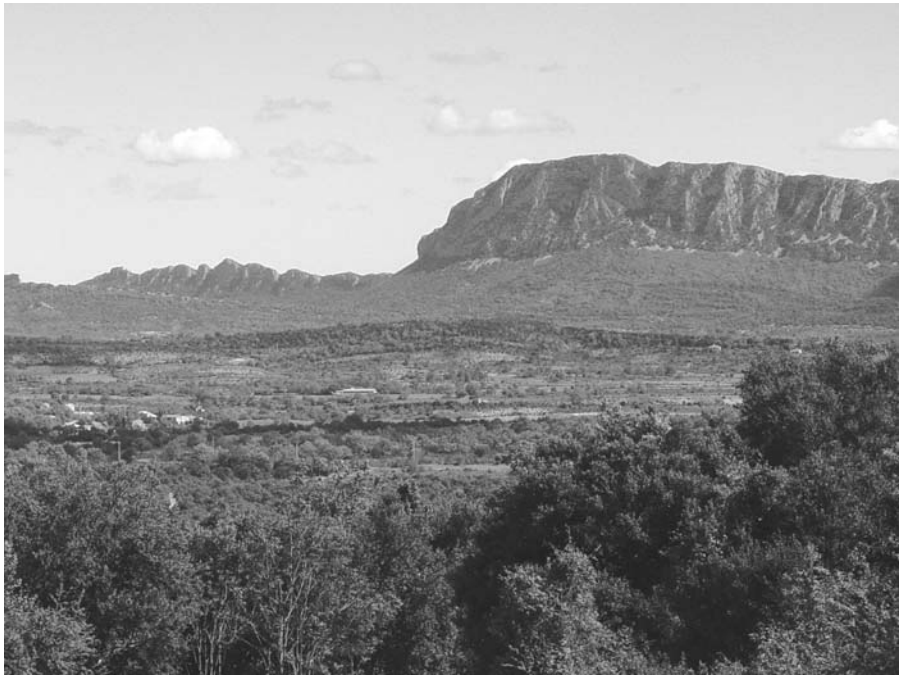

PLATE 1. The landscape where reciprocal transplants of thyme chemotypes were carried out on a local scale. In the foreground is the St. Martin-de-Londres basin where, below 250 m, the four non-phenolic chemotypes predominate. In the background is Pic St. Loup (658 m) on the slopes of which the phenolic carvacrol phenotype is the majority chemotype. To the left are the low hills where the other phenolic chemotype, thymol, is the majority chemotype. Photo credit: J. D. Thompson.

close to the Mediterranean Sea, in the region where the carvacrol chemotype is the majority chemotype in natural and semi-natural habitats. This site experiences a typical Mediterranean climate with mild freezing temperatures in winter and hot, dry summers (Table 1). The experimental field site within the non-phenolic zone or "non-phenolic site" was east of the Rhône Valley at 800 m elevation at the Mévouillon experimental field site of La Chambre d'Agriculture (Rhône-Alpes). This site occurs in the upper reaches of the Ouvèze valley to the north of the Mont Ventoux (i.e., ~200 km distant from the Montpellier experimental field site) where only non-phenolic chemotypes have been reported to occur naturally (Granger and Passet 1973, Thompson et al. 2003). This site experiences a mix of Mediterranean and Alpine influences on its climate with strong freezing temperatures in winter and hot summers which are frequently but not always associated with an extended drought (Table 1). Soils at the two sites show no significant differences in available nitrogen and soil moisture retention but do differ in overall levels of organic matter, due to greater quantities of available carbon at the phenolic site and higher concentrations of phosphorus at the non-phenolic site, where the pH is slightly lower (Table 2). The use of these sites enabled us to grow replicated clonal transplants of all six chemotypes in semi-controlled conditions in parts of the natural distribution of *T. vulgaris* where only either phenolic or non-phenolic chemotypes are naturally present.

In total, 30–50 cuttings were made on each of ~20 clones of known chemical composition (analyzed in Thompson et al. 2003) of each chemotype in January–February 1999. Cuttings of each clone were established in rows in irrigated perlite in the glasshouse in a large basin covered with plastic. Due to the need for mass production of cuttings (many do not root or insufficiently root for transplantation and thus a total of nearly 5000 cuttings were made) all replicates of each clone were placed in rows. However, we randomized the position of clones of the different chemotypes during this stage. Then, for 15 clones that produced large numbers of rooted cuttings, 15 cuttings of similar size were transplanted to individual one-third-liter plastic pots in April 1999 and maintained in a single randomized block outdoors during summer and autumn. The 15 clones of each chemotype were from three to six original populations (depending on the chemotype) and were composed of a roughly equal number of female and hermaphrodite clones (the species is gynodioecious) for each chemotype.

Five cuttings of each clone were transplanted into a single randomized block at each of the two transplant sites. At each site the plantation was made up of a series of parallel lines 1 m apart in which cuttings were randomly planted 35 cm distant from each other. The plantations were established in February 2000 at the

TABLE 1. Climate data for the period 1970–2005 for the two geographic-scale experimental field sites (CEFE and Séderon) and populations at the landscape level (Prades-le-Lez and St. Martin-de-Londres).

Scale and site	Total rainfall (mm)			
	Annual		June–August	
	Mean ± SE	Min–max	Mean ± SE	Min–max
Geographic scale				
CEFE (P)	807.4 ± 254.4	368.5–1358	107.9 ± 54.5	18.7–214.5
Sédéron (NP)	1022.1 ± 217.8	674–1418	167.9 ± 77.7	55–425.9
Landscape scale				
Prades-le-Lez (P)	853.6 ± 276.5	448.1–1688.8	116.4 ± 56.5	32.8–242.6
St. Martin-de-Londres (NP)	1122.7 ± 323.9	680.1–2051	149.6 ± 72.7	29.5–315

Notes: For Prades-le-Lez, data were only available for 1980–2005 (with two missing years in 1990–1991). Key to abbreviations: P, phenolic zone; NP, non-phenolic zone.

phenolic site and in March 2000 at the non-phenolic site. This experimental design of two sites, each with five replicates of each of the 15 clones for six chemotypes thus involved a total of 450 plants at each site. Plants were watered during the week which followed transplantation. Following this, no supplementary watering or fertilizer or protection from herbivores was made. The only maintenance was occasional weeding in spring to maintain homogeneous conditions across each plot. At neither site did large herbivores cause any damage, however plants were open to herbivory by several snail species, the small galling Diptera *Janetiella thymicola* and other insects. None of these were observed to produce significant damage on plants.

We monitored plants during six years (from June 2000 to June 2006 inclusive) after their transplantation to the experimental sites in early 2000. Survival of the initial transplants was recorded in June (phenolic site) or July (non-phenolic site) 2000. The number of surviving transplants at this date was used as the number of original plants in order to disregard the small number of transplants which died in each site due to transplantation shock. From then on plants were checked for regrowth and survival in late autumn (November or early December), after winter (early March), and in June of each year until June 2005. Thus, from the first recording (date 1) of survival in November 2000 we recorded survival on a total of 15 occasions, until June 2005. The study thus encompassed one year of growth in pots (cuttings) and then six growing seasons, a total time that is more than half the length of an average lifetime for a thyme plant that survives the initial seedling stage in controlled conditions (J. D. Thompson, *unpublished data*). Care must be taken in recording survival of woody plants such as thyme since although at some periods (especially in late summer) plants may look dead, they are merely in a state of vegetative dormancy. Indeed, during the experiment we occasionally noted a plant as dead, only to discover it alive on a subsequent recording. We thus did not attempt to quantify mortality on a monthly basis, preferring to attribute its occurrence to one of three seasons (capacity to survive and regrow after summer drought, winter survival, or spring growth to flowering) in each year of the study. Transplanting of clones and subsequent recording of flowering, size and survival were slightly later (except the autumnal monitoring) at the non-phenolic site due to the phenological differences between the two sites.

Size measurements were made on all surviving plants in June of each year from 2001 to 2005. Size was estimated using an index of plant biomass, $B = (h + w)/2$ (where h is height of the plant and w its maximum width). Plant biomass in thyme is closely correlated with size (Linhart et al. 2005) and this index has thus been used in other studies to assess performance variation (e.g., Thompson et al. 2004). Each year we recorded whether plants flowered.

TABLE 2. Values (mean ± SE) and results of one-way ANOVA for soil properties at each of the two experimental field sites.

Variable	Phenolic site	Non-phenolic site	$F_{1,7}$
Soil moisture (g/100 g soil)	24.14 ± 0.17	24.25 ± 0.30	0.11 ns
Organic carbon (g/kg soil)	13.80 ± 0.40	12.01 ± 0.16	17.32***
Total N (g/kg soil)	1.35 ± 0.04	1.48 ± 0.05	4.69 ns
C:N ratio	10.22 ± 0.13	8.14 ± 0.18	90.6***
Organic matter (g/kg soil)	23.90 ± 0.68	20.78 ± 0.28	18.0*
pH	8.39 ± 0.01	8.32 ± 0.02	14.2*
Phosphorus (g/kg soil)	0.040 ± 0.002	0.079 ± 0.003	109.0***

Note: Eight soil samples were randomly taken within the zone where transplants were installed and were sent for analysis to the Institut National de la Recherche Agronomique laboratory at Arras (France).

