N O T E

Freeze Susceptibility and Supercooling Temperature of *Plectris aliena* (Coleoptera: Scarabaeidae) Third Instars[1]

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Temperature is a density-independent abiotic mortality factor, although the response of insects to cold temperature varies between and within species (Bale 1989, Agric. Zool. Rev. 3: 157–192). The abundance of the overwintering stage of an insect depends in part on its cold hardiness, or ability to survive low- temperature exposure aboveground or in subterranean habitats (Bale 1989). Freeze-tolerant insects can survive the freezing of tissues at temperatures below 0°C, while freeze-susceptible insects perish when their tissues freeze (Chapman 1998, Pp. 509–528, *In* The Insects: Structure and Function, 4th ed., Cambridge Univ. Press, New York). Insects that are freeze susceptible avoid freeze mortality by supercooling, or maintaining liquid water below the freezing point (Chapman 1998). For many freeze-susceptible insects native to tropical or temperate regions, the supercooling point (the sub-0°C temperature at which the tissue water spontaneously freezes) is very low, often below −20°C, but this can be influenced (increased or decreased) by life stage, acclimatization, feeding, water content, and cuticular surface moisture (Bale 1989, Chapman 1998). Freezing of freeze-susceptible insects can be induced at a temperature above the supercooling point through ice inoculation by internal or external factors, such as food in the gut or surface contact with an ice-nucleating agent (Bale 1989).

Plectris aliena Chapman (Coleoptera: Scarabaeidae) is native to South America and, although it has been in the United States since the early 1900s (Chapin 1934, Proc. Biol. Soc. Wash. 47: 33–36), the insect only became a severe problem for sweetpotato growers in Columbus Co. in southeastern North Carolina beginning in 2006. Sweetpotato *Ipomoea batatas* (L.) Lamarck roots are rendered unmarketable

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by the feeding of *P. aliena* larvae, or white grubs. *Plectris aliena* appears to be univoltine in North Carolina, and the insect overwinters as third-stage larvae (Brill and Abney 2012, J. Integr. Pest Manage. 4(2): C1–C8).

The effects of cold temperature on *P. aliena* have not been documented. The objective of this study was to determine if third-instar *P. aliena* grubs are freeze susceptible and, if so, their supercooling point. This study provides a better understanding of the potential range limitations of the insect into areas north of its current distribution.

Third instars were collected on 22 and 29 January 2011 from a field near Chadbourn and were placed individually in 0.5-L plastic cups with field soil and without food. They were then transported to the laboratory at North Carolina State University and held in a dark (light:dark, 0:24) refrigerator at 7°C, which was the average soil temperature at 20 cm at the collection location (WatchDog™ loggers, Spectrum Technologies, Aurora, IL). Grub burrowing behavior was assessed 10–17 d after collection to ensure only healthy larvae were used in experiments. Larvae were warmed gradually over a period of 4 h to 15°C in an incubator with photoperiod set to simulate southeast North Carolina in late January (light:dark, 10:14). A larva was considered to exhibit normal, healthy behavior if it was able to burrow into field soil within 5 min of placement on the soil surface (George et al. 2007, J. Econ. Entomol. 100: 431–439). Once assessed, healthy larvae were held at 15°C, the initial starting temperature in the test chamber for experiments, for up to 3 h prior to experimentation.

Surface contact thermometry was used to determine the temperature at which larval freezing occurred. Grubs were removed from their plastic cups and dipped in 95% ethanol for 3 s to remove excess soil, which did not affect behavior (Brill et al. 2013, J. Agric. Urban Entomol. 29: 10–15), then blotted on clean filter paper and weighed. A 24-gauge copper–constantan thermocouple (Omega Engineering, 5SRTC-TT-T-24-36, Stamford, CT) was placed directly against the surface of each grub to measure insect temperature. The grubs and thermocouples were individually wrapped in 4×4-cm cheese cloth and then 6×6-cm aluminum foil. Paraffin film (Parafilm "M," Bemis, Neenah, WI) was used to seal the point where the thermocouple wire protruded from the aluminum foil against cold bath solution penetration.

Because insects can freeze more quickly when in contact with surface moisture, either on their body or in the soil (Lee and Denlinger 1991, Insects at Low Temperature, 1st ed., Chapman and Hall, New York, NY), the effect of moisture conditions on the freeze-mortality temperature of the grubs was determined by creating "wet" and "dry" treatments. The cheesecloth was dipped into distilled water for the "wet" treatment; unmoistened cheesecloth was used in the "dry" treatment ($n = 6$ grubs in wet cheesecloth and $n = 6$ grubs in dry cheesecloth).

