



**Original article (Orijinal araştırma)**

**Resilience of the date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), following periods of exposure to subzero temperature<sup>1</sup>**

Hurma böceği, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae)'nın sıfır altındaki sıcaklıklara esnekliği

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**Abstract**

The date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), is a cold-sensitive agricultural pest that goes into hibernation inside infested seeds, ready to breed as temperature increases. To assess their resilience following exposure to cold, specimens reared from infested *Phoenix canariensis* Chabaud seeds were exposed prolonged subzero temperature in a laboratory setting at Charles Sturt University (Albury, Australia) in 2018. Specimens which had been allowed to tunnel into *P. canariensis* seeds as well as those held in vials were exposed to -8°C for durations between 5 min and 7 h. Unprotected beetles survived a 75-min exposure to -8°C, but the beetles in their brood galleries inside the seeds survived for over 7 h.

**Keywords:** Biogeography, climatic extremes, insect pests, physiology

**Öz**

Hurma böceği, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), sıcaklık arttıkça üremeye hazır hale gelen ve bulaşık tohumların içinde kışı geçiren soğuğa duyarlı tarımsal zararlıdır. Soğuğa karşı dayanıklılıklarını değerlendirmek için, bulaşık *Phoenix canariensis* Chabaud tohumlarından elde edilen örnekler, 2018 yılında Charles Sturt Üniversitesi (Albury, Avustralya)'ndeki bir laboratuvar ortamında uzun süreli sıfırın altındaki sıcaklıklara maruz bırakılmıştır. *Phoenix canariensis* tohumlarının içinde galeri açmalarına izin verilen örnekler ve şişelerde tutulan örnekler 5 ila 420 dakika arasında -8°C'ye maruz bırakıldı. Korunmasız böcekler -8°C'ye 75 dakika hayatta kalabilirken, tohumların içindeki gizlendikleri galerilerindeki böcekler 7 saatten fazla yaşamışlardır.

**Anahtar sözcükler:** Biyocoğrafya, iklim aşırı uçlar, zararlı böcekler, fizyoloji

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## Introduction

The spermatophagus date stone beetle, *Coccotrypes dactyliperda* Fabricius, 1801 (Coleoptera: Curculionidae), is a pest in commercial or domestic date (*Phoenix dactylifera* L., Arecaceae) plantations throughout Northern Africa and the Middle East where it can cause extensive fruit losses (known as the July drop) (Mostafa, 1997; Anber et al., 1998; Helal, 1998; Bar-Shalom & Mendel, 2001; Ali et al., 2002; 2003). They have since infested ornamental palms, primarily Canary Island date palm (*Phoenix canariensis* Chabaud) throughout the Mediterranean and beyond.

Closely associated with the date palm complex and originally endemic to the Middle East and North Africa, *C. dactyliperda* has become a true cosmopolitan species that can be found in most subtropical and temperate zones (Spennemann, 2018a). As with other crypto-parasites, the entire life cycle of *C. dactyliperda* occurs inside the seed. Mated females start to deposit eggs 3 to 5 d after inhabiting a new seed. Unmated *C. dactyliperda* females are able to deposit eggs that produce male offspring and then proceed to mate with these to produce offspring of mixed sex. Depending on the size of the seed, multiple broods and even generations of beetles can hatch and reproduce inside a seed before emerging. A single date seed can concurrently contain large numbers of eggs, larvae, pupae and imagines in excess of 80 individuals (females, males, pupae, larvae and eggs) (see review by Spennemann, 2019a and references cited therein).

*Coccotrypes dactyliperda* is a cold-sensitive species. During the winter imagines of female *C. dactyliperda* enter a hibernation or dormancy period inside the seeds in which they hatched. Hibernation in the northern hemisphere is usually from November or December to February (El-Bahria Oasis, Egypt) (Ali et al., 2003), or even to May (Abd-Allah & Tadros, 1994 cited after Boraei, 1994; Helal, 2014). The commencement and termination of hibernation are linked to both temperature and photoperiod.

