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Shifted phenology in the pine processionary moth affects the outcome of tree–insect interaction

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Abstract

In the Mediterranean and temperate regions, an increase in the frequency and intensity of drought events has been recorded, probably due to climate change. In consequence, trees will more frequently experience hydric stress, a condition that can be expected to affect insect–tree interactions, while adaptation mechanisms may be further in course. The effect of tree water stress on the performance of two allochronic populations of *Thaumetopoea pityocampa* was here studied. Namely, we compared a unique population of this insect, in which the larvae develop in the summer (SP), with the typical population having winter larval development (WP), to test the adaptation hypothesis to host plant status. Larvae of each population were fed on needles of young potted *Pinus pinaster* plants under two water supply regimes: (i) well-watered (control) and (ii) subjected to 3 months of drought stress. Compared to control, stressed plants had higher amounts of soluble sugars, phenols, and higher C/N ratio, whereas water content and chlorophylls concentrations were lower. In general, *T. pityocampa* larvae had lower performances on water-stressed plants, as shown by lower survival rates, lower needle consumption, and longer development times. Yet, the detrimental effects of tree stress were only significant for the WP larvae, while SP larvae were able to overcome such conditions. Results demonstrate that tree water stress can negatively affect *T. pityocampa* populations. Furthermore, the evidence is also provided that responses to the physiological condition of the host trees may occur at the population level, as a result of adaptation mechanisms driven by climate change.

Introduction

Global climate change, characterized by increased temperatures and temporal variability of precipitation (IPCC, 2013), is resulting in a higher frequency and intensity of

drought events (Hoerling *et al.*, 2012). In particular, prolonged summer/autumn drought is predicted to occur more often in Mediterranean regions (IPCC, 2007). Warming and drought decrease soil water availability and increase atmospheric evaporative demand, leading to growth and physiological changes in water-stressed trees (McDowell *et al.*, 2008). Effectively, in many regions, increased tree mortality is often associated with drought, and sometimes jointly with insect outbreaks (Anderegg *et al.*, 2015). However, although demographic increase of insect populations can result directly from the positive effects of temperatures on development

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rates or survival (Ayres & Lombardero, 2000), it is not so straight forward to predict how physiological changes in host trees due to drought may affect insect populations (Jactel *et al*., 2012; Anderegg *et al*., 2015).

Tree responses to water stress can alter their attractiveness and physical and nutritional value for herbivorous insects (Mattson & Haack, 1987; Jaleel *et al*., 2009). For example, water stress may reduce tree tissue water content and turgor, decrease transpiration and photosynthesis leading to the accumulation of osmolytes such as amino acids and carbohydrates (Rouault *et al*., 2006; Netherer & Schopf, 2010) that can increase the performances of herbivorous insects (Mattson & Haack, 1987; Caldeira *et al*., 2002). On the other hand, secondary metabolites such as phenols may increase in water-stressed trees, then negatively affecting the performance of herbivorous insects (Koricheva *et al*., 1998; Huberty & Denno, 2004; Kolb *et al*., 2016).

Experimental evidence has shown that insect performance may increase (Mattson & Haack, 1987), decrease (Huberty & Denno, 2004; Branco *et al*., 2010), or remain unaffected (Koricheva *et al*., 1998; Huberty & Denno, 2004) in response to water-stressed host trees. Numerous studies have suggested that different insect feeding guilds could show different responses to drought stress, due to different evolutionary and physiological mechanisms (Koricheva *et al*., 1998; Inbar *et al*., 2001; Huberty & Denno, 2004; Rouault *et al*., 2006). Generally, bark beetles and wood borers benefit from severe drought (Koricheva *et al*., 1998; Huberty & Denno, 2004), sap suckers (e.g., aphids) are impaired (Huberty & Denno, 2004; Rouault *et al*., 2006), whereas the effect on leaf miners, leaf chewers, and gall makers is more uncertain (Larsson, 1989; Koricheva *et al*., 1998; Huberty & Denno, 2004). More intra-specific differentiation and adaptation mechanisms at the population level can modify locally the interactions between herbivorous insects and water-stressed trees. Recent works have highlighted adaptation mechanisms at the population level, due to selective pressures in regard to environmental changes, concerning, for example, the response to temperature (Santos *et al*., 2011*b*). Phenotypic traits associated with plant feeding, such as the size of mandibles and their strength (Zovi *et al*., 2008), or physiological mechanisms allowing insects to cope with plant chemicals (Després *et al*., 2007) may be under strong selective pressure in response to changes in host plants. Within the context of climate change, it is critical to understand if and how insect species are able to cope with drought-stressed hosts (Kolb *et al*., 2016), and if the outcome of such interactions can vary depending on the pest population considered.

