

THERMOCOUPLE DESIGN FOR MEASURING TEMPERATURES OF SMALL INSECTS

A.A. Hanson¹ and R.C. Venette^{2*}

¹Department of Entomology, University of Minnesota, St. Paul, MN 55108, USA.

²USDA, Forest Service Northern Research Station, St. Paul, MN 55108, USA.

*Corresponding author e-mail: rvenette@fs.fed.us

Abstract

Contact thermocouples often are used to measure surface body temperature changes of insects during cold exposure. However, small temperature changes of minute insects can be difficult to detect, particularly during the measurement of supercooling points. We developed two thermocouple designs, which use 0.51 mm diameter or 0.127 mm diameter copper-constantan wires, to improve our ability to resolve insect exotherms. We tested the designs with adults from three parasitoid species: *Tetrastichus planipennis*, *Spathius agrili*, and *S. floridanus*. These species are <3 mm long and <0.1 mg. Mean exotherms were greater for fine-gauge thermocouples than thick-gauge thermocouples for the smallest species tested, *T. planipennis*. This difference was not apparent for larger species *S. agrili* and *S. floridanus*. Thermocouple design did not affect the mean supercooling point for any of the species. The “cradle” thermocouple design developed with the fine gauge wire was reusable and allowed for easy insect recovery after cold exposure.

Keywords: supercooling point, exotherm, parasitoid

INTRODUCTION

Contact thermocouple thermometry is commonly used to measure the surface body temperature of an insect during assessments of insect cold tolerance (5). Thermocouples are often attached to the bodies of insects with an adhesive, such as high vacuum grease or petroleum jelly (3, 9). As an insect is subjected to low temperatures, it will eventually reach a temperature at which body fluids begin to freeze (i.e., the supercooling point). Freezing is evident from a sudden increase in body temperature (i.e., an exotherm) as the heat of crystallization is released (3, 6, 9). The increase in temperature during an exotherm can be very apparent for larger insects as a result of their large mass and often high amount of fluids. However, the exotherms of minute insects, such as many insect parasitoids, (e.g., families: Braconidae, Eulophidae, and Encyrtidae) can often be small and difficult to discern from small sensor variations (AAH, personal observation). In addition, the adhesive used to attach the insect to the thermocouple often damages an insect (e.g., blocks spiracles), which is problematic when insect development after cold exposure is of interest.

Carrillo et al. (3) developed a method to control the cooling rate of insects placed inside a -80°C reach-in freezer. The insects were first placed inside polystyrene cubes with

dimensions designed to give desired and relatively constant cooling rates as the cooling rate can affect the supercooling point (10). For example, 20.3-cm cubes gave a theoretical cooling rate of approximately 1°C/min at the center of the cube when the interior temperature of the cubes was approximately <0°C. This cooling rate is standard when supercooling points are measured (10). However, the realized cooling rate could be slightly different, based in part on how close the insect was to the center of the cube. In addition, as the insect and thermocouple were lowered into the center of the cube, the thermocouple would often touch the side of the cube and dislodge the insect (4, RCV personal observation). Thus, our goal was to design a thermocouple that would help to reliably place the insect near the center of the cube, detect exotherms of small insects (2-3 mm long; <0.1 mg), recover an unadulterated insect, and would be durable for multiple uses. We developed two new thermocouple designs, which we call the coil and cradle, and used them to measure exotherms of three parasitoids: *Spathius agrili* Yang (Hymenoptera: Braconidae), *S. floridanus* Ashmead (Hymenoptera: Braconidae) and *Tetrastichus planipennis* Yang (Hymenoptera: Eulophidae). These parasitoids attack emerald ash borer, *Agilus planipennis* Fairmaire (Coleoptera: Buprestidae) an invasive insect in North America that infests ash (*Fraxinus* spp.) trees (8). Each insect was placed in a gelatin capsule, and the capsule was placed on thermocouples of differing thickness. With the insects restrained directly over the junction of the two thermocouple metals, we expected larger exotherms from thinner wire than the thicker wire, especially for smaller insects.

MATERIALS AND METHODS

Thermocouple construction: Two styles of thermocouples were constructed: a coil and cradle design. The coil design was constructed with 0.51 mm diameter (24 AWG) copper-constantan wire (accuracy = $\pm 0.5^\circ\text{C}$). Wire was threaded through a 35-ml plastic syringe barrel (Fig. 1A). Approximately 7 cm of the plastic insulation was stripped from the end of the wire. The copper and constantan wires were wound tightly around each other for the entire length of the exposed wire. The wound wire was then formed into a spiral inside interior of the syringe tube (Fig. 1B).