Although the extant literature is silent on resilience of *C. dactyliperda* following exposure to subzero temperature, some resilience can be inferred from the spatial distribution of the species (Spennemann, 2018a). In North Africa and the Middle East, where *C. dactyliperda* is considered an endemic, the temperature can drop below zero, for example,  $-1.7^{\circ}\text{C}$  (Tripoli, Libya),  $-4.0^{\circ}\text{C}$  (Jerusalem, Israel),  $-6.1^{\circ}\text{C}$  (Tunis, Tunisia) and  $-8.0^{\circ}\text{C}$  (Amman, Jordan). In the south of France, where *C. dactyliperda* has become naturalized, the temperature can drop to  $-13.0^{\circ}\text{C}$  (Toulon, France) (NCDC, 2018). Annual minimum temperatures vary and are thus of indicative value only (Figure 1). Although temperature extremes in North Africa and the Middle East can drop to these minima, the average minima are higher by  $5\text{-}8^{\circ}\text{C}$  (Ageena et al., 2013; World Bank Group, 2019). There is no evidence that any of the areas where date palm and the date stone beetle occur have multiday events with temperature below  $-5^{\circ}\text{C}$ .

Over recent decades, the Middle East and North Africa have experienced warming trends. Significantly for *C. dactyliperda*, these are accompanied by fewer cold days and nights, and shorter cold periods (Zhang et al., 2005; Almazroui et al., 2012; Ageena et al., 2013; Donat et al., 2014; El Kadi, 2016). Also, on a broad spatial scale, this trend is predicted to continue if not accelerate (Samuels et al., 2011), although it is not uniform on a finer scale. Indeed, the opposite trend is likely to occur in some smaller areas (Matouq et al., 2013).

Although it is understood that adult beetles in their galleries will be somewhat protected from low temperature, at least for short periods, compared to beetles that are fully exposed, there are no reliable experimental data of the ability of *C. dactyliperda* to withstand exposure to subzero temperature. Cooperband et al. (2016) found for another scolytinid species (*Euwallacea fornicatus* Eichhoff, 1868) (Coleoptera: Curculionidae) that 4-h exposure to a temperature between  $-5$  and  $-1^{\circ}\text{C}$  led to mortality of 100% of larvae, 95.7% of pupae and 69.2% of adults. Longer exposure killed all adults.