Among defoliators, *Thaumetopoea pityocampa* (Denis & Schiffermuller) (Lepidoptera: Notodontidae), the winter pine processionary moth (PPM) is one of the most damaging defoliators of conifers in Europe, and a threat to human health (Battisti *et al*., 2017). The species has one generation per year. Over its wide distribution range across the Mediterranean Basin, adult emergence, mating, and egg laying normally occur in the late summer months. Larvae hatch about 1 month later and live gregariously for several months. They feed on pine needles, preferentially the mature ones grown during the previous years (Jacquet *et al*., 2014), throughout the fall and winter. By late winter/early spring, they leave the nest and search for a pupation site in the soil. Adult emergence occurs the following late summer months (Démolin, 1969). This phenology, characterized by the development of larvae in winter, is typical of the PPM throughout its range, and corresponds to the so-called 'winter populations' (WP).

This typical *T. pityocampa* phenology is modulated accordingly to climate along its latitudinal and altitudinal range, still keeping summer oviposition. By contrast, a unique population displaying a shifted life cycle, in which adults emerge in the spring and the larvae develop throughout the summer (Santos *et al*., 2007) forming the so-called summer population (SP), was discovered in Portugal (in Mata Nacional de Leiria – MNL) ca. 20 years ago. This SP population is genetically differentiated from the local sympatric WP from which it is hypothesized to originate (Santos *et al*., 2007, 2011*a*; Burban *et al*., 2016). The two sympatric populations are reproductively isolated by time as adults of the two populations have non-overlapping, heritable periods of activity (Branco *et al*., 2017). It represents one of the best documented cases of true allochronic speciation (Taylor & Friesen, 2017).

Since its discovery, the SP has been expanding to most parts of the core MNL area (Godefroid *et al*., 2016). In this region, SP and WP populations co-occur in the same stands, feeding on the same host plants (*Pinus pinaster* Aiton). In consequence of this shift in the phenology, the different developmental stages of the SP are subjected to different climatic conditions as compared to the WP. In particular, SP larvae develop under high summer temperatures, when trees are generally water stressed, while WP larvae occur in fall and winter, when temperatures are much lower and rainfall is frequent (fig. 1).

In this study, we capitalize on the occurrence of the two genetically differentiated populations of PPM, to investigate the potential adaptive response to host mediated-water stress. To achieve this, we first analyzed the effect of drought on needle chemical and physical characteristics, and then compared the performance of the two PPM populations on control and water-stressed host trees during the first larval stages.

We hypothesize that SP larvae can feed more efficiently on tougher needles, and thus perform better than the WP larvae on water-stressed pines. To test our hypothesis we developed an experimental design in order to (i) determine if larval performances were affected by a moderate water stress of the pine host, and (ii) compare the capacity of the two PPM populations to develop on drought-stressed trees, which may underline adaptation mechanisms in the context of climate change scenarios.

Materials and methods

Tree material and treatments

Five-year-old trees of *P. pinaster*, about 1.8 m high, obtained from a forest nursery (Aliança Florestal, Portugal) were transplanted to pots (volume 25 l, height 35 cm) filled with a substrate of turf, vermiculite 2:1 (v/v), and nutrients (Projar Professional). During 2 months, trees were kept outside, well-watered, and fertilized weekly (NPK 4:3:6 and micronutrients) (Bio-Grow Biobizz) until the experiments started.