Wrapped larvae were placed individually in 50-ml centrifuge tubes, which were then placed in a programmable test chamber with a 0.5L:1L ethylene glycol:water mixture (Thermo Scientific, NesLab Rte10, Waltham, MA) set to 15°C and progressively cooled at -0.5°C min^{-1}. The onset of freezing was verified by the appearance of an exotherm spike (i.e., release of the heat of crystallization; Lee and Denlinger 1991), and the temperature of each grub was recorded at 0.5-min intervals using a multichannel data logger (Omega Engineering, HH147U, Stamford, CT). The freezing temperature of each insect was recorded as the

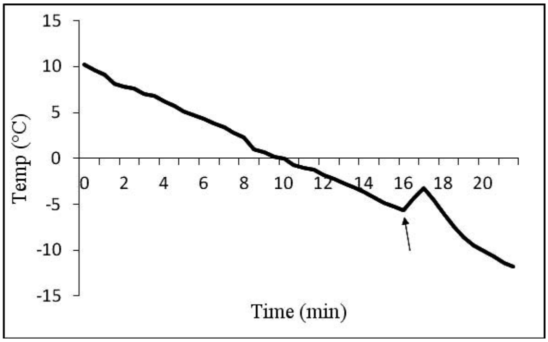

Fig. 1. Typical freezing curve for an unnucleated *P. aliena* larvae. The arrow indicates the supercooling point for one insect.

lowest reading reached before the release of latent energy during freezing, as indicated by the exotherm spike (Lee 1991, Pp. 17–46, *In* Insects at Low Temperature, Chapman and Hall, New York, NY). Mortality was determined after insects were allowed to thaw at room temperature for 24 h following exposure to cold temperatures. *t* tests using GraphPad Software, Inc. (http://www.graphpad.com) were conducted online to determine the effect of wet and dry conditions on supercooling point.

Plectris aliena is freeze susceptible. All grubs (100%) died within 24 h after freezing. The average supercooling point for third-instar *P. aliena* grubs was −5.3°C. There were no significant differences in mortality temperature between grubs wrapped in wet cheesecloth and grubs wrapped in dry cheesecloth ($P = 0.55$; df = 10; $t = 0.6202$). The following were the temperatures (°C) for the six grubs in dry cheesecloth: −5.2, −4.2, −6.3, −5.4, −5.1, −5.0 and for the six grubs in wet cheesecloth: −5.7, −6.5, −5.2, −5.9, −4.8, −4.6. Grubs weighed an average of 0.4 g. A graph of a typical freezing curve is given in Fig. 1.

Determining the effect of cold temperature on *P. aliena* third instars is important to further understand how winter temperatures could affect *P. aliena* populations in North Carolina and potential limitations on *P. aliena* population range expansion north of the currently affected area. The average freeze mortality temperature of −5.3°C obtained in this study is similar to that reported by Hoshikawa et al. (1988, Appl. Entomol. Zool. 23: 273–281) of inoculative freezing point mortality of −5.1°C and −6.2°C, respectively, for two scarab species, *Anomala testaceipes* Motschulsky and *Popillia japonica* Newman, where inoculation was achieved by wrapping the insect with cotton soaked in muddy water. Those larvae also had high supercooling temperatures (−6.8°C and −7.0°C) without inoculation; Hoshikawa et al. (1988) attributed the high supercooling temperatures (compared with other non–scarab beetle larvae with supercooling temperatures as low as −20.4°C) to the presence of large quantities of dark material in their rectum, which could have acted as an ice nucleator. When the hindguts of *P. aliena* white grubs used for these experiments were dissected, dark material was also present. This material was most likely ingested soil that remained in the digestive tract (Tashiro 1987, Turfgrass Insects of the United States and Canada, Cornell Univ. Press, Ithaca, New York). Root food sources were not offered to the white grubs used in these studies prior to experimentation. Soil in the hindgut of *P. aliena* may provide ice nucleation and thus elevate the temperature at which grubs die from freezing; this could also explain the lack of differences in mortality temperature between the grubs wrapped in wet and dry cheesecloth.

Soil temperatures in Columbus Co., NC, where *P. aliena* is an economic problem, and 160 km north of Columbus Co. (where the North Carolina county with the greatest sweetpotato production is located), are often above 0°C in the winter. The lowest soil temperatures in Columbus Co. recorded from the Border Belt Tobacco Research Station in Whiteville, NC, at a 10-cm depth were 6.2°C, 5.5°C, 3.6°C, and 3.9°C in January for 2008, 2009, 2010, and 2011, respectively. Hu and Feng (2003, J. Appl. Meteor. 42: 1139–1156) reported that at a 10-cm depth of measurement, the average minimum soil temperature in January for North Carolina ranges from 5°C to 8°C and that the average minimum soil temperature at that depth in January in the United States is −2.0°C. Even if *P. aliena* grubs can move to a depth of at least 30.1 cm during the winter in North Carolina (Brill and Abney 2012), acute temperature alone will not cause grub mortality.

This study shows that *P. aliena* is freeze susceptible, but also suggests that the geographic distribution of *P. aliena* in the United States will not be limited by acute mortality caused by low winter soil temperatures, because the temperature at which grubs froze in this study is still lower than average winter soil temperatures at 10 cm in the United States. These data indicate that there is a potential for *P. aliena* to expand its range further north of the affected area in North Carolina, at least as indicated by acute freezing mortality. However, the effects of long-duration exposure to cold, but not freezing, temperatures on overwintering *P. aliena* grubs may warrant research attention.