* $P <$ corrected 0.05 thresholds for multiple tests; *** $P < 0.001$; ns, not significant.

TABLE 1. Extended.

Monthly maxima of warmest month (°C)		Monthly minima of coldest month (°C)	
Mean ± SE	Min–max	Mean ± SE	Min–max
35.5 ± 1.86	31–40.5	−6.8 ± 2.27	−13–−3
32.47 ± 1.91	27–36.2	−16.43 ± 3.28	−23–−11
37.3 ± 1.5	34.7–42	−9.3 ± 2.5	−14–−5.3
35.7 ± 2.4	30.3–41.2	−10.5 ± 3	−19–−6

Monthly values of minimum and maximum temperature and total rainfall were obtained from Meteo France for a climate station near the town of Séderon, 8 km distant and at the same elevation as the non-phenolic experimental field site. Climatic data for the phenolic experimental field site were extracted from records at this site. These data provide an estimation of climatic differences between the phenolic and non-phenolic transplant sites during the course of our study.

Local landscape scale: transplants in natural populations

In June 2000 and 2001, seeds were collected from four phenolic and four non-phenolic populations of *Thymus vulgaris* located either within or outside of the St. Martin-de-Londres basin in Southern France and used as a basis for paired transplant trials (Table 3; also see Plate 1). Soils at the phenolic sites are stony, shallow fersiallitic brown calcareous soils with frequent rock outcrops, those in non-phenolic sites are less stony (sometimes rendziniform) deeper calcareous brown soils (see Gouyon et al. 1986). The climate in the St. Martin-de-Londres basin differs from surrounding areas (e.g., Prades-le-Lez and CEFE weather stations; Table 1) primarily because of more extreme freezing temperatures in winter associated with a marked temperature inversion relative to the surrounding landscape where phenolic chemotypes predominate.

Seeds were removed from the calyces by rubbing them on a plastic corrugated board and then scarified by automatic shaking in Eppendorf tubes containing fine sand for 45 minutes. Seeds were germinated in early September in seed trays filled with a 1:1:1 mix of humus, sand, and calcareous garden soil. This mix has been used for thyme seed germination and seedling cultivation in our previous work (Thompson and Tarayre 2000, Thompson et al. 2004). In addition, cuttings of plants of known chemotype were grown at the CEFE-CNRS experimental gardens and used to make up an additional transplant pair (Table 3).

Transplantation was done in November during a period of rainy weather. In each transplantation site, small plants were planted in a single randomized block made up of three to eight parallel lines and marked with a color-coded plastic ring at their base. Each block was located in an area of the population where thyme plants had been sampled for seeds. Each plantation site consisted of small plants from both that site (home chemotype) and its paired phenolic or non-phenolic counterpart (away chemotype). The whole procedure was carried out in 2000 and 2001, depending on the site, with a total of eight pairs of transplants and 2158 propagules planted (Table 3). Distances between reciprocal transplant sites varied from 2- to 8 km (Table 3). In all sites, survival was monitored each year around 1 March, 1 June, and 1 December to ascertain survival through winter, flowering, and autumnal regrowth, respectively. Both sites of pair 2 and the phenolic site of pair 3 were destroyed in late 2001 (due to a fire at the

TABLE 3. Seedling numbers in reciprocal transplants planted at the landscape scale in autumn 2000 (pairs 1, 2, 3, 4, and 8) or 2001 (pairs 5, 6, and 7).

Pair	Distance (km)	Site location	Chemotype†	No. "home" propagules	Site origin	No. "away" propagules
1	2	Gardiol	T	70	Aéroport	65
		Aéroport	L/U	65	Gardiol	67
2	8	Ferrière	T	52	Cabane	51
		Cabane	L	48	Ferrière	54
3	8	Ferrière	T	68	Grèzes	69
		Grèzes	G	68	Ferrière	65
4	3	Tourrière	C	35	Grèzes	30
		Grèzes	G	31	Tourrière	34
5	3	Tourrière	C	85	Fraicinède	87
		Fraicinède	A, U, L	82	Tourrière	74
6	8	Hortus	T	70	Fraicinède	80
		Fraicinède	A, U, L	69	Hortus	72
7	5	Hortus	T	84	Grèzes	83
		Grèzes	G	87	Hortus	92
8‡	8	Ferrière	T	95	Cabane	69
		Cabane	L	70	Ferrière	87

† Majority chemotype from Thompson et al. (2003).

‡ In this pair, propagules were cuttings prepared with those used in the transplant experiment at the geographic scale: phenolic propagules were a mix of thymol (T) and carvacrol (C) chemotypes; non-phenolic propagules were a mix of linalool (L), thuyanol (U), α-terpineol (A), and geraniol (G) chemotypes.

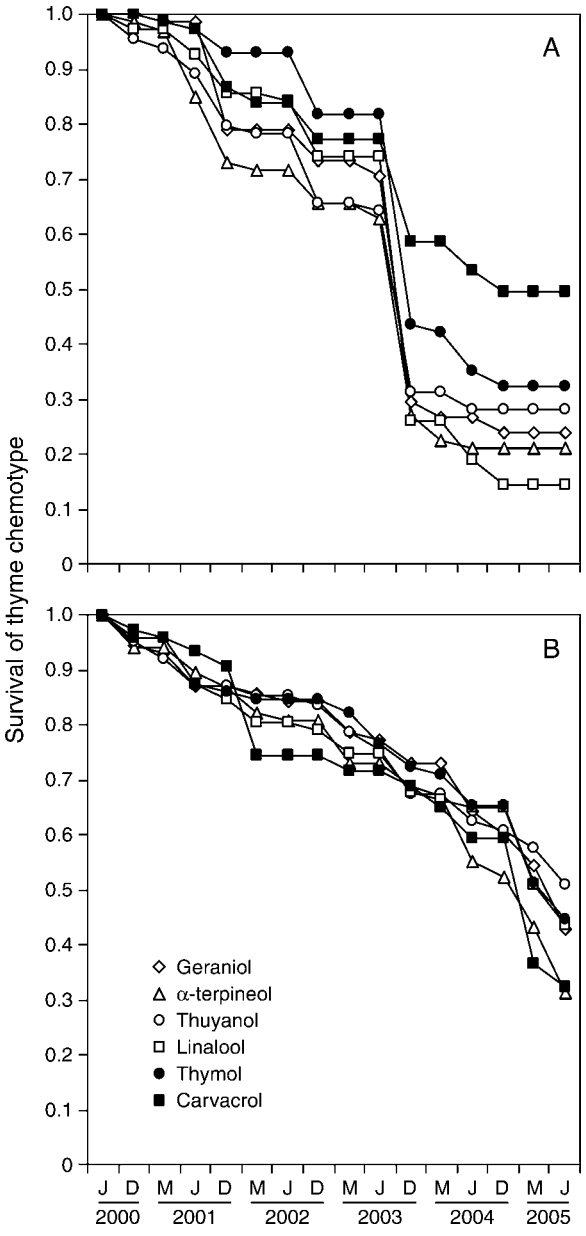

FIG. 1. Proportional survival of all plants of each chemotype of *Thymus vulgaris* in two experimental field sites: (A) the phenolic site and (B) the non-phenolic site. Chemotypes are geraniol, α-terpineol, thuyanol, linalool, thymol, and carvacrol. Survival was recorded three times each year: in mid-March (M), mid-to-late June (J), and during the last week of November or first two to three days of December (D).

Ferrières site and sheep grazing at the Cabane site), and the Gardiol site of pair 1 was destroyed in early 2002 as a result of severe grazing. Monthly values of minimum and maximum temperature and total rainfall were obtained from Meteo France for a climate station near the towns of St. Martin-de-Londres (for the non-phenolic sites) and Prades-le-Lez (within the area where phenolic chemotypes are predominant). These data provide an estimation of climatic differences between the phenolic and non-phenolic transplant sites during the course of our study.

Data analyses

Survival and mortality rates of transplants in both the experimental field sites and natural populations were treated as binomial data since each plant could have one of two different values at a given time: alive or dead. We used PROC GENMOD with a LOGIT function in SAS (SAS Institute 2001) using chemotype and site as fixed factors. For the study of seedling transplants in natural populations we included transplant pair as a fixed factor. This provides more biologically realistic tests of variation related to site, chemotype and their interaction; tests which are also more conservative due to the removal of degrees of freedom from the residual variation. Mortality rates were calculated for each time period based on the number of surviving plants at the start and end of that time period. Due to the large number of comparisons made, we only report significant mortality rates at $P < 0.01$ or $P < 0.001$ (i.e., we do not report those at $P < 0.05$).

Plant size variation in the two experimental fields was first analyzed in repeated measures ANOVA using PROC GLM in SAS (SAS Institute 2001) with chemotype, site, and interaction between them as fixed effects and plant genotype as a random effect nested within chemotype to produce a mixed-model ANOVA. Analyses were subsequently made on size values in each year and for each site. We employed a priori contrasts to test for significant differences among phenolic and non-phenolic chemotypes in each of the two transplant sites. Finally, for each year, we carried out mixed-model ANOVA on the size of plants which either survived to flowering in the subsequent year or which died in the subsequent 12-month period.