Trees were then transferred to a greenhouse and randomly assigned to two different hydric treatments: (i) non-limiting water input (control) and (ii) moderate water stress. Ten replicates per treatment were used. As the larvae of the two *T. pityocampa* populations develop in different seasons, the feeding experiments were performed accordingly in July-August 2015 for the SP trial and in October–November 2015 for the WP trial. For both trials, the onset of the tree water stress started 3 months before larval hatching (i.e., in
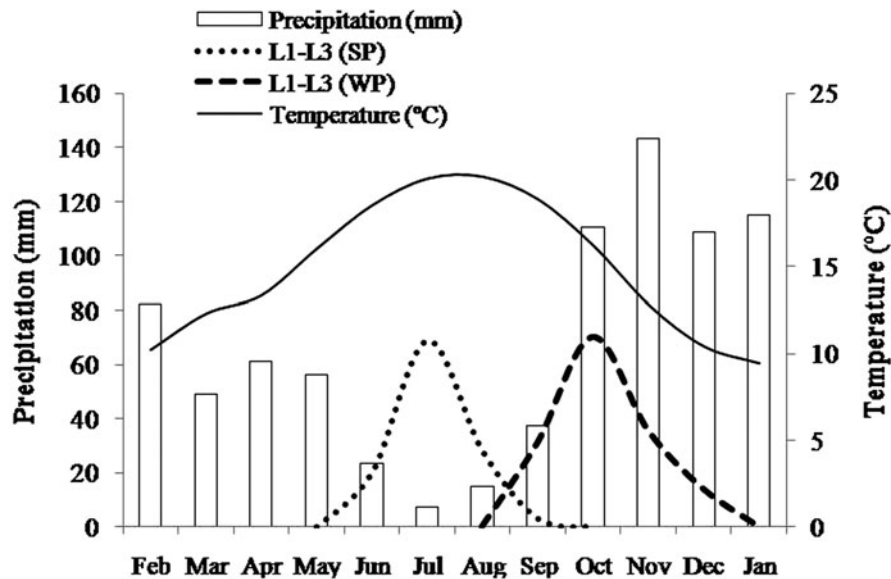
Fig. 1. Distribution of rainfall and mean monthly temperature in the studied area (meteorological station of Monte Real, Leiria, data for the period 1986–2016). Distribution curves of the young larval stages (L1 to L3) for the two populations, SP and WP, based on the respective males flight period (Santos *et al.*, 2011*a*); the embryonic development (ca. 1 month), and the total period for L1, L2, and L3 development (ca. 2 months).

April 2015 for the SP trial and July 2015 for the WP trial). Plants were kept in greenhouse conditions: 14/10 h (light/ dark), mean air temperature (±SE) 26C ± 2°C, relative humidity 50 ± 2%, during the SP trial; and 12/12 h (light/dark), 23 ± 2°C and 60 ± 2% during WP trial.

Water stress was imposed through the incomplete replacement of the water transpired from the plant, which was estimated by weighting the tree pots weekly. Replacement of the water lost was achieved by successively reducing this amount by 10% per week, until moderate water stress was attained as defined by the leaf water potential, and then maintained at that level. Water from the pots was only lost through tree transpiration as the top of the pots was closed with a white plastic sheet to avoid direct evaporation from the soil. Well-watered trees were watered to soil field capacity once or twice per week, depending on air temperature. Air temperature and relative humidity (reference sensor) inside the greenhouse were continuously monitored with an Em50 datalogger (Decagon Devices Inc, Washington, USA).

Plant water status

In both SP and WP trials, tree water condition was monitored by measuring the predawn needle water potential (ψ_{pd}) in a pair of 1-year-old needles (i.e., grown the previous year) for all trees, using a Scholander pressure chamber (PMS-1000, PMS Instruments, Corvalis, Oregon, USA). The water potential was evaluated twice for each trial, 1 month previously to the beginning of the test and at the start of the feeding experiments. Tree water status was also determined by measuring the needles relative water content (RWC), a parameter that reflects the metabolic activity in tissues and is used as an index for dehydration tolerance (Anjum *et al.*, 2011). Ten needles per tree were collected at dawn and immediately weighted to obtain their fresh weight (FW); next they were placed in distilled water for 24 h in the dark, to achieve turgid

weight (TW) and finally samples were dried at 80°C for 48 h to obtain their dry weight (DW). RWC values were calculated as: RWC = (FW − DW)/(TW − DW) × 100.

