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High winter survival rate of acorn ants inside artificial nest sites (Hymenoptera: Formicidae)

Sławomir MITRUS

Institute of Biology, University of Opole, Oleska 22, 45-052 Opole, Poland – smitrus@uni.opole.pl

Abstract
Although most species of ants overwinter underground to avoid low temperatures, the acorn ants of the genus *Temnothorax* remain in nests situated at ground level. During a field experiment, I studied the winter mortality of acorn ants in nest sites situated aboveground, as well as in sites experimentally buried in the soil. Despite the low air temperatures (even reaching –19°C, recorded 1.5 m above the ground), the survivorship was very high: all of the 18 queens used in the experiment survived, while the survival rate of workers was 61.9-100%, and for most colonies it exceeded 95%. The rate of survival in the nest sites aboveground and those experimentally buried in the soil was similar. Such a high survival rate in the nests situated at ground level could have resulted from the presence of snow cover during the strongest frost. The artificial nest sites used in the study provided safety for the ants; however, the real mortality during winter, e.g. connected also with predation or destruction of the nest sites, is a subject that needs further study.

Keywords: Hymenoptera, Formicidae, *Temnothorax crassispinus*, winter mortality, snow cover, social insects, overwintering, cavity-nesting ants.

Introduction

Winter mortality can be crucial for invertebrates living in temperate climate zones, but for most species good data on the winter survivorship is not available (Leather et al. 1995; Rodenhouse et al. 2009; Bradshaw & Holzapfel 2010). The individuals of some species migrate before winter to avoid the low temperatures, while others spend the period underground where conditions are more favourable or have evolved to tolerate freezing (Lee & Denlinger 1991; Leather et al. 1995; Bradshaw & Holzapfel 2010). Typically, a high rate of winter mortality is connected with low temperatures, but other factors can also affect the survivorship. For example, a strong frost can have different results depending on whether a thick snow cover is present or there is a lack of such cover (Leather et al. 1995; Mitrus 2016).

Ants have strong influence on their ecosystems (Wilson & Hölldobler 2005; Czechowski et al. 2012). Most ants living in temperate zones overwinter below ground level to avoid the low temperatures. However, overwintering deeply in the soil comes at the cost of a delayed emergence in the spring (see Włodarczyk 2021). Besides the limited numbers of nest sites, this is probably the reason why some species overwinter in nests situated at ground level, like the acorn ants of the genus *Temnothorax* (Herbers 1989; Herbers & Johnson 2007). Overwintering in nests on the ground could result in high levels of mortality, and I previously showed that experimental burying of the nest sites could considerably increase the survivorship (Mitrus 2013). However, other experiments have shown that the survival rate of *Temnothorax* ants can also be really high during mild winters (Mitrus 2015; Honorio et al. 2021) or when the nest sites are covered by snow (Mitrus 2016).

In this paper, I present the results of an experiment comparing the winter mortality of acorn ants that overwintered above ground to those that overwintered in nests experimentally buried in the soil. It is a similar experiment to the one that was conducted in the winter of 2011/12. However, the winter mortality can considerably vary between years, particularly when the winter weather differs considerably. Thus, data on the overwintering in different years is necessary to understand the ecology of the species, as well as the effects of climate change (see Sinclair et al. 2003; Christensen et al. 2013).

Materials and method

On 19 November 2020, near Opole (GPS: 50°37'29"N 18°06'33"E), I collected 27 acorns containing colonies of the ant *Temnothorax crassispinus* before the first winter frost. All of these colonies contained a brood in the early stages of development and workers (13-424; mean: 125.0; median 118). In addition, 20 of the 27 colonies had a queen. I transferred the colonies to plastic boxes (ca. 10 cm × 10 cm × 2 cm) with a thin plaster base and with artificial nest sites placed on top. For the nest sites, I used a beech woodblock measuring 7.5 cm × 2.0 cm × 2.0 cm, drilled lengthwise to form a 4 mm hole and closed on one side with a beech plug. The hole was reduced on the other side using a beech splinter. The final volume of the resulting cavity was approximately 0.9 cm^3. The boxes with the artificial nest sites were kept in a thermostatic cabinet while maintaining a daily cycle: LD (light:darkness) 10:14 h, at temperatures of 15°C and 7°C, respectively. When most of the artificial nests had been settled by the ants, the temperature was gradually decreased; therefore, the final culture regime was LD 10:14 h, 5°C and 3°C, respectively. The ants were fed on a half of frozen Dubia roach (*Blaptica dubia*; length: approximately 12 mm) and honey, with water provided ad libitum. Any dead ants found in the boxes were counted and removed (0-11 workers, median 0; and one queen).