RESULTS

Geographic scale: transplants of cloned material in experimental field sites

Survival and mortality rates.—For total survival covering the duration of the experiment we detected a significant site × chemotype interaction for the comparison of all six chemotypes ($\chi^2 = 22.0$, df = 5, $P < 0.001$), which reflects a differential survival of chemotypes in the two sites, with markedly greater variability of chemotype survival in the phenolic site than in the non-phenolic site (Fig. 1). Indeed, in the phenolic experimental field site there were significant differences ($\chi^2 = 25.3$, df = 5, $P < 0.001$) in overall survival of the six chemotypes (Fig. 1A) with a significantly enhanced survival ($\chi^2 = 6.9$, df = 1, $P < 0.01$) of phenolic chemotypes relative to non-phenolic chemotypes. In contrast, in the non-phenolic experimental field site there was no significant difference in overall survival of the six chemotypes ($\chi^2 = 8.0$, df = 5, $P > 0.1$; Fig. 1B)

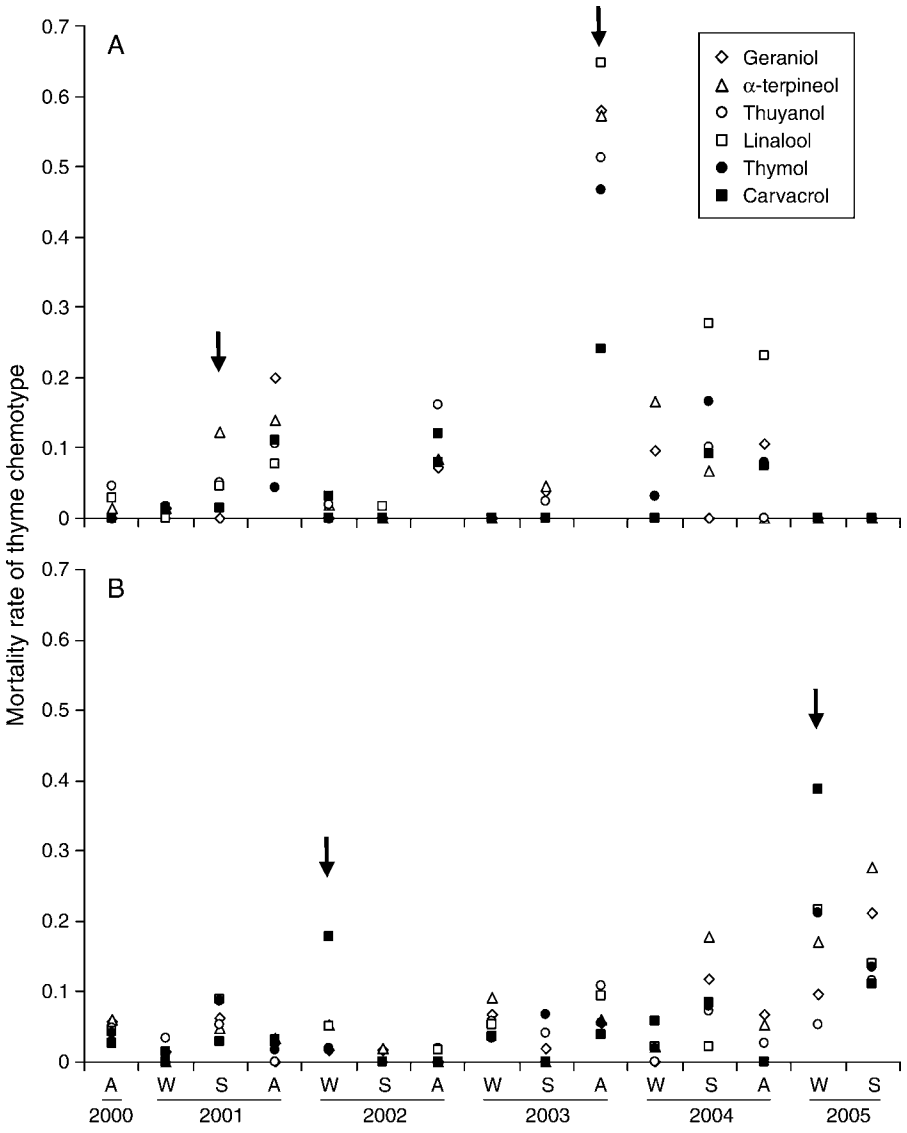

FIG. 2. Mortality rates of six *Thymus vulgaris* chemotypes in two experimental field sites: (A) the phenolic site and (B) the non-phenolic site, from 2000 to 2005. Arrows indicate significant differences (at least $P < 0.01$) among chemotypes for a given period: S, spring, based on surviving plants in June; A, late summer and autumn, based on surviving plants at the end of November; W, winter, based on surviving plants in mid-March.

and thus no significant variation between phenolic and non-phenolic chemotypes ($\chi^2 = 0.6$, df = 1, $P > 0.1$).

Significant differences in survival rates were such that the rank order of chemotypes and the timing of differences were not the same in the two sites. In the phenolic experimental field site (Fig. 2A), the analysis of all six chemotypes showed significant differences in mortality rates in spring 2001 ($\chi^2 = 17.0$, df = 5, $P < 0.01$) when the α-terpineol chemotype had a notably higher mortality rate than the other chemotypes, and in autumn 2003 ($\chi^2 = 23.2$, df = 5, $P < 0.001$) when the carvacrol chemotype had a mortality rate less than half that of all other chemotypes (Fig. 2A). The mortality of non-phenolic chemotypes was significantly greater than that of phenolic chemotypes ($\chi^2 = 15.0$, df = 1, $P < 0.001$) during autumn 2003 at this site. Indeed, during autumn 2003, mortality rates of all chemotypes were higher than at any other period during the experiment at this site. In the non-phenolic experimental field site, the analysis of all six chemotypes showed significant differences during the winter periods of 2001–2002 (df = 5, $\chi^2 = 19.4$, $P < 0.01$) and 2004–2005 (df = 5, $\chi^2 = 18.3$, $P < 0.01$), when the mortality rate of the carvacrol was notably higher than that of the other chemotypes (Fig. 2B). Indeed, the survival of non-phenolic chemotypes was significantly greater than that of phenolic chemotypes ($\chi^2 = 9.1$, df = 1, $P < 0.01$) during winter 2004–2005. At both sites the thymol and linalool chemotypes had intermediate mortality rates during episodes of high mortality.

TABLE 4. Mixed-model ANOVA of size variation of six thyme chemotypes in the two experimental field sites over five years, with *F* values and associated probabilities.

Source of variation	2001		2002		2003		2004		2005	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Che	5, 86	4.87***	5, 87	5.06***	5, 89	3.23*	5, 90	2.59*	5, 103	1.20 ns
Gen(Che)	84, 84	1.91**	84, 84	2.01***	83, 83	1.77**	76, 56	1.26 ns	74, 51	0.89 ns
Site	1, 88	464.90***	1, 89	288.30***	1, 89	91.90***	1, 65	24.40***	1, 62	40.40***
Site × Che	5, 88	4.51***	5, 89	4.21**	5, 89	3.64**	5, 63	2.09 ns	5, 59	2.00 ns
Site × Gen(Che)	84, 568	2.68***	83, 498	2.33***	82, 426	1.97***	54, 163	1.97***	47, 113	0.99 ns

Note: Key to abbreviations: Che, chemotype; Gen, genotype.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

We also compared mortality rates among sites for each chemotype (no figures shown). For the phenolic chemotypes, mortality was significantly higher in the phenolic site than in the non-phenolic site during autumn 2002 ($\chi^2 = 17.3$, df = 1, $P < 0.001$) and autumn 2003 ($\chi^2 = 36.3$, df = 1, $P < 0.001$) and significantly higher in the non-phenolic site during winter 2004–2005 ($\chi^2 = 31.2$, df = 1, $P < 0.001$). Significantly higher mortality rates in the phenolic site compared to the non-phenolic site were observed for the thymol chemotype ($\chi^2 = 27.4$, df = 1, $P < 0.001$), and, to a lesser extent, the carvacrol chemotype ($\chi^2 = 10.4$, df = 1, $P < 0.01$) in autumn 2003, and for the thymol chemotype in autumn 2002 ($\chi^2 = 11.0$, df = 1, $P < 0.001$). Mortality were significantly higher in the non-phenolic site during the winter 2001–2002 for the carvacrol chemotype ($\chi^2 = 8.4$, df = 1, $P < 0.01$) and during the winter 2004–2005 for both the carvacrol ($\chi^2 = 24.5$, df = 1, $P < 0.001$) and thymol ($\chi^2 = 8.8$, df = 1, $P < 0.01$) chemotypes.