Foliar analyses

We assessed relevant needle biochemical (chlorophyll, soluble sugars, phenols, carbon, and nitrogen), and physical (hardness) characteristics using 1-year-old fresh needles from controlled and stressed trees from SP and WP trials. The needles for chemical analysis were collected when feeding trials started, and were stored at −80°C. For chlorophyll and soluble sugars, three samples from each tree were analyzed from different tree canopy expositions, while for phenols, carbon, and nitrogen, one composite sample per tree, comprising needles collected from different tree orientations was used. Still, in both cases, representative samples were obtained for each tree.

Chlorophyll content (*a*, *b*, and total) was determined using a modified version of Arnon (1949) method. Extractions were made from 0.1 g of fresh material with ethanol 80% (v/v). After centrifugation at 13,000 rpm for 5 min, absorbance was read at 663 and 645 nm for chlorophyll *a* and *b*, respectively. Soluble sugars (glucose, fructose, and sucrose) were measured as described by Stitt *et al.* (1989) based on the enzymatic method of Jones *et al.* (1977) using 0.1 g of fresh needles. Total phenols content were determined based on the Folin–Ciocalteu method (Folin & Ciocalteu, 1927) using 0.5 g of dried (48 h at 60°C) fine powered needle. Calibration curves were created using standard gallic acid solutions and the amount of total phenols was expressed in mg of gallic acid equivalents per g of dry weight.

Total needle nitrogen concentration was analyzed by a modified Kjeldahl analysis (Berthelot reaction, modified) (Searle, 1984) using 0.5 g of fine-milled needles. Samples were digested with 5 ml sulfuric acid (98%) and selenium as

a catalyst for 3 h at about 350°C. On cooling, it was diluted to 50 ml with distilled water and shaken thoroughly. Quantifications were made by molecular absorption spectrophotometry in the visible region (660 nm). The N-concentration of the digests was determined on a continuous flow analyzer (Skalar SanPlus System, Breda, The Netherlands), while total needle carbon (C) was determined by quantification of CO_2 by infrared after combustion at 1200°C (Analytik Jena EA 4000, Jena, Germany). Results were expressed as a percentage of dry matter in both cases, and the C/N ratio was determined.

Needle toughness was measured with a penetrometer that determined the strength necessary to rip the needle epidermis, as an indicator of the strength that larvae would need when feeding on the needles. Ten pairs (1-year-old) needles per tree for each plant treatment and trial (SP and WP), were measured for toughness in the middle of the needle top surface. Values were expressed in units of force (Newton).

PPM population feeding trials

Larvae of the two PPM populations were obtained from egg masses collected in the field, from *P. pinaster* trees. For SP populations, egg masses were collected at the end of June, in Nazaré (39°36′50.70″N; 9°04′25.80″W), and for WP, collections were made at the end of September, in Setúbal Península (38°34′42″N; 9°07′35″W). Egg masses were individually placed in glass test tubes and kept at room temperature (22–28°C) until larval hatching. For both populations, 30 newly hatched larvae were used in the trials that started at the beginning of July for SP and at the end of September for WP. The feeding experiments were carried out in glass Petri dishes (140 mm in diameter) where larvae were fed, following the feeding protocol described in Hódar *et al.* (2002). Each Petri dish corresponded to one tree of each treatment. In total, each PPM population feeding trial consisted of ten replicates per treatment. At each time, four pairs of needles (1-year-old), inserted into the wet floral foam to avoid excessive loss of water, were offered to the larvae. The Petri dishes were placed in rearing chambers with controlled temperature (25 ± 2°C) and relative humidity (60 ± 2%) within the range of optimal conditions for larval development (Démolin, 1969), at the entomological laboratory of Forest Research Centre, Lisbon University. Larvae were inspected every 3–4 days for food replacement, cleaning, feces removal and counting of dead larvae. Larval performance was estimated by larval survival, consumption rate, feces production and development time (number of days to reach the 2nd instar) for each treatment. The development time until 3rd instar was not estimated due to the low number of individuals reaching this stage, especially on the WP trial. The consumption rate was determined by measuring the total length (mm) of needles consumed. Feces production was calculated based on the feces dry weight. Both consumption and feces produced were then divided by the number of individuals alive at each sampling date, to obtain average values per individual. The feeding trials were conducted until larvae reached the 3rd instar, after which they become urticating and thus difficult to handle.