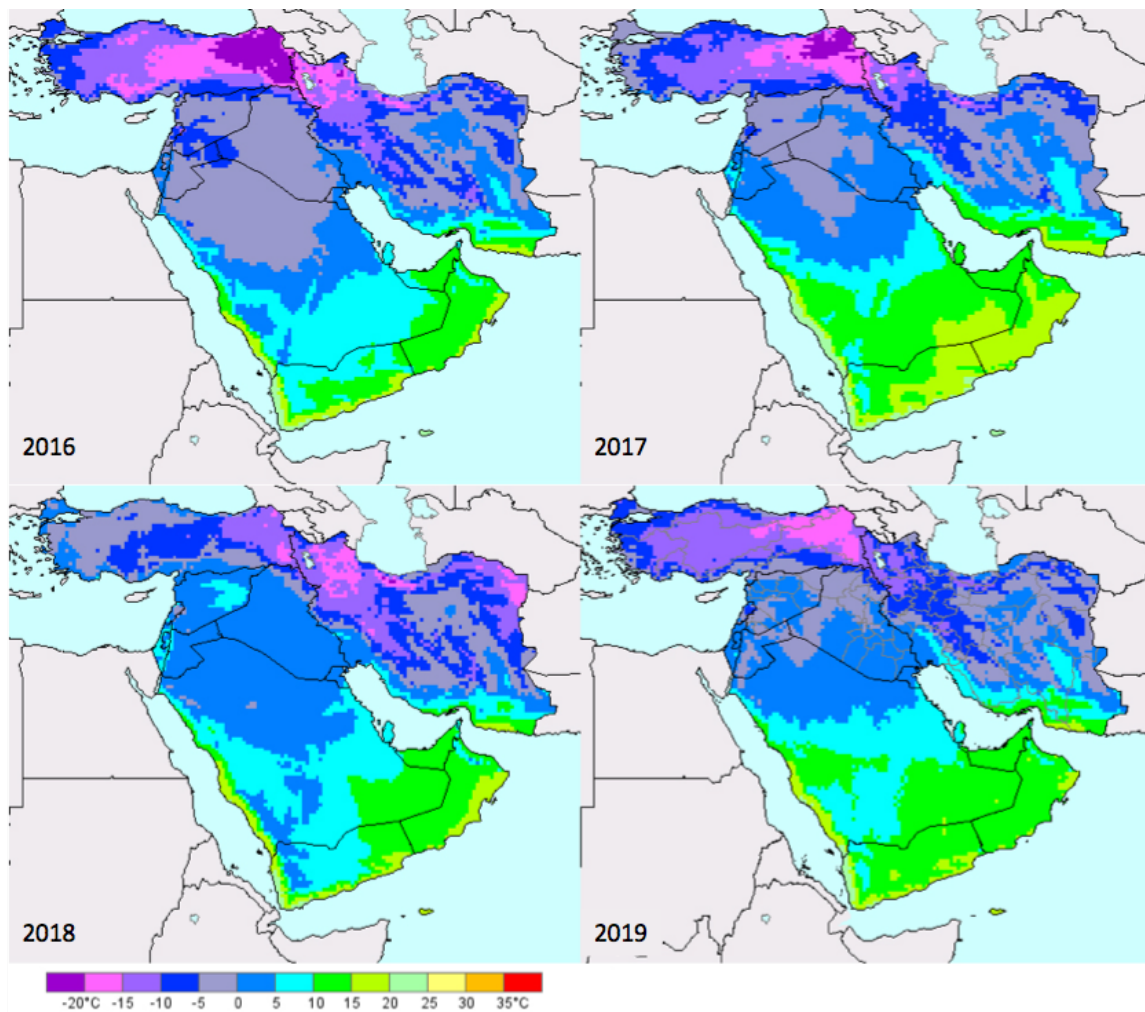


Figure 1. Minima for the Middle East for the winters of 2016-2019. Data: USAF 557<sup>th</sup> Weather Wing, via International Production Assessment Division, Foreign Agricultural Service, United States Department of Agriculture.

The resilience of *C. dactyliperda* following exposure to subzero temperature has implications of the future spread of the beetle into currently uncolonized areas. Knowledge of death threshold temperature informs on past and present biogeography of the species. This paper reports the results of an experiment exposing date stone beetles to subzero temperature, both on their own and inside seeds.

## Materials and Methods

The beetles used were came from a *C. dactyliperda* population bred for multifactorial experiment designed at assessing food choices and emergence times (Spennemann, 2019b). The original beetle population stemmed from Canary Island date palm seeds collected at Alma Park, NSW, Australia (Spennemann et al., 2018a). The experiment was conducted in October 2018 in the PC2 laboratory of the Peter Till Laboratories, Faculty of Science, Charles Sturt University (Albury, Australia).

To assess the ability of *C. dactyliperda* to withstand subzero temperature, two sets of samples were prepared: 1) 16 vials with 25 ml air volume, containing 25 specimens each without any substrate; and 2) 16 same-sized vials populated with 10 specimens each that had been allowed to tunnel into 10 fresh *P. canariensis* seeds for 49 h. One set of beetles and one set in other Canary Island date palm seeds in a food preference experiment were used as controls.

The sample vials were placed on an expanded polystyrene tray in a laboratory freezer at -8°C. After 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, 360 and 420 min, one sample of each set was removed from the freezer, and left to thaw on a laboratory bench at room temperature (about 20°C) for 60 min before assessing the viability of the beetles.

The samples with beetles in the seeds were placed between moistened sheets of tissue in a germination chamber at 28°C for 1 week. Experience has shown that live beetles will cause frass to emerge from entrance hole in infested seeds, which is readily apparent in a germination chamber (Spennemann et al., 2018b). After 1 week, the seeds were dissected to expose and assess the viability of the beetles (found to crawl from opened seed or on filter paper when shaken from the seed). The sample vials with specimens without any substrate were emptied on the center of 100-mm circular filter paper. Survival was defined as the ability of the beetle to crawl from the center of the filter paper to its edge, i.e., a distance of at least 50 mm (i.e., 25x a beetle's body length). To meet the phytosanitary requirements of the PC2 laboratory, any specimens found alive were killed in 90% alcohol.