For non-phenolic chemotypes, mortality rates were significantly higher in the phenolic site compared to the non-phenolic site after the summer drought in three consecutive years, 2001 ($\chi^2 = 26.3$, df = 1, $P < 0.001$), 2002 ($\chi^2 = 22.8$, df = 1, $P < 0.001$), and 2003 ($\chi^2 = 121.2$, df = 1, $P < 0.001$), and significantly higher in the non-phenolic site during spring 2005 ($\chi^2 = 19.3$, df = 1, $P < 0.001$). Indeed the mortality rates of the geraniol chemotype ($\chi^2 = 37.2$, df = 1, $P < 0.001$), the α-terpineol chemotype ($\chi^2 = 30.7$, df = 1, $P < 0.001$), the thuyanol chemotype ($\chi^2 = 17.7$, df = 1, $P < 0.001$), and the linalool chemotype ($\chi^2 = 37.2$, df = 1, $P < 0.001$), were significantly higher in the phenolic site than in the non-phenolic site in autumn 2003. The mortality rates of the geraniol ($\chi^2 = 19.0$, df = 1, $P < 0.001$) and thuyanol ($\chi^2 = 8.2$, df = 1, $P < 0.01$) chemotypes in autumn 2001, the thuyanol chemotype in autumn 2002 ($\chi^2 = 7.0$, df = 1, $P < 0.01$), and the linalool chemotype during spring 2004 ($\chi^2 = 9.1$, df = 1, $P < 0.01$) and autumn 2004 ($\chi^2 = 9.7$, df = 1, $P < 0.01$) were also significantly higher in the phenolic site. The α-terpineol chemotype showed a significantly higher rate of mortality in the non-phenolic site during spring 2005 ($\chi^2 = 7.2$, df = 1, $P < 0.01$).

Size variation.—Size was recorded each year towards the end of the flowering period, when size is maximized in a given year, in each experimental site. Each year, all plants alive at this time produced some flowers: we never observed a vegetative plant in either site at this time of the year. Repeated-measures ANOVA produced a significant effect of chemotype ($F_{5,83} = 2.33$, $P < 0.05$), site ($F_{1,53} = 146$, $P < 0.001$), site × genotype(chemotype) interaction ($F_{47,107} = 1.97$, $P < 0.01$), significant variation among years ($F_{4,104} = 115.2$, $P < 0.001$), and a significant year × site interaction ($F_{4,104} = 6.86$, $P < 0.001$).

Significant differences among sites in plant size were detected in all five years of the study, with a significant site × chemotype interaction in the first three years (Table 4). These differences are primarily due to the significantly greater size of phenolic chemotypes relative to non-phenolic chemotypes in the phenolic experimen-

TABLE 5. Mixed-model ANOVA of size variation of six thyme chemotypes in the two experimental field sites over five years, with *F* values and associated probabilities.

Source of variation	2001		2002		2003		2004		2005	
	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Phenolic site										
Che	5, 87	5.42***	5, 88	4.95***	5, 93	3.71**	5, 69	1.72 ns	5, 78	1.28 ns
Gen(Che)	84, 298	4.51***	83, 251	3.75***	82, 209	2.76***	56, 63	2.19**	54, 56	1.12 ns
Contrast P/NP	1, 298	79.75***	1, 251	63.70***	1, 209	43.49***	1, 63	11.37**	1, 56	5.76*
Non-phenolic site										
Che	5, 88	1.77 ns	5, 88	2.96*	5, 90	1.38 ns	5, 90	1.87 ns	5, 89	0.90 ns
Gen(Che)	84, 270	3.02***	84, 247	3.60***	83, 217	3.14***	74, 100	2.22***	67, 57	1.03 ns
Contrast P/NP	1, 270	2.45 ns	1, 247	5.11*	1, 217	2.36 ns	1, 100	2.11 ns	1, 57	0.07 ns

Note: Key to abbreviations: P, phenolic chemotypes; NP, non-phenolic chemotypes; Che, chemotype; Gen, genotype.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

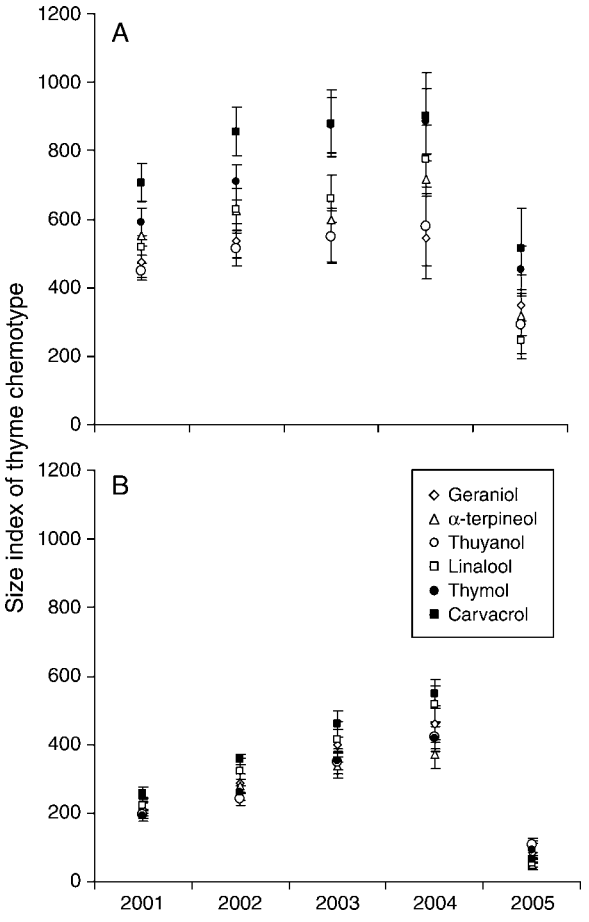

FIG. 3. Size index (mean ± SE) of six *Thymus vulgaris* chemotypes in two experimental field sites: (A) the phenolic site and (B) the non-phenolic site, from 2001 to 2005. Size was estimated using an index of plant biomass, $B = (h + w)/2$, where h is the height of the plant and w is its maximum width.

tal field site and the lack of any significant differences among chemotypes in the non-phenolic experimental field site (Table 5, Fig. 3). Indeed, in the phenolic site the contrast between phenolic and non-phenolic chemotypes was significant in all five years, primarily as a result of the greater size of carvacrol plants (during the first three years of the study), whereas it was never significant in the non-phenolic site (Table 6, Fig. 3). The genotype × site interaction and the effect of genotype were significant for most chemotypes in the first three years of the study, but diminished or disappeared after the high rates of mortality and loss of genotypes in autumn 2003 (Table 7). In both sites, the probability of survival from 2003 to 2004 was closely related to plant size: the average size in 2003 of plants which survived to flowering in 2004 was significantly greater than that of plants which died in this period (Table 8, Fig. 4). In the phenolic site this relation varied among chemotypes (site × size interaction in Table 8, Fig. 4), a result which may simply be due to the significant differences in mean size of chemotypes at this site.

Climatic data.—The experimental field sites experienced major differences in their climatic regimes due to differences in winter temperatures, the intensity of summer drought and occurrence of dry spring periods during the experiment (Fig. 5).

First, minimum winter temperatures are always lower, with a different timing of the onset of sharp freezing events, in the non-phenolic site. At the phenolic sites minimum winter temperatures never dropped below −10°C, at the non-phenolic site minimum temperatures dropped below −15°C in four of the five winters encompassed by this study, reaching −18°C on several occasions. On average, there was a 9°C difference in minimum winter temperature between the two sites, with a 14°C difference in December 2004. These differences in extreme temperatures reflect the long-term pattern of generally colder winters in the non-phenolic sites (Table 1, Table 9). Inspection of the graphs shows that the harsher the winter, the greater the difference in minimum temperature between the two sites.

In addition, extremely low freezing temperatures occurred in December at the non-phenolic experimental field site, but not at the phenolic experimental field site where temperatures reached minimum values in January or February (Fig. 5). At the non-phenolic site, extreme minimum temperatures in December occurred in 2001 (−17.2°C) and 2004 (−17.5°C). These two winters were the only two periods when a significant difference in mortality of phenolic and non-phenolic chemotypes was detected. Inspection of long-term climatic data show that this occurrence of severe freezing in early winter (December) is common in sites where non-phenolic chemotypes occur, but very rare or unknown in sites within the range of phenolic chemotypes (Table 9). Although the other winters (except the mild winter of 2000–2001) had equivalent very low temperatures in January and/or February, their minimum temperatures

TABLE 6. Scheffé means comparisons of plant size in the six thyme chemotypes in the two experimental field sites over five years.

Site and chemotype	2001	2002	2003	2004	2005
Phenolic site					
Geraniol	c	c	d	a	a
α-terpineol	b	bc	d	a	a
Thuyanol	c	c	d	a	a
Linalool	bc	bc	cd	a	a
Carvacrol	a	a	ab	a	a
Thymol	b	b	bc	a	a
Non-phenolic site					
Geraniol	a	cd	a	a	a
α-terpineol	a	cd	a	a	a
Thuyanol	a	d	a	a	a
Linalool	a	bc	a	a	a
Carvacrol	a	ab	a	a	a
Thymol	a	cd	a	a	a

Note: Chemotypes that do not share a common code letter in a given year have a significantly different size.

TABLE 7. Statistical significance of genotype × site interaction for each of the six thyme chemotypes grown in two experimental field sites.