Statistical analyses

Independent sample *t*-tests were performed to compare needle water potential between treatments (stressed and control), for each date of measurement. Levene's test was used to test for equal variances between groups. Whenever equal variances were not assumed, degrees of freedom were adjusted. RWC, chlorophylls, soluble sugars, and toughness were analyzed through Generalized Estimating Equations (GEE), with normal distribution and identity link function. Each tree was a replicate and needles from the same tree were repeated measurements accounting for within-subject effect. Consumption and feces production by the larvae were also analyzed through GEE, with normal distribution and log link function. Each rearing Petri dish was a replicate and observations on different dates were considered repeated measurements. Generalized linear models (GLM) with log link function were used to test for differences between treatments of C/N values and larvae development time. A two-way ANOVA was used for analyzing the effects on total phenols. For all models, the factors tree treatment and trial were considered as categorical predictable variables. Linearly independent pairwise comparisons among the estimated marginal means were conducted using Least Significant Difference test. For GLM and GEE models, statistics are presented in the form of Wald χ^2. Degrees of freedom were 1 in all cases. The mean number of days of larval survival for each trial was estimated by the Kaplan–Maier method. Individuals alive at the end of the period were used as censored ones. The Log Rank (Mantel–Cox) test was used to compare mean survival between groups of larvae from different treatments. Statistical analyses were conducted with IBM SPSS statistics 23 software.

Results

Tree parameters

Two months after the stress treatment started, that is 1 month before the beginning of the feeding trials, the needle predawn water potential (Ψ_{pd}) was already significantly lower in stressed than in control trees, for both SP and WP trials (fig. 2) (Trial SP: *T*-test = −7.344, df = 10.52, *P* < 0.001; Trial WP: *T*-test = −5.120, df = 9.98, *P* < 0.001). Differences increased until the onset of the feeding trial (0 month) (fig. 2) (Trial SP: *T*-test = −12.489, df = 18, *P* < 0.001; Trial WP: *T*-test = −6.873 df = 9.79, *P* < 0.001). Levene's test further evidenced unequal variance with higher variance on the stressed trees in comparison with control ones for most cases.

Accordingly, needles RWC was higher for control (Trial SP = 94.4 ± 0.25%; Trial WP = 92.7 ± 0.17%) than for stressed trees (Trial SP = 80.9 ± 0.16%; Trial WP = 77.6 ± 0.52%). Differences between tree treatments, considering both trials, were statistically significant (Wald χ^2 = 2107.193, df = 1, *P* < 0.001).

Tree treatment produced significant effects on all biochemical compounds analyzed, as well as on needles toughness (tables 1 and 2). Consistently, total soluble sugars, phenols, and toughness were higher in water-stressed trees, in both trials. An opposite trend was found for chlorophylls, which were higher on control trees than on stressed ones (table 2). The effect of the trial (SP or WP) was only significant for the carbon/nitrogen ratio and not for the remaining parameters (table 1). C/N was higher on stressed trees but differences were only significant for the SP trial. The interaction trial × treatment was never significant (table 1). Total soluble sugars were represented mainly by sucrose accounting for 67 and 74% in control and stressed trees, respectively.
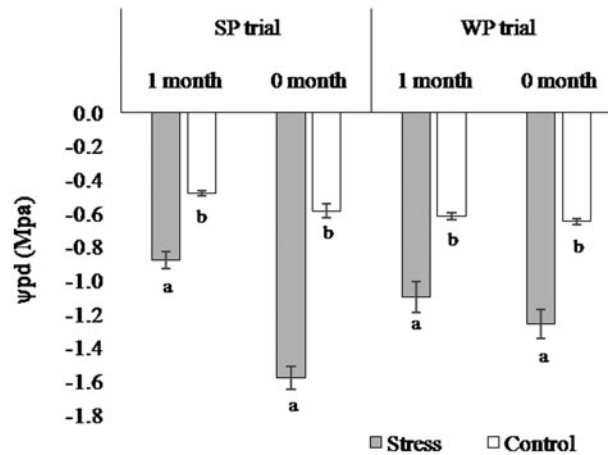
Fig. 2. Predawn needle water potential (Ψ_{pd}, MPa), (mean ± SE), on drought-stressed and control trees, of trials SP and WP, measured 1 month and just (0 month) before the larvae feeding experiment started.

Table 1. Results of statistical analysis on needles' traits from two treatments (stressed and control trees) and two trials (SP and WP).