Statistical analysis of the small sample was limited to exploratory analysis (Tukey, 1980). Survivorship curves were calculated based on straight percentages.

## Results and Discussion

No mortality was observed in any sample during the first 75 min. Thereafter, the survivorship curves diverged (Figure 2). Unprotected beetles showed a gradual increase in mortality, with 72% alive after 180 min and all dead after 240 min. Beetles, partially protected from the cold in their galleries, began to die after 150 min. By 180 min, survivorship had dropped to 70%, dropping to 60% at 300 min and reaching 20% at the termination of the experiment after 420 min (7 h). None of the beetles in the seeds kept at room temperature died.

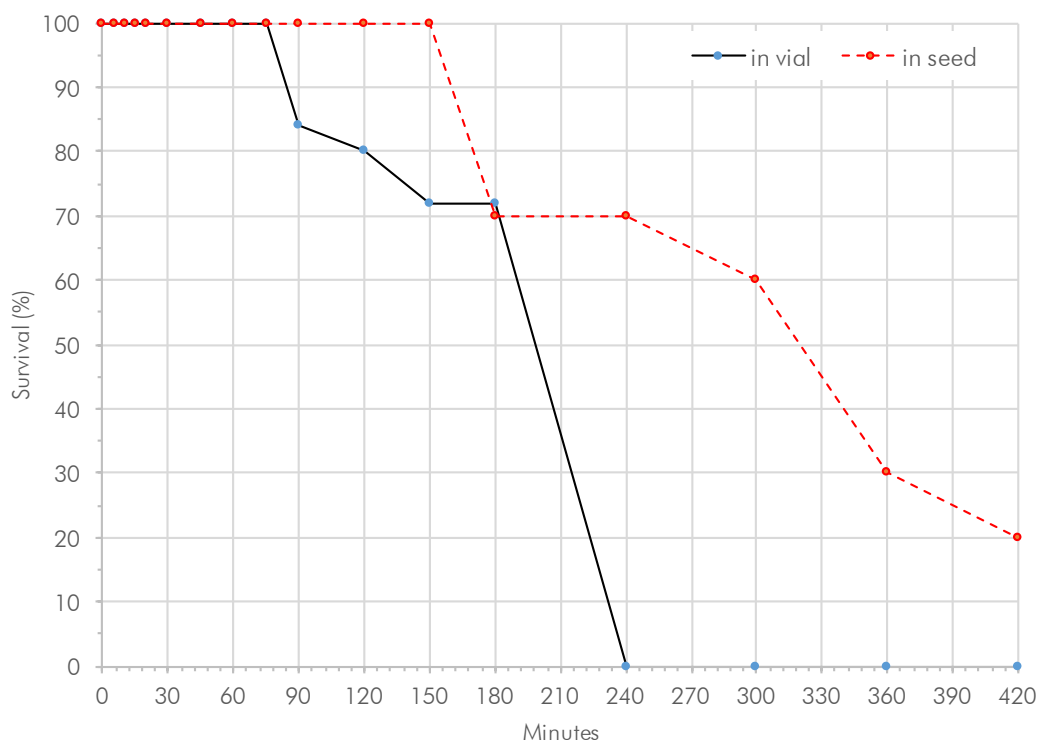


Figure 2. Survivorship curves (in %) for date stone beetles exposed to the air and tunneled into seed.

The total time between exposure of the seeds to the beetles and termination of the experiment was 8 d (2 d penetration and 6 d post cold exposure). Given tunneling speeds observed among siblings of the beetles used for the experiment (unpublished data), it can be assumed that after 24 h a rudimentary tunnel would have been excavated, with the tunnel being at least twice the length of the beetle after 48 h. As fertilized females start to deposit eggs between 1 and 3 d after entering a new seed, with an incubation period of 4-6 d (see review by Spennemann, 2019a and references cited therein), it can also be assumed that few, if any, of the females would have deposited eggs at the time of exposure to subzero temperature. This was confirmed by dissecting some seeds (Figure 3).