On 27 November, after the first slight frost, I closed the entrances to the artificial nests with a piece of metal mesh (woven stainless steel mesh: wire diameter 0.2 mm; hole size: 0.31 mm) to prevent the ants from escaping. At that time, 7 workers from one colony were outside of the artificial nest site, and one colony did not accept the nest site. Thus, in the field experiment, I used 26 nest sites containing ant colonies: 19 with a queen and 7 without a queen; workers: 12-408, mean: 125.7; median: 117. The colonies were randomly divided into two groups. On 4 December, the nest sites were placed in the field; at that time, the temperature in the research area was about 2°C. The nest sites from the first group (N = 13) were carefully buried in the soil at a depth of about 5 cm, while those from the second group (N = 13) were placed on the ground. Each the artificial nest sites was attached to a small stick poked into the ground. The distances between the nest sites amounted to 15 cm. Previously calibrated AZ 88128 data loggers were placed near the nest sites: two data loggers on the ground, and another two about 5 cm below ground level. Additionally, two data loggers were placed on tree trunks at 1.5 m above ground level. Using the data loggers, the temperature was monitored at one-hour intervals.

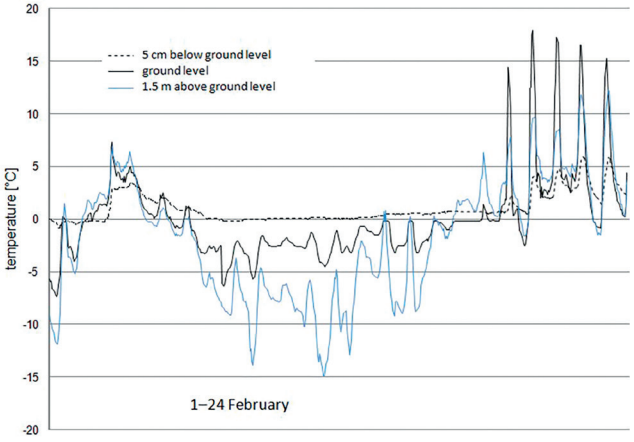

Fig. 1 – Changes in temperature recorded near the nest sites with the *Temnothorax crassispinus* ant colonies localised at ground level, as well as near the nest sites about 5 cm below ground level and 1.5 m above ground level. The experiment lasted from 4 December 2020 to 24 February 2021, but only the temperatures for February are presented. The higher temperatures recorded at ground level and 5 cm below ground level, compared to the temperatures 1.5 above ground level, were probably affected by heating from the sun; the data loggers situated 1.5 m above ground level were attached to trees on the north side, so they were not affected by the sun.

During the winter the nest sites were checked irregularly. After a period with snow and strong frost (reaching as low as –19.4°C at 1.5 m above ground level in the second half of January and –14.8°C on 12 February), a sudden thaw started in the middle of February. On 22 February, I found that the research area was partially dug up, probably by animals, e.g. I noticed that three data loggers were now at ground level (whereas at the beginning of the experiment two data logger were situated on the ground and two were 5 cm below ground level). Therefore, on 24 February I finished the experiment. I then found 24 of the 26 nest sites. Based on data from the data loggers, the experiment was partially destroyed on 20 February – I assumed that the data logger found at ground level, which had previously been located 5 cm below the ground, was dug up at the same time when the experiment was partially destroyed; initially, the pairs of data loggers were situated in similar positions. As a result, I ultimately had three groups of nest sites during the experiment:

- Nest sites at the ground level during the experiment, i.e. from 4 December 2020 to 24 February 2021 [N = 11 colonies; 3 queenless, 8 queenright];
- A group that remained 5 cm below ground level during the experiment [N = 7; 1 queenless, 6 queenright];
- Nest sites that were initially, on 4 December 2020, 5 cm below ground level, but that were dug up, probably around 20 February 2021 [N = 6; 2 queenless, 4 queenright].

On 24 February, during the nest collection, the temperature in the research area was about 4°C. The nest sites were transported to a laboratory, and were placed in a climatic chamber at 5°C. Then, the temperature was gradually increased to reach 11°C. On 26 February, the nest sites were carefully opened and the live ants were counted.

I used the median test to compare the survival rate of workers in the three experimental groups. The Spearman

Table 1 – Percentage of workers of the acorn ant *Temnothorax crassispinus* that survived winter in the artificial nest sites. During the field experiment (4 December 2020 to 24 February 2021, in southern Poland), the ant colonies were situated in nest sites at ground level and were experimentally buried ca. 5 cm below the ground. Additionally, a third group is presented: colonies in the nest sites that were experimentally buried 5 cm below ground level, but were dug up, probably by animals, around 20 February 2021. In brackets: number of colonies analysed.