Needles trait		C/N	Sugars	Chlorophylls	Phenols	Toughness
Treat.	Test	4.772	12.640	9.853	14.532	36.456
	P value	*0.029*	*<0.001*	*0.002*	*0.001*	*<0.001*
Trial	Test	12.373	1.125	1.018	0.199	1.108
	P value	*<0.001*	0.289	0.313	0.658	0.293
Treat.	Test	0.517	0.070	0.277	1.713	1.353
x Trial	*P* value	0.472	0.791	0.598	0.199	0.245

For C/N, sugars, Chlorophylls and Toughness, statistical test corresponds to Wald χ^2 (df = 1) obtained with Generalized linear models; for phenols, a two-way ANOVA was used ($F_{1,36}$). For all models, the factors treatment (Treat.), trial, and its interaction term were considered. In italic P-values <0.05.

Table 2. Physical and chemical parameters analyzed on stressed and control trees and on SP and WP trials (mean ± standard error).

Trial/Treatment	C/N	Sugars (μmol.g^{-1})	Chlorophylls (mg.g^{-1})	Phenols (mg.g^{-1})	Toughness N (x100)
SP					
Control	0.94 ± 0.08[a]	15.6 ± 0.5[a]	0.77 ± 0.03[a]	51.6 ± 2.4[a]	3.47 ± 0.15[a]
Stress	1.14 ± 0.07[b]	19.4 ± 1.2[b]	0.66 ± 0.03[b]	61.6 ± 3.9[b]	4.01 ± 0.11[b]
WP					
Control	1.24 ± 0.09[b]	16.9 ± 0.6[a]	0.72 ± 0.03[a]	48.1 ± 4.6[a]	3.22 ± 0.10[a]
Stress	1.37 ± 0.08[b]	20.2 ± 1.4[b]	0.65 ± 0.02[b]	68.6 ± 4.0[b]	4.02 ± 0.08[b]

Different letters mean statistically significant differences between treatments determined by multiple pairwise comparisons through Least Significant Difference (α = 0.05).

Overall, glucose accounted for only 6% and fructose to 23.5% of total soluble sugars content.

Larval parameters

Log Rank (Mantel–Cox) test showed an overall significantly lower survival of larvae fed with stressed trees in comparison with control ones (χ^2 = 71.837, df = 1, *P* < 0.001). Still, pairwise comparisons (i.e., stress vs. control groups) for each PPM population evidenced significant differences only for WP, with the survival of the larvae fed on stressed trees decreasing sharply as from the first days of feeding (fig. 3). By the end of the trial, mortality reached almost 100% for larvae fed on stressed trees, but was also very high for the control

ones. Kaplan–Meyer estimates for the average number of days of survival for SP larvae were 21.1 ± 0.7 and 19.6 ± 0.6 days, respectively, for larvae fed on control and stressed trees. However, estimated survival (expressed as the number of days) was lower for the WP larvae than for the SP, especially for those fed on needles from stressed host trees (18.8 ± 0.7 and 11.5 ± 0.5 days, on control and stressed hosts, respectively).

The mean development time needed to reach the 2nd instar was shorter for larvae fed on control trees (12.3 days ± 0.5) than for larvae fed on stressed trees (13.8 days ± 0.6). However, differences were not significant (Wald χ^2 = 3.685, *P* = 0.055). Similarly, differences between PPM population feeding trials were borderline (Wald χ^2 = 3.630, *P* = 0.057), with larval development being on average faster in SP than
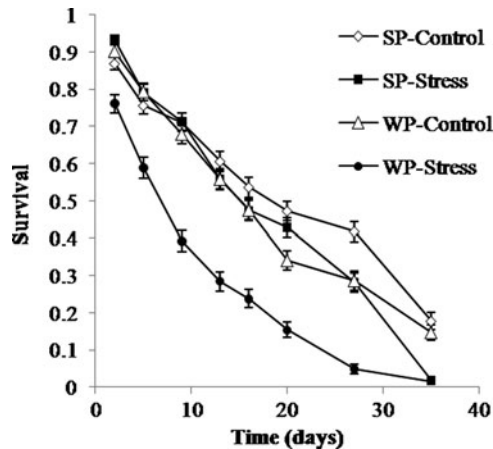
Fig. 3. Larvae survival estimates (±SE) of the two phenotypic divergent populations of *T. pityocampa* (SP and WP) reared with pine needles of drought stress and control trees.
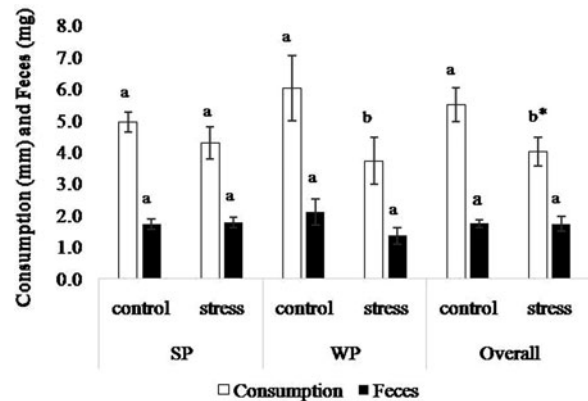