	2001		2002		2003		2004		2005	
Chemotype	df	*F*	df	*F*	df	*F*	df	*F*	df	*F*
Geraniol	14, 102	3.16***	14, 84	2.90**	14, 75	5.61***	9, 29	5.05***	8, 18	2.35 ns
α-terpineol	14, 86	4.01***	13, 71	3.41***	12, 61	0.92 ns	8, 17	1.93 ns	7, 10	2.49 ns
Thuyanol	14, 78	1.01 ns	14, 73	1.77 ns	14, 55	1.56 ns	9, 19	2.15 ns	8, 17	0.84 ns
Linalool	14, 94	1.19 ns	14, 85	2.14*	14, 73	2.00*	6, 18	2.17 ns	4, 12	4.46*
Carvacrol	14, 105	5.50***	14, 88	2.47**	14, 79	1.68 ns	11, 46	2.16*	10, 34	1.07 ns
Thymol	14, 103	2.35**	14, 97	1.60 ns	14, 83	1.89*	11, 34	0.98 ns	10, 22	0.47 ns

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

in December were never below −12°C. Finally, the period during which minimum temperatures did not drop below 0°C was two to three months longer at the phenolic site.

Second, the intensity of summer drought is greater at the phenolic site, both in terms of temperature and precipitation. Maximum temperatures during the three month summer period (June–August) were repeatedly more than 2°C higher at the phenolic site. This was particularly apparent during summer 2003 when maximum summer temperature at the phenolic site (40.5°C) was 5°C higher than at the non-phenolic site (36.5°C). At the phenolic site this was the highest recorded temperature for the 40-year period of records at this site and clearly extreme in nature (Table 1). These extreme temperatures were associated with a typical summer drought at the phenolic site during June–August (66 mm of precipitation). In contrast, the non-phenolic site experienced 175 mm of rain in this period.

Finally a period of mortality in spring 2001 at the phenolic site was associated with a very low precipitation for the two months (April and May) between census dates in March and June. At the non-phenolic and phenolic sites rainfall in these two months was respectively 213 mm and 75 mm in 2001, 197 mm and 152 mm in 2002, 144 mm and 126 mm in 2003, 83 mm and 128 mm in 2004, 161 mm and 78 mm in 2005.

Local landscape scale: transplants in natural populations

Seedling mortality rates showed significant variation among transplant pairs at all dates during the experiment, except for the springs of 2002 and 2003 and autumn 2002. We detected a significant site × chemotype interaction during winter in the first year of the study, and during autumn 2002 (Table 10), a significant effect of site during winter 2000–2001, autumn 2001, winter 2001–2002, and autumn 2003 and a significant effect of chemotype during autumn 2001. As for the transplants into experimental field sites, the most important rates of mortality occurred following the intense summer drought of 2003, and to a lesser extent after the summer 2001 (Fig. 6). The exceptionally high mortality rates in autumn 2003 reduced sample sizes to almost zero or zero in most plots; hence there was no difference between chemotypes. This heavy mortality curtailed the experiment.

In the phenolic sites (Fig. 6A), the mortality rates of seedlings from non-phenolic plants was significantly higher than that of seedlings of phenolic plants during winter 2000–2001 ($\chi^2 = 11.5$, $P < 0.01$) and after the summer 2001 ($\chi^2 = 34.9$, $P < 0.001$). In the non phenolic sites (Fig. 6B), seedlings of phenolic plants had higher mortality rates than seedlings of non-phenolic plants during spring 2002 ($\chi^2 = 7.8$, $P < 0.01$) and seedlings of non-phenolic chemotypes had significantly higher mor-

TABLE 8. Statistical analysis of size variation in plants of the six thyme chemotypes that either survived or died (survival class) following a given summer period for the four years that this analysis was possible in the two experimental field sites.

	2001		2002		2003		2004	
Source of variation	df	*F*	df	*F*	df	*F*	df	*F*
Phenolic site								
Che	5, 355	2.54*	5, 328	0.54 ns	5, 167	2.78*	5, 104	0.90 ns
Gen(Che)	84, 293	4.52***	83, 245	3.46***	82, 205	2.62***	56, 59	2.26**
Survival class	1, 293	0.03 ns	1, 245	2.19 ns	1, 205	14.41***	1, 59	2.63 ns
Survival class × Che	5, 293	0.89 ns	5, 245	0.30 ns	5, 205	2.33***	3, 59	0.67 ns
Non-phenolic site								
Che	5, 336	0.90 ns	5, 310	1.93 ns	5, 293	0.34 ns	5, 137	1.45 ns
Gen(Che)	84, 264	3.22***	84, 239	3.43***	83, 210	3.13***	74, 92	2.10***
Survival class	1, 264	2.54 ns	1, 239	3.05 ns	1, 210	9.81**	1, 92	2.26 ns
Survival class × Che	5, 264	1.27 ns	5, 239	1.13 ns	5, 210	1.74 ns	5, 92	0.21 ns

Note: Key to abbreviations: Che, chemotype; Gen, genotype.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, not significant.

tality rates than seedlings of phenolic chemotypes in autumn 2001 ($\chi^2 = 8.7$, $P < 0.01$).

Variation in climatic factors among sites at the landscape scale (Fig. 7) was less than that between sites on a geographic scale; the maximum summer temperatures at the non-phenolic (St. Martin-de-Londres) and the phenolic (Prades-le-Lez) weather stations were both 41–42°C. In general, the major differences in climate between non-phenolic and phenolic sites at this landscape level involve differences in annual rainfall (higher in non-phenolic sites) and harsher winters at the non-phenolic sites (Table 1). However, differences in severe winter temperatures were not observed during this study (Fig. 7).

Discussion

Climatic cues are fundamental to the functioning of biological organisms. Despite the fact that natural events of strong selection are notoriously difficult to study (Grant and Grant 1987), we were able to detect ongoing selection on the performance of *Thymus vulgaris* transplants in our study. In particular, we observed differential mortality which was associated with extreme climate events. Modifications of the frequency and magnitude of such events may affect a range of ecological phenomena; including population abundance, species distribution and behavior, and community structure (Easterling et al. 2000). Less is known, however, concerning the adaptive response to such events (Agrawal et al. 2004, Davis et al. 2005). Our results illustrate how climatic selection pressures may contribute to the maintenance of population differentiation, providing novel insights into the nature of adaptive variation to climate extremes and the potential for plant populations to respond to predicted future climate change.

Natural selection during summer drought

In summer 2003, Europe experienced an extreme climatic event with temperatures of up to 6°C above normal and reduced precipitation, causing a dramatic reduction in productivity in forest ecosystems across Europe, with potential effects on a range of ecosystem functions (Ciais et al. 2005). In our experimental phenolic field site, maximum temperatures in 2003 were 4–5°C above those on record (38 years) for this site and other sites in the region. The non-phenolic experimental field site experienced only slightly higher temperatures (in the range of those normally experienced at the phenolic site) and some precipitation in the summer of 2003 and no subsequent differential mortality. In addition, it was equally as hot in the non-phenolic and phenolic natural populations studied at the local landscape scale in 2003 (maximum recorded temperatures for St. Martin-de-Londres and Prades-le-Lez weather stations were both between 41° and 42°C in August 2003).

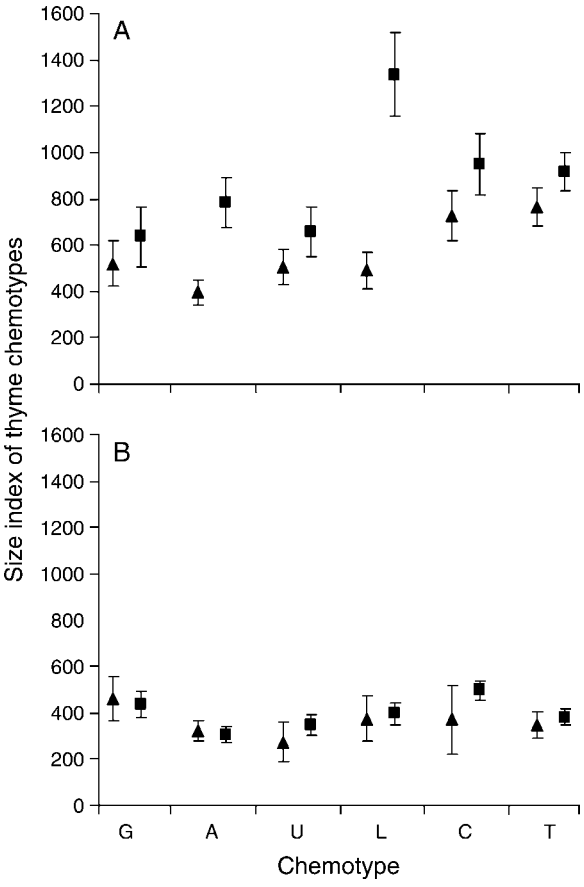

FIG. 4. Size index (mean ± SE) in June 2003 (prior to the severe summer drought of that year) of *Thymus vulgaris* chemotypes that either died (triangles) or survived (squares) the following 11 months in two experimental field sites: (A) the phenolic site and (B) the non-phenolic site. Chemotypes are geraniol (G), α-terpineol (A), thuyanol (U), linalool (L), thymol (T), and carvacrol (C). Size was estimated using an index of plant biomass, $B = (h + w)/2$, where h is the height of the plant and w is its maximum width.