Nest sites with colonies	Median (mean)	Min/max
At ground level [11]	98.0 (98.4)	96.3-100.0
Buried ca. 5 cm below ground level [7]	98.3 (96.2)	82.1-100.0
Buried ca. 5 cm below ground level, but dug up during the experiment [6]	100.0 (93.7)	61.9-100.0

rank correlation was used to test if the survival rate was correlated with the colony size, i.e. the initial number of workers. The statistical analyses were carried out using the software package Statistica, ver. 13.3 (TIBCO 2017). The threshold for significance was p = 0.05 throughout. All the probability values shown are two-tailed.

Results

During the experiment, the average temperature in the study area near the nest sites localised on the ground level was +1.37°C (median = +0.7°C; range –7.4 to +17.9°C; 25-75% quartiles –0.7 to +3.2°C), 5 cm below the surface of the ground the temperature was +2.26°C (median = +2.0°C; range –3.6 to +8.0°C; 25-75% quartiles +0.8 to +3.4°C), and 1.5 m above ground level the temperature was +0.34°C (median = +0.4°C; range –19.4 to +13.1°C; 25-75% quartiles –1.3 to +3.2°C) (see Fig. 1).

All of the 18 queens survived. The survival rate of the workers was 61.9-100% (Table 1). The lowest survival rates were 61.9% and 82.1% in colonies where the initial number of workers was 21 and 28, respectively. For the other colonies, the survival rate of workers exceeded 95%. The survival rate of workers did not differ among the experimental groups (median nest, $\chi^2 = 3.63$, df = 2, p = 0.16) and it was not correlated with the initial number of workers (Spearman rank correlations: Rs = –0.24, N = 11, p = 0.48; Rs = 0.61, N = 7, p = 0.63; and Rs = 0.65, N = 6, p = 0.16, for the three experimental groups).

Discussion

During the experiment, the winter survival rate of the acorn ant *Temnothorax crassispinus* was high: all the 18 survived, and for most colonies workers survival rate exceeded 95%. The lowest survival rate was recorded among the smallest colonies; however, for such colonies mortality just several individuals could result of considerable per cent change.

Unlike most ant species living in temperate climate zones which overwinter underground, the acorn ants of the genus *Temnothorax* spend the winter in nests on the ground where they are subjected to variable temperatures and snowfall (Herbers & Johnson 2007). Despite this, their survival rate during winter may be high: in earlier studies, a high survival rate was reported for the workers (average >80%, median >95%) and queens (73 out of 74) of *T. crassispinus* (Mitrus 2015) and for the workers of *T. nylanderi* (96% on average, Honorio et al. 2021), and well as for colonies of *T. nylanderi* (Honorio et al. 2020, 2021) and *T. curvispinosus* (Martin et al. 2021). However, during other studies, only 32.5% and 45.4% of the workers of *T. curvispinosus* (Herbers & Johnson 2007), and less than 50% of the workers of *T. crassispinus* survived (in nests situated on the ground surface, Mitrus 2013).

During this experiment, part of the nest sites were experimentally situated below ground level. This was a similar procedure to my research carried out during the winter of 2011/12, where the workers of *T. crassispinus* survived in higher numbers in the nests that were experimentally buried compared to the ones overwintering in nest sites on the surface (Mitrus 2013). In the current experiment, no such difference was found. However, during the winter of 2011/12 there was little snow, but during this experiment the snow cover was periodically thick and was present during the stronger frost. The minimal temperature of –19.4°C was recorded about 1.5 above ground level, but at the ground level the minimal temperature was –7.4°C, probably as an effect of the snow cover. Thus, the results of the study support the data that a high mortality of acorn ants is affected by low temperatures connected with the lack of a thick snow cover (see Mitrus 2016). It is known that climate change will affect the temperature during winter, but will also reduce the depth and duration of the snow cover (Christensen et al. 2013; Convey et al. 2015). Thus, in the future, even short periods with strong frost could result a higher mortality of the ants overwintering in nests situated at ground level.

An important factor in this study was the nest sites that were used. The acorn ants typically dwell in empty acorns and cavities in small sticks (Czechowski et al. 2012; Seifert 2018). Such sites could be easy crushed, e.g. accidentally walked on by larger animals, or predated, but used in the experiment nest sites were made of pieces of strong wood, which provided safety for the ants. A high survival rate during winter was also found for *T. nylanderi* during research conducted in France (Honorio et al. 2021) where 'strong' nest sites were used – in that study, the nest sites were prepared from cork. Thus, experiments like this one that give data about the mortality connected with atmospheric conditions, but related to the real mortality, e.g. connected also with predation or the destroying of nest sites during the winter, need to be examined in further studies.

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