Fig. 4. Consumption (in millimeters) and production of feces (in milligrams) by the larvae of both trials (SP and WP) on drought-stressed and control trees (mean ± SE). Different letters mean statistically significant differences between treatments determined by multiple pairwise comparisons through Least Significant Difference ($\alpha = 0.1$ and $\alpha = 0.05$ – indicated with *).

WP. The interaction term was not significant (Wald $\chi^2 = 0.193$, $P = 0.660$).

Tree stress had an overall significant negative effect on the needle consumption (Wald $\chi^2 = 4.439$, $P = 0.035$). Overall, average consumption was 5.48 ± 0.54 mm per larvae.day^{-1} in the control group and 4.00 ± 0.45 mm on the stressed plants (fig. 4). On average, there were no differences between the SP and WP trials (Wald $\chi^2 = 0.121$, $P = 0.728$) and the inter-action term plant treatment x trial was not significant (Wald $\chi^2 = 1.328$, $P = 0.249$). Yet, pairwise comparisons between the control group and stressed plants showed that differences were higher for WP ($P = 0.072$) than for SP ($P = 0.256$). Concerning the production of feces, no significant effect was found for treatment (Wald $\chi^2 = 1.731$, $P = 0.188$), population (Wald $\chi^2 = 0.003$, $P = 0.957$), or the interaction term (Wald $\chi^2 = 2.326$, $P = 0.127$).

Discussion

Overall, we observed a tendency for a negative effect of plant water stress on larval performance, reflected on a lower larval survival, slower development, and lower con-sumption rate. Yet, a negative effect was significant only for the WP, mainly resulting in a lower larval survival. SP larvae survived, on average, 2 and 8 more days than WP larvae when fed on control and stressed plants, respectively. Taking into account the shifted phenology and the recent and local origin of the SP (Leblois et al., 2017), it can be considered that SP larvae have been adapting to summer dryer conditions.

The negative effect on the performance of the larvae may be explained by changes in plant chemical and physical traits. Water-stressed plants registered higher amounts of soluble sugars, higher C/N ratio, and phenols; needles were tougher, whereas water content and chlorophylls concentrations were lower. Water-stressed trees reached similar stress values and chemical traits for SP and WP trials, which suggest that the differences found in insect performance are not due to a differ-ence in plant treatment. The leaf water potential of the stressed trees in both trials was in the range of values measured in *P. pinaster* trees under field conditions during the summer (e.g., Loustau et al., 1996).

Most of the results obtained in this work are supported by literature references. Several studies document effects of drought stress in plants reflected in leaf water potential, RWC, turgor, transpiration rate, and photosynthesis (Huberty & Denno, 2004; Jaleel et al., 2009). Consequently, many components of plant nutritional quality are modified with possible effects, either positive or negative, on the per-formance of associated insects. The outcome may also depend on how insects feed on plant tissues (Huberty & Denno, 2004). Several studies concluded that drought often increases the concentration of sugars and inorganic ions, as well as nitrogen compounds, such as amino acids, in pant tissues thus enhan-cing foliage nutritional quality for some herbivore insects (Huberty & Denno, 2004; Rouault et al., 2006; Netherer & Schopf, 2010; Jactel et al., 2012). Yet, in the system here studied, the possible positive effect of increased sugar concentration was insufficient to counteract the negative effects of plant drought stress on larvae. At the same time, our results con-firmed that a moderate water stress applied to *P. pinaster* trees led to a significant decrease in chlorophyll concentration and higher C/N ratio, similarly observed by Chen et al. (2015) for sorghum seedlings.

