

SCIENTIFIC NOTE

# Effects of substrate water content on maturation feeding of *Monochamus galloprovincialis* (Coleoptera: Cerambycidae)

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## Abstract

We test whether tissue moisture content affects settling and feeding behaviours of *Monochamus galloprovincialis*, a forest insect that feeds on multiple pine species and is a vector of tree disease. In a watering experiment using potted Aleppo pine trees, *Pinus halepensis* Miller (Pinaceae), water deprivation reduced mid-day shoot water potentials and corresponded to lower phloem water content. In short-term choice assays allowing prereproductive beetles to select among *P. halepensis* phloem for maturation feeding, beetles preferred to settle and initiate feeding on phloem with lower moisture content and over a 24-hour period consumed more phloem from oven-dried phloem punches. No differences in settling and feeding preferences between males and females were observed. In no-choice feeding assays where beetles were confined to either “dry” or “fresh” shoots (moisture differential ~10%) over a five-day period, beetles fed on fresh shoots excreted on average 38% more frass, potentially consistent with higher consumption requirements. Our data suggest that water input affects shoot water potentials of Aleppo pine and corresponding phloem water content, which influences feeding preferences of newly emerged *M. galloprovincialis*.

## Introduction

Conifer forests globally have been heavily impacted by pine wilt disease, caused by the pathogenic pine wilt nematode *Bursaphelenchus xylophilus* (Steiner and Buhrer, 1934) Nickle, 1981 (Parasitaphelenchidae), which damages and kills several economically and ecologically important pine species (Pinaceae) (Mota and Vieira 2008). Pine wilt nematode is associated with beetles in the genus *Monochamus* (Coleoptera: Cerambycidae) and is transmitted during maturation feeding (Naves *et al.* 2007). In Europe, *Monochamus galloprovincialis* (Olivier) (Coleoptera: Cerambycidae) is the primary vector of pine wilt nematode: the beetle is highly mobile and has a distribution spanning most of Europe (Naves *et al.* 2016; Haran *et al.* 2018). As a potential disease vector, patterns of host use by *M. galloprovincialis* are important for understanding and predicting factors that may drive pine wilt nematode spread.

We investigated whether the feeding preferences of *M. galloprovincialis* vary in response to water content within tree tissues. The majority of nematode transmission occurs during

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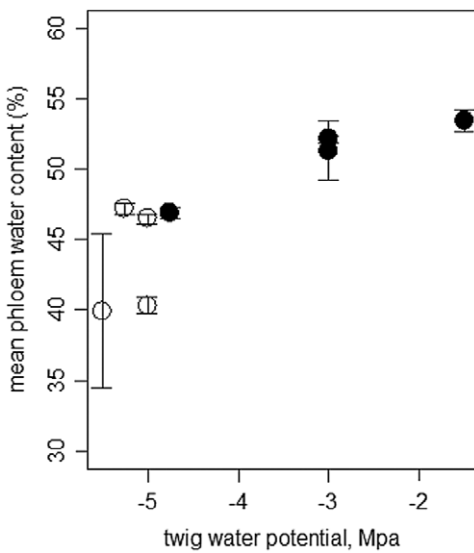
maturation feeding by new beetle adults in spring and early summer (Naves *et al.* 2007). Consequently, feeding by newly eclosed beetle adults before dispersal, mating, and oviposition may be the most important life stage for understanding whether tree water status has important epidemiological effects on *M. galloprovincialis* feeding preferences. We tested whether water deprivation reduces water potential and water content of tree material (bark and phloem) fed on by *M. galloprovincialis*, and we subsequently evaluated whether newly emerged *M. galloprovincialis* settling and feeding preferences and phloem consumption vary in response to substrate water content.