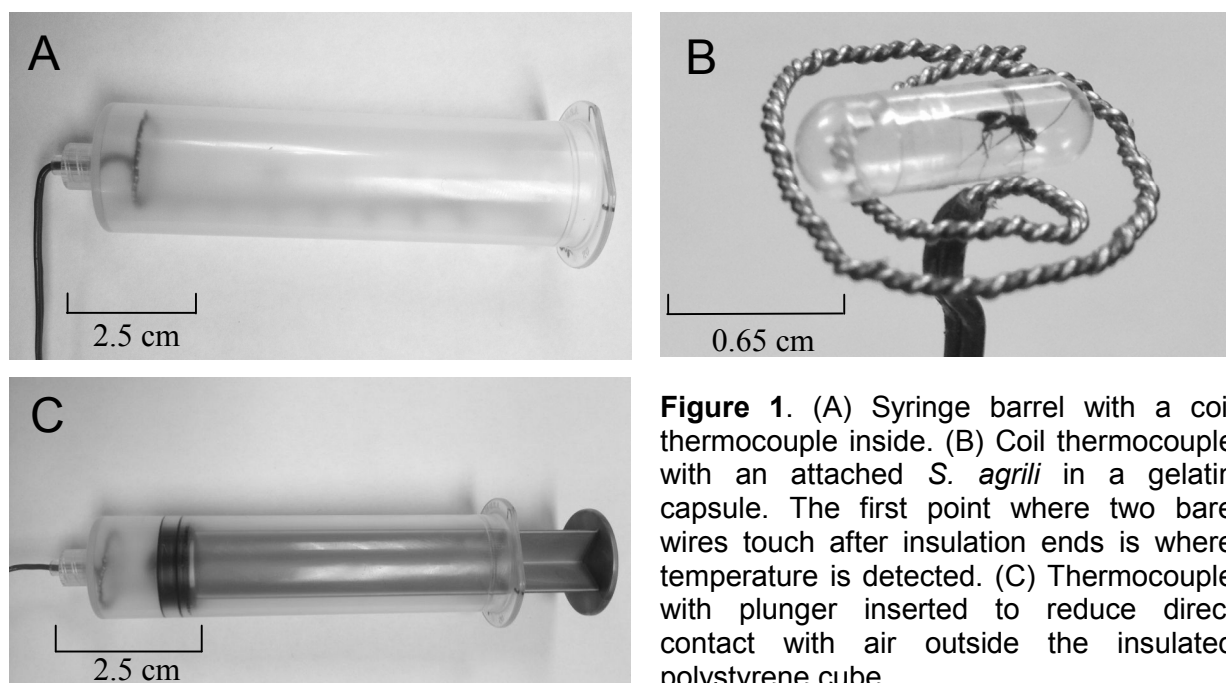


Figure 1. (A) Syringe barrel with a coil thermocouple inside. (B) Coil thermocouple with an attached *S. agrili* in a gelatin capsule. The first point where two bare wires touch after insulation ends is where temperature is detected. (C) Thermocouple with plunger inserted to reduce direct contact with air outside the insulated polystyrene cube.