This extreme summer drought had a dramatic impact on the survival of thyme chemotypes in our study. At the scale of the local landscape, the effects were so strong that ~75% of all seedlings that had survived till summer 2003 died in the following autumn, curtailing the experiment. At the geographic scale, phenolic transplants had significantly lower mortality rates than non-phenolic chemotypes following the 2003 summer drought at the experimental phenolic field site. Analysis of all six chemotypes showed that the carvacrol chemotype had a markedly higher survival rate after this summer drought stress (Figs. 1 and 2). In addition to higher survival of phenolic chemotypes relative to non-phenolic chemotypes in the phenolic experimental field site, we also observed a distinction in survival following summer drought between the two phenolic chemotypes, carvacrol and thymol. The former occurs in warmer and drier sites in nature and its natural distribution

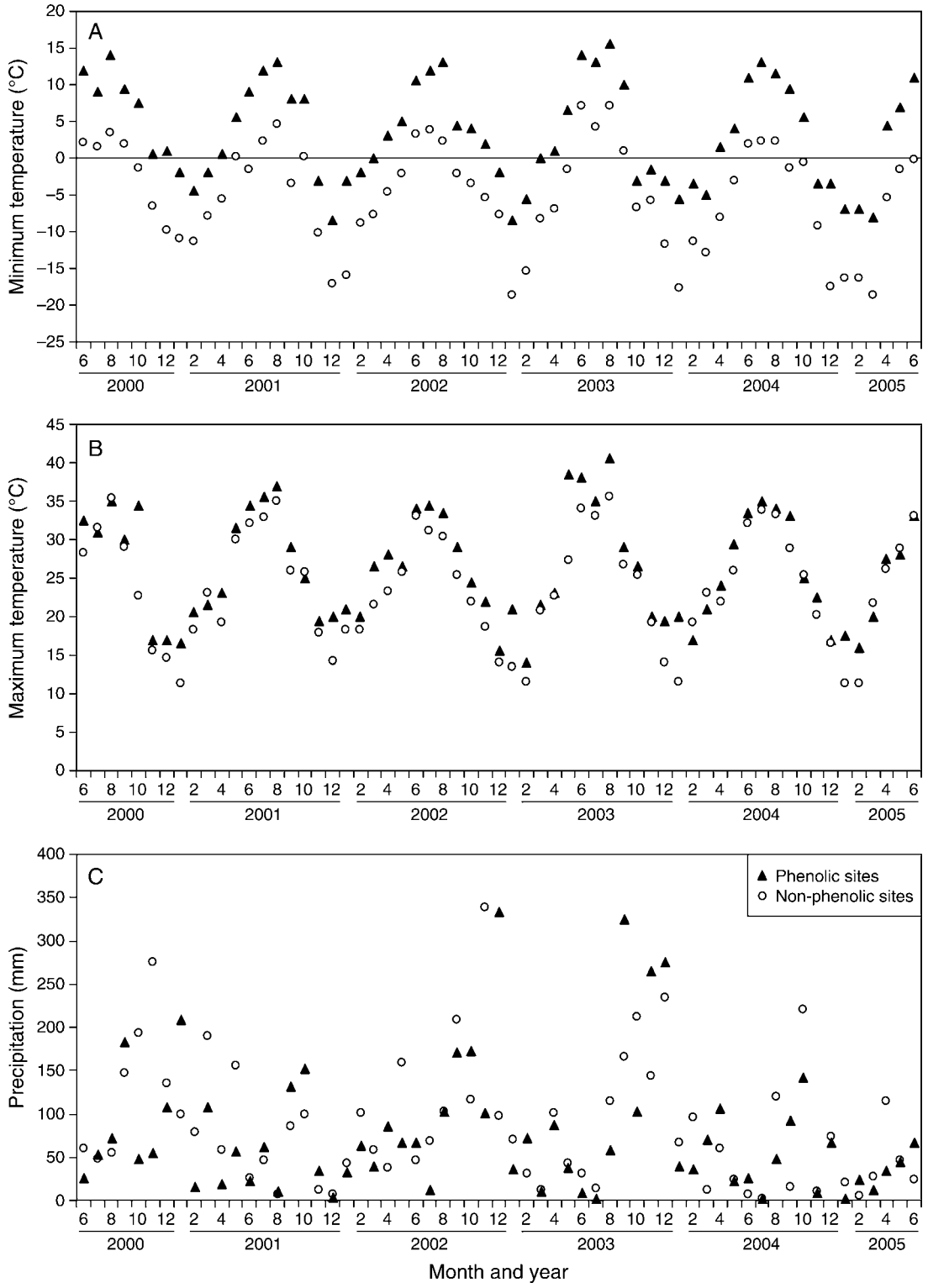

FIG. 5. Monthly climate data, 2000–2005, for the phenolic (solid symbols) and non-phenolic (open symbols) experimental field sites during the six years of study: (A) minimum temperature, (B) maximum temperature, and (C) total precipitation. Data for the phenolic site were recorded in the same field as the experiment. Data for the non-phenolic site are from the Meteo France meteorological station near the town of Séderon, 8 km distant at the same elevation. Months are identified by number: 2, February; 12, December.

TABLE 9. Number of winters with minimum temperatures below two threshold temperatures for eight weather stations in southern France.

Weather station	Available data	Minimum temperature throughout the winter		Minimum temperature in December	
		Below −10°C	Below −15°C	Below −10°C	Below −15°C
1) Séderon	41	41	15	26	9
2) St. Martin-de-Londres	51	29	8	10	2
3) Le Caylar	28	22	4	8	0
4) Le Vigan	41	9	0	3	0
5) Prades	25	9	0	1	0
6) CEFE, Montpellier	38	4	0	0	0
7) Salon de Provence	56	7	1	1	0
8) Valflaunes	15	4	0	1	0

Note: Three weather stations are known to be within the range of non-phenolic thyme chemotypes (sites 1–3); one occurs in a transition zone (site 4); and four occur within the range of phenolic chemotypes (sites 5–8).

encompasses the experimental gardens used in this study, whereas thymol occurs in less arid and cooler sites (Thompson 2002).

Our experiment, which is based on the performance of cloned genotypes of known chemical composition in different environments, thus provides strong support for the idea that individual chemotypes show local adaptation to summer drought. Differential resistance to summer drought stress may involve both resistance to drought stress per se and/or differential tolerance of extreme heat.

First, drought, exacerbated by excessive heat, is likely to be a principal cause of differential mortality at the experimental phenolic site. Gouyon et al. (1986) first concluded that, despite a strong concordance between chemotype distribution and soil type, the primary determining factor in chemotype distribution is likely to be moisture availability. At the experimental phenolic field site, plants with a phenolic chemotype, in particular carvacrol, showed more rapid growth and greater size than all other chemotypes from the moment of transplantation in 2000 to the summer drought in 2003. More rapid growth of seedling offspring from the phenolic chemotypes, particularly those with a carvacrol maternal phenotype, at this site has been documented in previous studies (Thompson et al. 2004, Linhart et al. 2005). We also detected that plants which survived the summer drought of 2003 had a greater vegetative size than plants which died after the summer drought of 2003 (Fig. 4), hence, size differences among and within chemotypes were attenuated or lost at this site in 2004 and 2005.

The greater size of phenolic plants, particularly carvacrol, may have facilitated their resistance to drought stress, since aboveground size may have been positively correlated with below-ground size and thus rooting depth. Rooting ability can vary markedly among species in Mediterranean plants (Kummerow 1981, Chiatante et al. 2006, Hummel et al. 2007) and our results provide a motivation to examine the occurrence of intra-specific variation in root traits in relation to local environmental conditions. Several functional traits also facilitate drought resistance in Mediterranean woody plants (Joffre et al. 1999). Although some of these traits, e.g., water-use efficiency, have been reported to show variation among populations that may be associated with local adaptation to drought (Heschel et al. 2002), there is a lack of information on intraspecific variation and local adaptation of root and leaf functional traits in relation to drought stress in Mediterranean species (Thompson 2005). The generally more rapid growth of plants at the phenolic experimental field site compared to the non-phenolic site may result from differences in soils (Table 2) and the fact that limitation on growth during winter is reduced at this site.

TABLE 10. Results of the statistical analyses (χ^2 and associated probabilities) of mortality rates in pairs of reciprocal transplants between phenolic and non-phenolic sites at the landscape level.

Source of variation	df	2001			2002			2003		
		Winter	Spring	Autumn	Winter	Spring	Autumn	Winter	Spring	Autumn
Site	1	39.2***	0.3 ns	87.4***	48.0***	ns	2.0 ns	1.9 ns	ns	41.2***
Chemotype	1	0.1 ns	1.0 ns	46.7***	1.1 ns	ns	0.2 ns	1.8 ns	ns	2.1 ns
Site × chemotype	1	17.4***	6.4 ns	4.8 ns	0.9 ns	ns	9.1**	0.9 ns	ns	1.2 ns
Transplant pair	4	285.0***	25.0***	78.0***	222.0***	ns	41.4	36.0***	ns	137.0***

Notes: Mortality rates for winter are those for the period between the end of November and early March, those for spring involve the period between the March and May, and those for autumn are from summer to end of November. Differences in mortality rates could not be analyzed statistically in spring 2002 and 2003 due to the absence of mortality in several plots.