We first examined whether water input was sufficient to drive variation in water potential of tree tissues. Potted *Pinus halepensis* Miller (Pinaceae), a preferred host of *M. galloprovincialis* (Naves *et al.* 2006), were used to determine water input–phloem water content relationships. Trees ( $n = 8$  total study trees) were 14 years old, approximately 3 m in height, and approximately 15 cm in basal diameter at the time of the test. Study trees were grown outside in Oeiras, Portugal, in 50-L pots containing field soil and received 15 L of water every other day. To induce water stress, four trees were randomly selected for water deprivation, and the four remaining trees continued receiving water normally. Deprivation occurred between 25 July and 10 August 2022, during which time environmental conditions were warm and sunny with a mean temperature of  $21.3 \pm 0.5$  °C, a relative humidity of  $73.5 \pm 1.2\%$ , and a day length of 14 hours (range: 14:25 [25 July]–13:54 [10 August]). This interval (~15 days) was selected because earlier studies have shown that two weeks is sufficient to detect differences in the water balance of conifer saplings under different drought treatments (Hartmann *et al.* 2013). No rainfall occurred during the deprivation period. After deprivation, mid-day water potentials (Mpa) of five terminal twigs taken from secondary branches of the lower canopy of each tree were measured using a pressure chamber (PMS Instruments, Corvallis, Oregon, United States of America). These subsamples were averaged to provide the mean shoot water potential. To measure the mean phloem water content, five bark and phloem subsamples were also collected from each tree using a metal punch (1.5 cm diameter, 5 mm thickness). The water content of replicate punches was estimated by  $[(\text{wet weight (mg)} - \text{dry weight})/\text{wet weight}] \times 100$ , and replicates were averaged to a single value for each tree. Welch's *t*-test, which allows for comparisons of two sample means with unequal variances, was used to compare differences in mean shoot water potentials between the deprivation and control group, and logistic regression was used to fit mean percent phloem water content to shoot water potential (function = “glm”, family = binomial). All statistical analyses were performed in R, version 4.2.2 (“Innocent and Trusting”; R Core Team 2022).

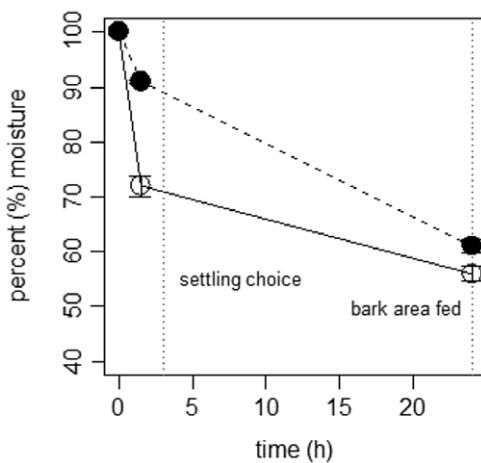
A significant difference was observed between mean shoot water potential in the water deprivation group ( $-5.2$  Mpa  $\pm$  0.11 standard error) and control group ( $-3.0$  Mpa  $\pm$  0.66 standard error; Welch's *t*-test = 3.147,  $P = 0.047$ ). Logistic regression indicated mean shoot water potential was a good predictor of mean phloem water content ( $\chi^2 = 15.365$ ,  $df = 1$ ,  $n = 8$ ,  $P < 0.001$ ,  $R^2 = 0.461$ ; equation: percent phloem moisture =  $0.384 + 0.120[\text{Mpa}]$ ; Fig. 1). Although the sample size was small, our test suggests short-term water stress is sufficient to cause significant changes in the water content of live host tree tissues.

A preference test was subsequently performed to analyse *M. galloprovincialis* settling and feeding preferences for bark and phloem of differing moisture contents. Test beetles were obtained from dead maritime pine trees (*Pinus pinaster* Aiton) near Troia, Portugal (coordinates: 38.42292° N, -8.82331° W); diapausing larvae were extracted from dead trees and placed individually in Petri dishes (55 mm diameter; DeltaLab, Rubi, Spain) lined with filter paper (Whatman; Cytiva Life Sciences, Marlborough, Massachusetts, United States of America) in a rearing chamber at 20–25 °C and 60% relative humidity until pupation.

Newly eclosed adults ( $n = 70$ ) that had not fed were sexed, measured (elytral length, mm), and isolated for 48 hours following sclerotisation and then placed in 8-L polyvinyl containers (dimensions: 34 cm long  $\times$  22 cm wide  $\times$  15.5 cm high; PlasticForte, Alicante, Spain, Lot no. 18191730) in the dark for two hours to acclimate before testing. Beetles were kept under the lid of a Petri dish in the centre of the container during acclimation. At the onset of the test, *P. halepensis* tissue punches containing both bark and phloem (1.5 cm diameter, 0.3 cm thickness) were placed