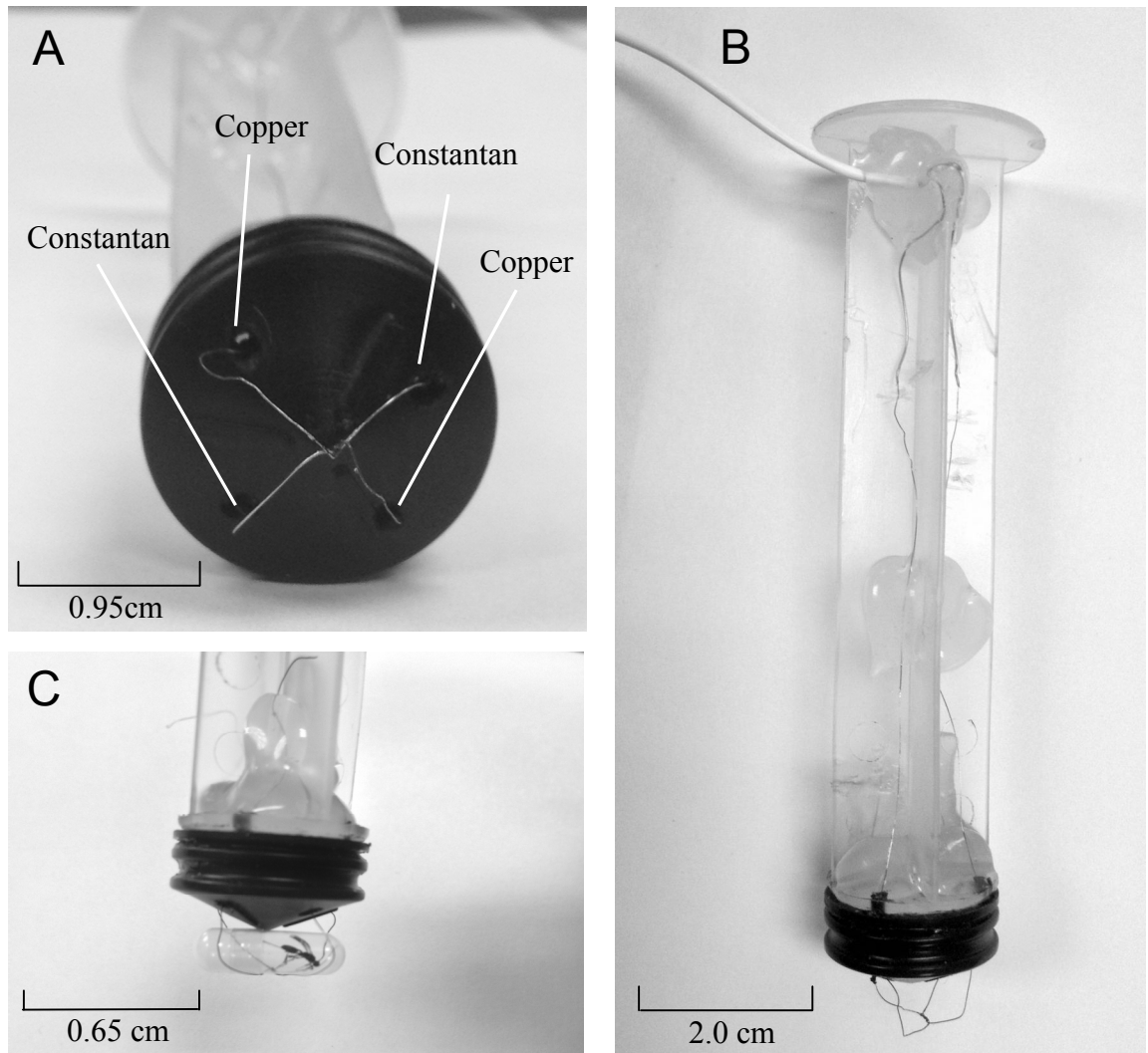


Figure 2. (A) Cradle thermocouple with holes made in the rubber plunger and the copper wire wrapped around the constantan. (B) Cradle thermocouple with glue holding bare wires in place. The split between the wires can also be glued to prevent wires from twisting. (C) Cradle thermocouple with an attached *S. agrili* in a gelatin capsule. Temperature is detected in the middle of the loops or “cradle” where the two wires are wound together.

The cradle design consisted of 0.127 mm (36 AWG) copper-constantan wire (accuracy = $\pm 0.17^{\circ}\text{C}$) and a 20 ml plastic syringe barrel. Four equally-spaced holes were melted through the plastic and rubber of the syringe plunger with a hot metal probe (Fig. 2A). Approximately 10 cm of insulation was stripped from the end of the thermocouple wire. The remaining insulation was split an additional 5 mm below the stripped wire. The constantan wire was threaded through one of the holes and through a hole on the opposite side so a 1cm loop of wire protruded from the rubber plunger. The loose end of the constantan wire was secured to the side of the syringe with thermoplastic glue (Adhesive Tech, Hampton, NH). The copper wire was threaded through one of the remaining holes in the plunger, wound tightly around the constantan wire three to four times, and threaded through the remaining hole (Fig. 2A). The copper and constantan wires were glued in place to ensure bare wires did not touch before the intended connection (Fig. 2B).