** $P < 0.01$; *** $P < 0.001$; ns, not significant.

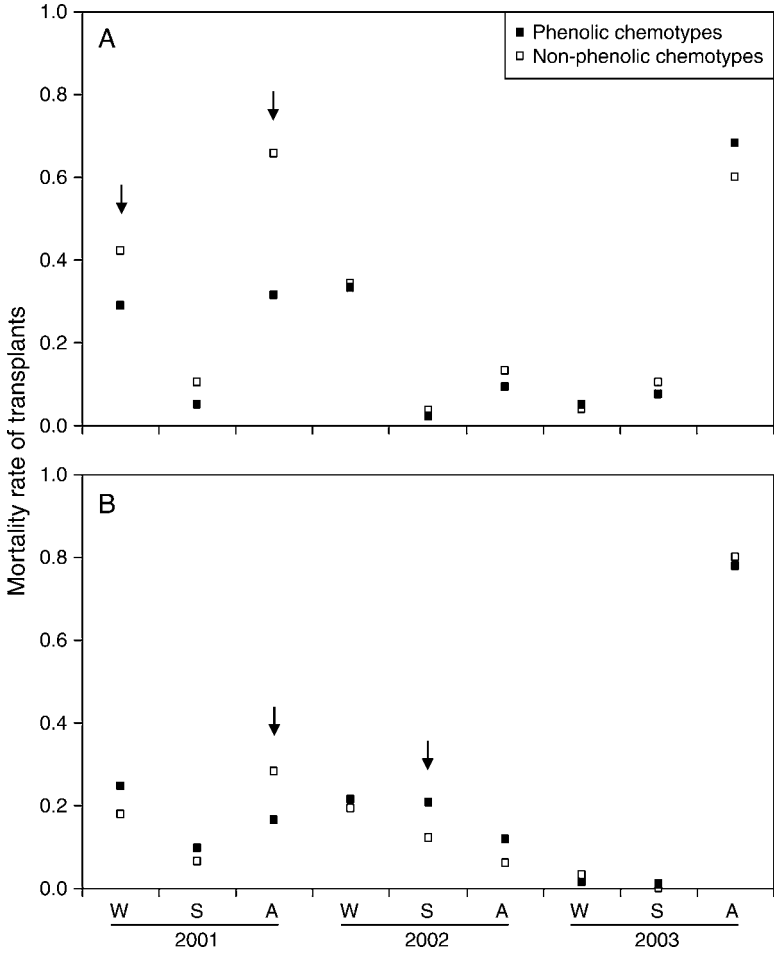

FIG. 6. Mortality rates for seedlings of phenolic (solid squares) and non-phenolic (open squares) chemotypes of *Thymus vulgaris* in reciprocal transplants at the landscape level, 2001–2003: (A) phenolic sites outside the St. Martin-de-Londres basin; (B) non-phenolic sites within the St. Martin-de-Londres basin. Arrows indicate significant differences ($P < 0.01$) in a given site for a given period: S, spring, based on surviving plants in June; A, late summer and autumn, based on surviving plants at the end of November; W, winter, based on surviving plants in mid-March.

Irregularity in the onset of rainfall during the spring may also cause an effective drought stress in the Mediterranean region. In 2001, a very dry spring at the phenolic site, we detected a significant difference in the mortality rate of the six chemotypes, with primarily a higher mortality of the non-phenolic α-terpineol chemotype. At the landscape scale, we also detected greater survival of seedlings of phenolic plants relative to seedlings of non-phenolic plants in phenolic sites during spring 2001. Drought periods prior to the typical summer dry period frequently occur in the Mediterranean region (in 1995 and 2006, similar dry springs have been observed) and may markedly reinforce selection against non-phenolic chemotypes on the more stony and shallower soils in the habitats of phenolic chemotypes. In general, the timing of the onset of summer drought can have a marked effect on plant strategy in the Mediterranean region where early-seasonal drought may select for avoidance traits, e.g., precocious reproduction, while later drought may select for resistance traits such as improved water-use efficiency (Heschel and Riginos 2005).

A second potential cause of differential survival of thyme chemotypes in autumn 2003 at the phenolic experimental site involves possible differences in thermotolerance mediated by monoterpene production and volatilization. The production of essential oils may have various functions in the abiotic Mediterranean environment (Thompson 2005). During summer, secondary compound biosynthesis may act as a "safety valve" to remove excess carbon or energy that cannot be processed by a plant under temperature and/or drought stress (Peñuelas and Lluisà 2004, Peñuelas and Munée-Bosch 2005) or to maintain enzyme systems in a state that allows for rapid reactivation with the return of favorable conditions (Banthorpe et al. 1972). In addition, due to their role in the stability of leaf cell membranes and their anti-oxidant activity, the emission

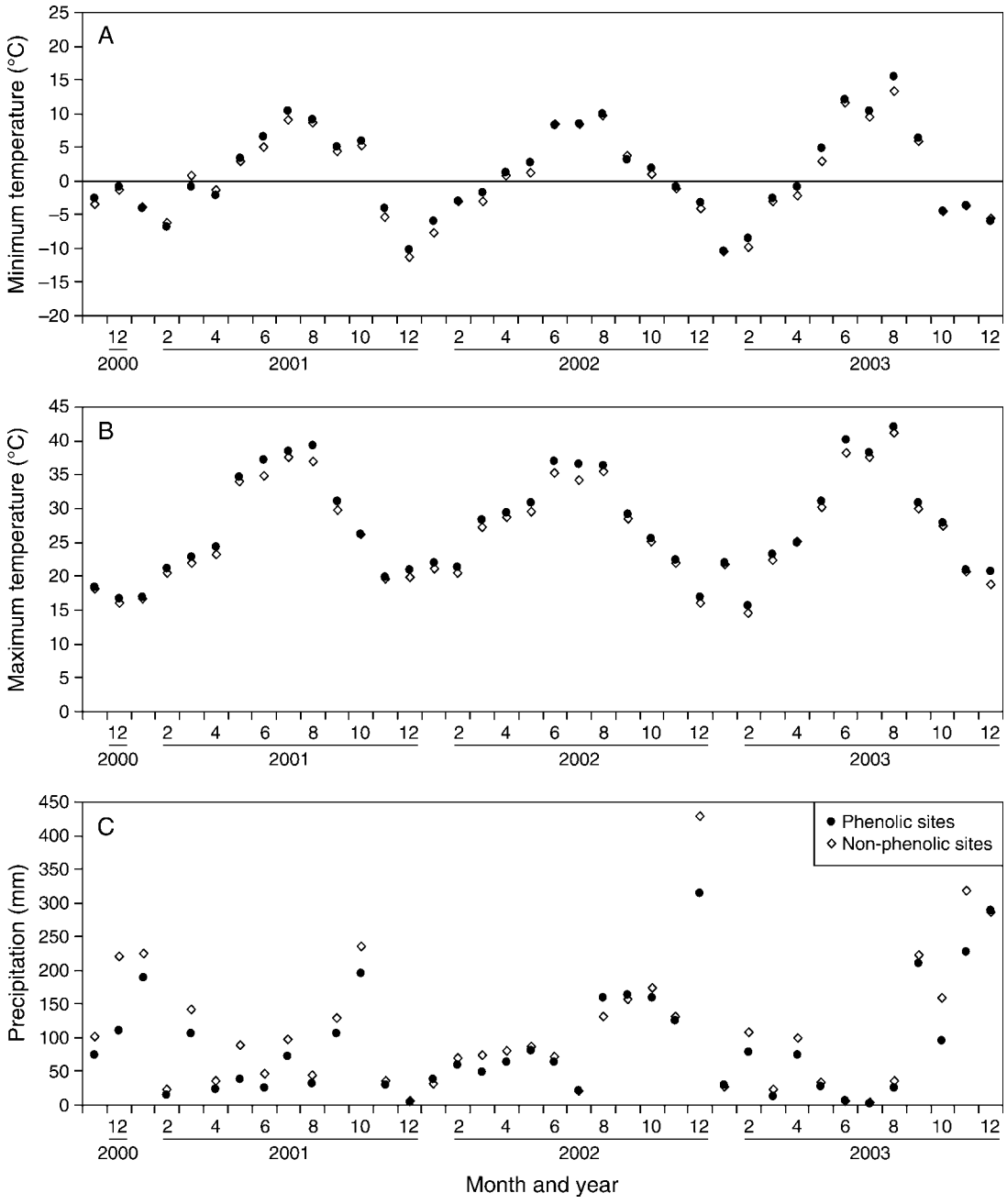

FIG. 7. Monthly climate data, 2000–2003, for phenolic (solid symbols) and non-phenolic sites (open symbols) close to natural populations used for transplants at the landscape level: (A) minimum temperature, (B) maximum temperature, and (C) monthly precipitation. Data were obtained from the Meteo France meteorological stations of Prades-le-Lez (in the zone where the carvacrol chemotype is in majority) and St. Martin-de-Londres (where non-phenolic chemotypes predominate).

of non-organ stored volatile organic compounds such as isoprene and various monoterpenes can improve leaf thermo-tolerance, as shown for Mediterranean oak and pine trees (Sharkey and Sangsaas 1995, Peñuelas and Lluisà 2004). Antioxidant activity has indeed been documented in vitro for the essential oil of other *Thymus* species (Vardar-Ünlü et al. 2003) and phenolic-dominated *T. vulgaris* oil (Sacchetti et al. 2005). Elsewhere, Copolovici et al. (2005) showed experimentally that volatilized α-terpineol is less efficient than α-pinene in stabilizing membrane structure and as an antioxidant for Mediterranean oak (*Quercus ilex*). Hence, although we have no precise experimental evidence for an antioxidant function of trichome-stored monoterpenes, we cannot rule out the possibility of differences among thyme chemotypes in their thermo-

tolerance that may be mediated by their chemical composition.