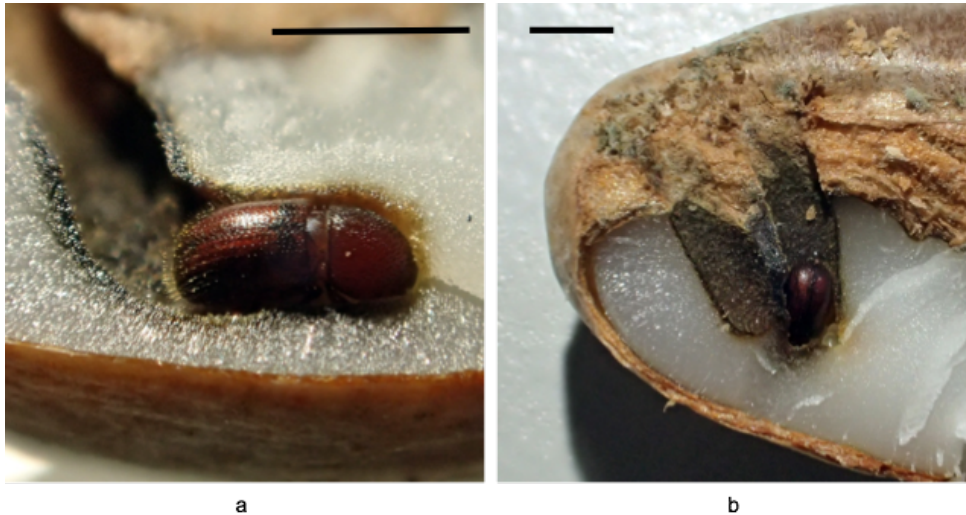


Figure 3. Brood galleries in Canary Island date palm seed with dead date stone beetles (*Coccotrypes dactyliperda*) after 4 h exposure to  $-8^{\circ}\text{C}$  following 7 d incubation in  $28^{\circ}\text{C}$ . Scale bar = 2 mm.

In seed exposed to shorter periods of subzero temperature, however, there was evidence of live beetles tending their eggs (Figure 4). One of these had at least 21 eggs (Figure 4b). Given the size of the seed and the nature of brood chambers, it is unlikely that a longer period of tunneling would have altered the outcome of the experiment. Although speculative, it can be assumed that adult beetles would have a lower mortality rate than eggs and larvae due to the latter's higher, unstructured moisture content and thus effects of ice crystal formation.



Figure 4. Brood galleries in Canary Island date palm seed with live date stone beetles (*Coccotrypes dactyliperda*) and their eggs after 3 h exposure to  $-8^{\circ}\text{C}$  following 7 d incubation in  $28^{\circ}\text{C}$ . Scale bar = 2 mm.

## Conclusions

The design of this experiment represents an extreme scenario. In the real world, it is unlikely that beetles would be crawling about at a time when the temperature dropped below 10°C, let alone into the subzero range. Moreover, it would be expected that some of the infested seed may be covered by leaf litter and thus further protected from the ambient air temperature. The exposure of unprotected *C. dactyliperda* beetles to such temperatures, however, underlines the resilience of the species at least after exposure to short periods of cold. Notably, 20% of the specimens survived a 7 h exposure to -8°C. This demonstrates that although cold winters will severely impact a hibernating population, sufficient numbers will survive to ensure the survival of that population.

The findings reported here have implications for the viability of *C. dactyliperda* populations in marginal environments. Although this beetle species was originally confined to the Middle East and North Africa, where it was closely associated with the date palm complex, it has been able to adapt to a range of host plants, in particular the Canary Islands date palm. Largely facilitated by the rapid and widespread horticultural dispersal of that ornamental palm (Spennemann 2018b, 2019c; Zona 2008), *C. dactyliperda* has been able to establish itself as a true cosmopolitan species in warm temperate zones (Spennemann, 2018a). The data presented here indicates that the cold tolerance of *C. dactyliperda* mirrors that of the Canary Islands date palm (Larcher & Winter, 1981). Therefore, the distribution of the Canary Islands date palm can act as a proxy for the maximum biogeographic dispersal of *C. dactyliperda*.

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