Supercooling point measurements: Thermocouple wires leading out of the syringes were connected to a multichannel data logger (USB-TC, Measurement Computing, Norton, MA). Supercooling points were measured for *S. agrili*, *S. floridanus*, and *T. planipennisi* adults reared from parasitized *A. planipennis* larvae at 18:6 h thermoperiod (26.5:22.5°C) and

photoperiod (L:D) at 60-70% RH. All parasitoids were reared by the US Department of Agriculture-Animal and Plant Health Inspection Service (Brighton, MI) and shipped via overnight courier to St. Paul, MN. Each insect was placed in a 5 x 14 mm clear gelatin capsule (Capsuline, Pompano Beach, FL). Capsules with insects to be cooled were held on the thermocouple with a small amount of high vacuum grease (Dow Corning, Midland, MI) at the first point of contact of the constantan and copper wires (Figs. 1C and 2C). For coil and cradle designs, the syringe plungers were pushed to the bottom of the syringe barrel leaving about 2 cm of open space, which was just enough room not to crush the insect (Fig. 1C). The thermocouples with insects were placed in polystyrene cubes calibrated to cool at a constant 1°C per minute inside a -80°C reach-in freezer and temperature was recorded every second (3). After all individuals had cooled to -30°C, the cubes were removed from the freezer and the parasitoids were placed in an 80% ethanol: water (vol:vol) solution for preservation. One run consisted of up to eight coil and eight cradle thermocouples containing a single species placed randomly in the freezer. At least two runs were performed per species. Supercooling points were determined as the lowest temperature experienced before the largest increase in temperature over 0.05°C, which was the approximate threshold where increases in temperature could be differentiated from slight background temperature variations. Exotherm height was determined as the difference between the supercooling point and the maximum temperature measured during the exotherm. In some cases we could not reliably detect an exotherm. A subset of parasitoids were later air dried for 1 wk at room temperature (~25°C) and weighed on a balance to obtain dry mass. However, individual *T. planipennisi* were <0.1 mg and could not be weighed with available equipment.

Data were analyzed using SAS 9.3 (11). Data were analyzed for normality (PROC UNIVARIATE) and homogeneity of variance (PROC GLM, Levene's test) to meet the assumptions for analysis of variance and linear regression. Supercooling points were normally distributed; potential effects of coil and cradle designs were tested with analysis of variance (ANOVA) blocked by run for each species (PROC GLM). Exotherm height for all three species and dry mass of *S. agrili* and *S. floridanus* were not normally distributed. A suitable transformation of exotherm height to correct for normality and unequal variance ($df = 5$; $F = 5.77$, $P > 0.01$) could not be found, so we used a Friedman nonparametric two-way ANOVA to test the effects of block and thermocouple on exotherm height. We applied a Bonferroni correction to account for family-wise error and maintain an overall α of 0.05 when comparing results from each ANOVA (7, 12). Logistic regression (PROC LOGISTIC) was used to evaluate the effects of block and thermocouple type on the proportion of tests in which an exotherm was detected for each species. The effect of block could not be evaluated for *S. agrili* due to quasi-complete separation. Linear regression (PROC REG) was used to determine if exotherm height on cradle thermocouples was correlated with dry mass (Box-Cox transformed (2) with $\lambda = -0.3$).

RESULTS

Mean supercooling points were not significantly different when measured on a coil or cradle thermocouple design across all species (Table 1; Fig 3A). Median exotherm height for *T. planipennisi* was significantly greater on the cradle thermocouples than on coil thermocouples (Table 1; Fig 3B). However, there was not a significant difference between coil and cradle exotherm heights for *S. agrili* or *S. floridanus* (Table 1). Supercooling points and exotherms were not significantly different between runs (Table 1).

Fig 3C illustrates the effect of thermocouple type on the proportion of tests in which an exotherm was detected. For *T. planipennisi*, exotherms were detected more frequently when measured with cradle thermocouples than coil thermocouples at Bonferroni adjusted $\alpha =$

0.0167 ($df = 1$; Wald $\chi^2 = 7.24$; $P < 0.01$); block had no effect ($df = 1$; $\chi^2 \leq 4.5$; $P \geq 0.03$). However, the proportion of tests in which an exotherm was detected was not affected by thermocouple type for *S. agrili* ($df = 1$; Wald $\chi^2 < 0.01$; $P = 0.94$) or *S. floridanus* ($df = 1$; Wald $\chi^2 = 1.85$; $P = 0.17$). Block had no effect for the latter species ($df = 1$; Wald $\chi^2 = 0.13$; $P = 0.72$). Dry mass was not a reliable predictor of exotherm height for *S. agrili* ($df = 1, 9$; $F = 1.57$, $P = 0.24$) or *S. floridanus* ($df = 1, 7$; $F = 0.94$, $P = 0.36$]. Mean masses were 0.7 mg ($n = 10$, SEM = 0.1) and 1.1 mg ($n = 10$, SEM = 0.1), for *S. agrili* and *S. floridanus*, respectively.