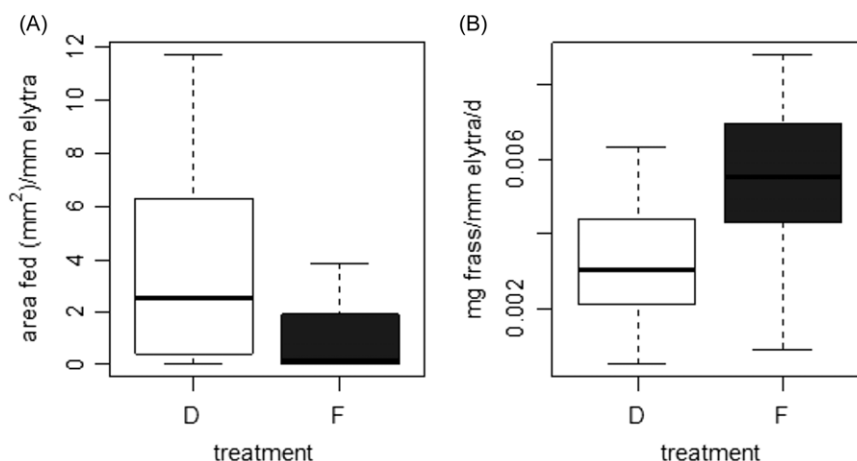


**Figure 1.** Relationship between mid-day twig water potential and mean phloem moisture (%) in droughted (open symbols) and watered (black symbols) *Pinus halepensis*. Bars show  $\pm$  one standard error of the mean.



**Figure 2.** Change-over-time in mean moisture content of oven-dried (open) and control (black) *Pinus halepensis* phloem punches. Bars show  $\pm$  one standard error of the mean. Vertical lines denote when settling preferences and phloem consumption were recorded.

at opposing corners of the container, with positions randomised in each test. Punches were placed such that the bark side faced upwards and beetles had to chew through the bark to access phloem (Supplementary material, Fig. S1). Phloem was collected at 1.5 m height on boles at a random aspect from trees growing in a nearby natural population. Just before the test, each punch was either (1) oven-dried at 50 °C for 90 minutes (dried) or (2) freshly excised (control). These two treatments resulted in a mean water content difference between oven-dried and control punches of 18% immediately following drying, but this difference eroded over time as water evaporated from all samples (Fig. 2). The assay was initiated by removing the Petri dish lids; during the assay period, beetles had unrestricted access to tissue punches for 24 hours. Assays were performed under red light at 25 °C and 30% relative humidity. After three hours, settling preferences were scored by recording beetle position within containers as either (1) settled on oven-dried punches, (2) settled on control punches, or (3) no choice. After 24 hours, phloem punches were collected, and the area of phloem consumed ( $\text{mm}^2$ ) from each punch was analysed using ImageJ software (Schneider *et al.* 2012; Supplementary material, Fig. S2). Area consumed was standardised to



**Figure 3.** The distribution of (A) area fed by newly emerged beetles on bark punches over a 24-hour period in a choice assay, and (B) mass of frass produced by beetles in a no-choice assay over a five-day period. D and F denote “dried” and “fresh” substrate, respectively.

elytral length (mm) to control for differences in beetle size. Welch’s *t*-test was used to test whether standardised mean area consumed ( $\text{mm}^2/\text{mm}$  elytra) differed between oven-dried and control phloem punches.

After three hours, only 47% (33 of 70) of beetles had settled on phloem punches, but of the beetles making a choice during that period, 69% (23 of 33) had settled on oven-dried punches. During the 24-hour period, 90% of beetles fed, and 41% of beetles consumed phloem from both oven-dried and control phloem discs. Beetles consumed more phloem area from dry phloem punches (mean =  $43.23 \text{ mm}^2 \pm 5.2$  standard error, range =  $0\text{--}160.7 \text{ mm}^2$ ) than from control phloem punches (mean =  $14.9 \text{ mm}^2 \pm 2.5$  standard error, range =  $0\text{--}84.2 \text{ mm}^2$ ). When area fed was corrected to beetle size (area fed/mm elytra), a significant difference was noted in area fed by beetles between oven-dried and control phloem punches (Welch’s *t*-test = 4.805,  $P < 0.001$ ; Fig. 3A).