On the other hand, a negative effect on insect performance can occur due to a decrease in leaf water content, increased leaf toughness or to an increase of secondary metabolites concen-tration. According to Henriksson et al. (2003), leaf water con-tent is one of the most important factors influencing larval growth of *Epirrita autumnata* (Lepidoptera). In our study, stressed plants revealed lower values of leaf water potential and RWC than control ones which could explain in part, the negative effects observed on larval performance. A compensa-tion mechanism was not observed for PPM, as consumption was lower when feeding upon needles of stressed plants than from non-stressed ones. We also found that stressed nee-dles were tougher than control ones, which could explain the lower larval consumption observed in water-stressed plants. Interestingly, a previous study (Santos et al., 2013) showed that SP eggs are generally bigger that WP eggs, suggesting that SP neonate larvae are also bigger, with stronger mand-ibles. This trait could allow SP young larvae to feed more efficiently on tougher needles, and thus perform better than

WP larvae on water-stressed pines. This could explain the significant difference in survival observed, since the first days when neonate larvae were present (fig. 3). A similar hypothesis was suggested by Zovi *et al.* (2008) who documented a case of local adaptation of *T. pityocampa* larvae to soft vs. tough needles. It is worth noting that overall mortality was very high by the end of the feeding trial, even in the control groups, which is explained by the high sensitivity of this insect species to laboratory rearing conditions during prolonged periods (Berardi *et al.*, 2015; Branco *et al.*, 2017). The production of feces was not affected by plant treatments. This particular outcome, lower consumption but unchanged feces production, suggests a reduction in digestibility of the pine needles following water stress. We could not calculate digestibility indices to confirm this hypothesis due to the gregarious behavior of this insect.

Concentrations of several classes of defensive secondary compounds such as cyanogenic glycosides, phenols, terpenoids, and alkaloids may also increase in plant tissues in response to moderate drought stress (Mattson & Haack, 1987; Kolb *et al.*, 2016). In both trials, total phenols were significantly higher in water-stressed plants compared to control ones which might have further accounted for the negative effect on larvae, especially on their survival. A higher detoxifying capacity of SP vs. WP larvae could have occurred thus explaining that the negative effect of stress was only significant for the WP. This hypothesis could be explored via dedicated experiments and analyses of differential expression of detoxification genes in both populations in stress vs. control groups.

The overall better performance of SP larvae on stressed plants by comparison to WP may indicate an evolutionary adaptation to cope with the changes resulting from host plant drought stress. In fact SP larvae feed during summer months, facing environmental conditions such as high temperatures, low air humidity, and low rainfall (fig. 1), all contributing to plant drought stress, whereas WP larvae feed during fall and winter, when the tree's physiological parameters are supposed to be less stressed. Previous studies demonstrated that the SP has already undergone some ecological divergences in relation to the WP, namely regarding the upper thermal tolerance limits of the larvae (Santos *et al.*, 2011*b*) and of the eggs (Rocha *et al.*, 2017), as well as some reproductive traits, such as fecundity and egg size (Santos *et al.*, 2013). Also, SP larvae had a faster development than WP, with more larvae reaching the 3rd instar in both treatments. Overall, results suggest that the SP is better adapted to develop on stressed hosts and might have a competitive advantage over the WP in some specific climatic conditions.

Conclusions

Under present climate change scenarios, characterized by rising temperatures and moderate to severe drought stress episodes, plant drought stress will accordingly increase and modulate plant–insect interactions. This work proved that water stress affected negatively the performance of young larvae of the pine defoliator species studied. A major finding revealed that the SP population, with summer larval feeding, was less affected by stressed trees than the WP population, indicating that some adaptation to the physiological status of the host plant has occurred following the phenological shift. This could be due to stronger mandibles, ability to detoxify secondary compounds, and/or capacity to withstand feeding upon low water contents foliage. The present study

also highlights the relevance to understand evolutionary and adaptation mechanisms that might occur in the context of global climate change and how this will affect species predictions under different scenarios.

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