Figure 3. Mean ($\pm 95\%$ CI) supercooling points (A), and median exotherm size ($\pm 95\%$ CI) (B) and proportion ($\pm 95\%$ CI) of exotherms detected (C) for adult parasitoids. Asterisk in (B & C) denotes a significant difference between values for the coil and cradle design for that species. Sample sizes were identical for supercooling point and exotherm measurement.

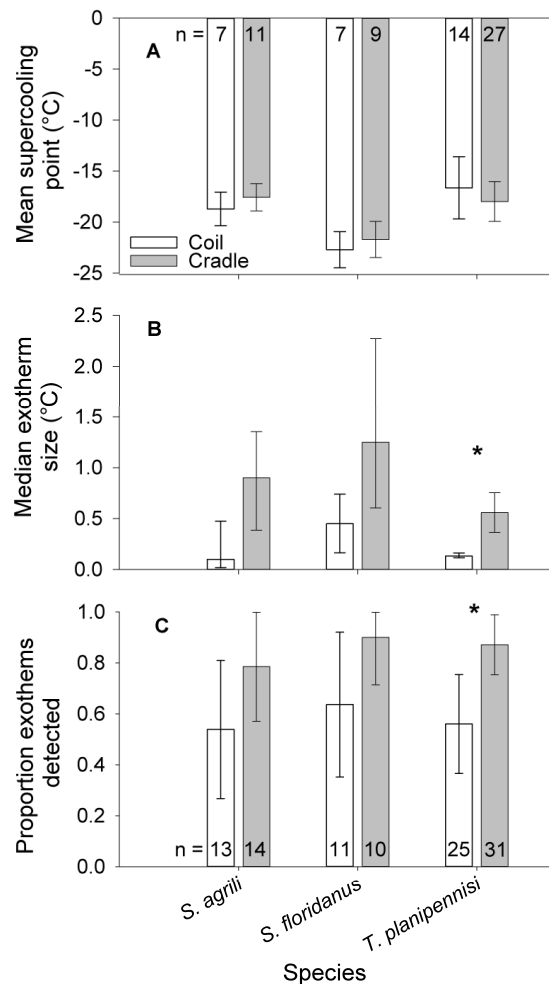


Table 1. Effects of block (run) and thermocouple type (coil vs. cradle) on supercooling point and exotherm height.

Response	Species	Run			Thermocouple type		
		<i>df</i>	<i>F</i>	<i>P</i>	<i>df</i>	<i>F</i>	<i>P</i>
Supercooling point	<i>S. agrili</i>	1, 15	0.01	0.93	1, 15	1.72	0.21
	<i>S. floridanus</i>	1, 13	6.54	0.02	1, 13	1.85	0.20
	<i>T. planipennisi</i>	3, 36	0.10	0.96	3, 36	0.58	0.45
Exotherm height	<i>S. agrili</i>	1, 15	6.54	0.17	1, 15	3.72	0.19
	<i>S. floridanus</i>	1, 13	5.22	0.04	1, 13	3.05	0.10
	<i>T. planipennisi</i>	3, 36	3.02	0.04	3, 36	13.57	<0.01*

* indicates significant p-value at Bonferroni adjusted $\alpha = 0.017$.

DISCUSSION

Our designs worked well to measure supercooling points of small insects. Both designs gave similar supercooling points, which suggests that design type and wire thickness do not bias the supercooling point measurement. For *T. planipennisi*, the smallest insect tested, we recorded larger exotherms and detected more supercooling points with the cradle design than the coil design. Because these larger exotherms were more readily distinguishable from random fluctuations in temperature readings, supercooling points were detected that might not have been observed with thicker gauge wire. This increase in efficiency can lead to fewer samples with no recorded supercooling point and less time required processing additional individuals to reach desired sample sizes. Because of the increased sensitivity of finer gauge

wire, exotherms may also be detected if the insect is not in direct contact with the thermocouple wire, which can occur with the insect inside a gelatin capsule.

Each thermocouple design has particular advantages. The coil design uses thicker gauge wire that maintains its shape when handling. Repairs were typically only needed when plastic insulation cracked. The cradle design stabilizes finer gauge wire that tends not to form to a desired shape without support. Cradles were prone to become broken or misshapen between uses. In both cases, insects were easily recovered after exposure to cold and could be observed without interference from grease. We found these designs to be extremely useful to measure supercooling points and recommend the cradle for small insects such as small parasitoids (e.g., <3 mm long) and the coil for larger insects when exotherms are easier to detect.

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