A no-choice assay was performed to test whether feeding rate differed on fresh or dried host material when beetles were confined to a single feeding substrate. Similar to punches, *P. halepensis* shoots were collected from between 1 and 2 m height in the crown of trees in a nearby natural population. Seventy newly emerged and starved adult beetles were each placed into a 500-mL plastic deli cup with an 11-cm shoot ( $\sim 2$  cm diameter, range 1.5–3.0 cm) subjected to one of two treatments: (1) oven-dried at  $50^\circ\text{C}$  for 24 hours (“dried”) or (2) harvested immediately before use (“fresh”). This treatment resulted in a 10% moisture differential after 24 hours; the subsequent rate of moisture loss was minimal under laboratory conditions ( $20^\circ\text{C}$ ). After confinement for five days, survival was recorded for each individual, shoots were removed, and frass was collected, dried, and weighed (0.1 mg) to estimate feeding rate. Frass production was standardised to beetle size by dividing mass of frass produced for each beetle by elytral length (mg frass produced/mm elytra/day). A two-sample Student’s *t*-test was used to test whether standardised frass production differed between oven-dried and control phloem punches. Frass production was also compared between males and females using the same test. Linear regression was used to test whether frass production ( $y = \text{mg frass}$ ) varied with beetle body size ( $x = \text{mm elytra}$ ) using the “lm” function in the R base package.

All beetles survived the five-day assay period and fed on shoots. Beetles feeding on fresh shoots produced  $5.4 \text{ mg} \pm 0.3 \text{ mg}$  standard error of frass/mm elytra/day, and beetles feeding on oven-dried shoots produced  $3.3 \text{ mg} \pm 0.2 \text{ mg}$  standard error frass/mm elytra/day ( $t_{69} = -4.561$ ,  $P < 0.001$ , Fig. 3B). Frass production varied significantly depending on beetle size ( $F_{1,68} = 11.698$ ,  $P = 0.001$ , equation: frass weight, five days =  $-0.184 + 0.037[\text{elytral length, mm}]$ ,  $R^2 = 0.163$ );

longer elytral length was associated with a higher mass of frass produced, although mean body size did not differ significantly between treatment groups ( $t_{69} = 1.882$ ,  $P = 0.175$ ). Males and females did not differ significantly in frass production ( $t_{69} = 0.004$ ,  $P = 0.984$ ).

Our experiments indicate that tree water deficit and physiological water stress (water potential) are linked to the water content of phloem tissues (% water; Fig. 1). Phloem and twig water content had consequences for selection of feeding substrates by newly eclosed *M. galloprovincialis* and its subsequent feeding rate on host material (Fig. 2A, B). Changes in both primary and secondary metabolite profiles of *P. halepensis* tissues may explain these patterns. For example, water stress and water content can affect the nutritional content of conifer tissues fed upon by herbivores (Sudachkova *et al.* 2002). In the field, *P. halepensis* exposed to moderate levels of drought exhibits elevated shoot nitrogen and starch concentrations, suggesting that water-stressed trees may have higher nutritional value than nonstressed individuals (Villar-Salvador *et al.* 1999) because tissues contain less water per unit mass. Drier tissues can also express reduced concentrations of quantitative defences, such as monoterpenes (McDonald *et al.* 1999), although water stress may also be associated with increased production of monoterpenes *in planta* (Blanch *et al.* 2009).

In the present study, oven-drying of phloem punches and twigs may have resulted in the volatilisation of secondary metabolites such as monoterpenes, which could also affect host preferences and feeding behaviours. This is a limitation of our study design. Water deficit has variable effects on the volatile secondary metabolite profiles of conifers (Lusebrink *et al.* 2011; Kopaczyk *et al.* 2020), and both monoterpene concentration and emissions have consequences for host selection (Chénier and Philogène 1989) and feeding behaviours of *Monochamus*, with some terpenoids functioning as feeding stimulants and others functioning as repellents (Fan and Sun 2006). Our experimental design did not allow us to determine whether the reported differences in feeding behaviours were due to changes in water content alone or were mediated *via* interactions between water content and primary or secondary metabolite profiles, but such interactions are an important area of future investigation. Additionally, our tests of settling preference used punches of bark and phloem from the bole in a laboratory setting, and although beetles fed upon this material, maturation feeding in the field often takes place on tertiary twigs and branches in the canopy.

Further studies could expand on our results by concurrently investigating the possible effects of *P. halepensis* water stress on carbon:nitrogen ratios of feeding substrates (*i.e.*, nutritional content) and secondary metabolite (monoterpene) profiles and relating this to *M. galloprovincialis* host selection preferences during maturation feeding under field conditions.

**Supplementary material.** To view supplementary material for this article, please visit <https://doi.org/10.4039/tce.2024.30>.

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**Competing interests.** The authors declare they have no competing interests.

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