Freezing resistance, phenology, and cold acclimation

During the winters of 2001–2002 and 2004–2005 at the non-phenolic experimental field site and in the winter 2000–2001 in the natural non-phenolic populations, we observed enhanced survival of non-phenolic chemotypes relative to phenolic chemotypes, primarily because of high mortality of the carvacrol chemotype. Plants which showed high survival rates after intense summer drought (phenolic chemotypes, in particular carvacrol) are thus those which show low survival after severe freezing. It is commonly thought that low temperatures induce cellular responses that minimize disruption of plant water status and maintain the integrity of crucial cell structures and functions in a similar way to those involved in resistance to drought (Thomashow 1999, Verslues et al. 2006). During drought there is a decrease in water uptake due to reduced water availability, while following freezing the formation of extra-cellular ice causes cellular dehydration. Many genes responsive to freezing stress may thus also be responsive to drought (Guy 2003, Blödner et al. 2005). However, molecular studies show that resistance to cold temperature is due to a mechanical constraint whereas drought incurs osmotic and ionic cellular dysfunction (Mahajan and Tuteja 2005). In our study species, natural populations do not incur both severe drought stress in summer and severe freezing in winter. Indeed, populations normally only experience one or the other. Hence, plants show reduced performance after drought or freezing depending on which of these stress factors is the more important selective force in the wild. The absence of a correlated response also suggests that in thyme the physiological mechanisms and functional traits involved in adaptation to drought and freezing may not be the same.

An important feature of frost resistance in thyme chemotypes involves the timing of freezing events and acclimation to freezing temperatures during winter. The ability of plants to acclimate and resist severe freezing stress following mild frost (i.e., not intense enough to cause mortality) is common and is an ecophysiological trait that depends on a suite of genes (Sakai and Larcher 1987, Chen 1994, Thomashow 1999, Guy 2003, Beck et al. 2004). These genes may not be the same as those that determine non-acclimated freezing tolerance (Stone et al. 1993). The ability of a plant to survive freezing temperatures may thus depend on the magnitude, timing and duration of freezing, which should be considered in relation to the phenology and stage of development of the vegetation (Sakai and Larcher 1987). As Inouye (2000:457) commented, "the effects that below freezing temperature (frost) can have at times of the year when it is unusual are an interesting ecological phenomenon that has received little attention."

In our study, we discovered differential requirements for cold acclimation among different populations of *T. vulgaris*. A key point here involves the timing of freezing events in relation to vegetative activity in natural environments. In four of the five winters at the non-phenolic experimental field site, minimum temperatures of less than −15°C occurred. However, we only detected a significant mortality difference among chemotypes in two of these four winters. In both these winters the onset of severe freezing (less than −15°C) occurred early, i.e., in December. This is precisely the timing of freezing temperatures which causes higher mortality and reduced re-growth of phenolic chemotypes relative to non-phenolic chemotypes in controlled conditions (Amiot et al. 2005). These authors showed that severe freezing in January or February does not cause differential mortality of the chemotypes; only in December did they observe a differential resistance to freezing.

Inspection of temperature records for the experimental field sites used in this study, and other meteorological stations which occur in the range of the phenolic and non-phenolic chemotypes shows that whereas severe winter freezing in December (less than −15°C) is common in sites where non-phenolic chemotypes occur, it is extremely rare or unrecorded at phenolic sites (Table 9). Prior to December, autumn temperature minima are such that frost hardening of plants can occur in sites where non-phenolic chemotypes naturally occur but not in sites where phenolic chemotypes naturally occur. However, even when they experience this frost hardening in autumn (i.e., when transplanted to non-phenolic sites), carvacrol plants have weak resistance of severe freezing in December. The reason for this probably lies in the fact that autumn is an important period of regrowth after summer drought for this chemotype. Hence, in December, the carvacrol chemotype is likely to be in a state of active vegetative growth and plants may have little resistance to freezing (Inouye 2000). Observations of the vegetative activity of a range of Mediterranean woody shrubs have shown that although many species limit their autumnal growth to October and November, some species maintain vegetative activity through December (Gratani and Crescente 1997). Severe freezing is frequent in January and February at sites where phenolic chemotypes occur (Table 9) hence they may have evolved tolerance of such temperatures at this time of winter (Amiot et al. 2005). A key feature of freezing resistance in thyme populations may thus be the timing of freezing in relation to autumnal regrowth.

The differential vegetative activity and acclimation ability of *T. vulgaris* chemotypes may be related to differences in seasonal production of secondary compounds. Quantitative variation in monoterpene production in relation to drought (Gershenzon et al. 1978) and other environmental factors (Lincoln and Langenheim 1979) has been reported, as has seasonal variation in monoterpene production (Staudt et al. 2002). In *T.*

vulgaris, the production of phenolic monoterpenes (carvacrol and thymol) declines markedly in winter and peaks in late spring (Passet 1971, Kaloustian et al. 2005), variation that may be associated with the acclimation of these chemotypes to late-winter freezing. Other *Thymus* species, e.g., *T. serpyllum* and *T. pulegioides,* and other aromatic labiates such as *Satureja montana* and *Monarda fistulosa,* which contain primarily phenolic monoterpenes occur in cold winter climates but have aboveground parts that may die back in winter and potentially allow plants to avoid leaf metabolism during winter (K. Keefover-Ring, E. Grøndahl, and B. Ehlers, *unpublished data*). In *T. vulgaris*, preliminary investigations suggest that phenolic monoterpenes may be more self-toxic if trichomes are damaged by freezing (Y. B. Linhart, *unpublished data*). However, damage to trichomes during freezing is most prevalent in non-phenolic chemotypes (Amiot et al. 2005), a feature which may allow them to volatilize essential oils, hence reducing any toxic effects.

Climatic selection and spatial chemotype distribution: past, present, and future

Climatic selection can act directly on plants or indirectly by reducing potential herbivores, seed predators and parasites (Inouye 2000). In our study, stress associated with summer drought (and heat) and winter freezing is directly and differentially experienced by the different chemotypes in experimental sites and not via an effect on herbivores or parasites. The concordance of experimental results in our study in field conditions with those in controlled conditions (Amiot et al. 2005) and with climate data provides strong evidence for local adaptation of thyme chemotypes to the stress imposed by drought and extreme temperatures in summer and early-winter. This contemporary adaptive variation probably evolved as chemotypes initially colonized different environments, where herbivory may have been different in the past due to abiotic conditions. Thyme chemotypes show marked differences in their susceptibility to generalist herbivores (Linhart and Thompson 1995, 1999) and a specialist parasite (J. Amiot and J. D. Thompson, *unpublished manuscript*), with a generally higher susceptibility of non-phenolic chemotypes. The situation in thyme is thus reminiscent of the cline in the frequency of cyanogenic and acyanogenic forms of *Trifolium repens* and *Lotus corniculatus* (Daday 1954*a*, *b*, 1965, Foulds and Grime 1972*a*, *b*, Dirzo and Harper 1982): the least resistant chemotypes to freezing having the most toxic monoterpenes.

A fascinating perspective here concerns the gradual warming of temperature minima observed in non-phenolic sites in the St. Martin-de-Londres basin over the last 50 years (Amiot et al. 2005) which may allow colonization of non-phenolic populations by phenolic plants. In parallel, exacerbated stress due to hotter, drier summers may increase selection favoring the phenolic chemotypes in sites where they currently occur. Such selection may also act on within chemotype variation in drought resistance and eliminate weaker genotypes of the carvacrol chemotype in arid sites (as the loss of genotypic variation in plant size since 2003 in our study would suggest). Local adaptation both between and within chemotypes may thus be ongoing. Our study thus points to the critical importance of moving away from treating species as homogeneous collections in studies of variation in sensitivity to drought and temperature stress (Sakai and Larcher 1987, Lo Gullo and Salleo 1993, Nardini et al. 2000, Peñuelas and Lluisà 2004, Cavender-Bares et al. 2005, Valladares and Sánchez-Gómez 2006). Indeed, we argue that within-species responses to climate change should be incorporated into management and policy guidelines concerning biodiversity dynamics in the Mediterranean flora which contains species of very diverse historical climate origin (Suc 1984, Quézel and Médail 2003, Thompson 2005).

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