Too Hot to Move: Temperatures during Transportation Might Reduce the Survival of Salvinia Weevils (Coleoptera: Curculionidae)

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Source: Florida Entomologist, 102(4) : 701-707

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.102.0405

Too hot to move: temperatures during transportation might reduce the survival of salvinia weevils (Coleoptera: Curculionidae)

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Abstract

The biological control agent, *Cyrtobagous salviniae* Calder and Sands (Coleoptera: Curculionidae) (salvinia weevil), is being used for management of the highly invasive fern *Salvinia molesta* Mitchell (Salvinaceae) in Louisiana and Texas, USA. The weevils and plants are transported from the nurseries and rearing facilities to the field release sites in plastic totes. Despite the increased transport of weevil-infested plants during the warmer months, limited data exist on the impact of heat stress and survivability of adult *C. salviniae*. Therefore, research was conducted to determine temperatures inside totes during summer transport, and to determine the upper temperature threshold for adult weevil survival. Field data demonstrated that temperatures within the totes were capable of exceeding 35 °C, and the type of lid used to secure plant material influenced internal temperature. In addition, there were no differences in temperature within the totes. Growth chamber trials determined the upper lethal time to kill 50 and 90% of the test population (ULt_{50} and ULt_{90}) at 35 °C was 27.5 and 42.8 hours, respectively, while at 40 °C, the ULt_{50} and ULt_{90} was 15.0 and 25.0 hours, respectively. As the temperature increased to 50 °C, the calculated ULt_{50} and ULt_{90} values were 5.0 and 11.0 minutes, respectively. These data provided evidence that *C. salviniae* mortality occurs more rapidly as the temperature increases, especially > 45 °C, and that extreme temperatures can occur within transportation totes.

Key Words: *Cyrtobagous salviniae*; heat stress; *Salvinia molesta*; thermal tolerance

Resumen

El agente de control biológico, *Cyrtobagous salviniae* Calder y Sands (Coleoptera: Curculionidae), está siendo utilizado para el manejo de la maleza altamente invasiva, *Salvinia molesta* Mitchell (Salvinaceae), en Luisiana y Texas, E.U.A. Los gorgojos y las plantas son transportados desde los criaderos hacia los sitios de liberación en envases de plástico. A pesar del mayor transporte de plantas infestadas con gorgojos durante los meses calientes, hay datos limitados del impacto del estrés por calor y sobrevivencia de adultos de *C. salviniae*. Por consiguiente, se hizo una investigación para determinar las temperaturas dentro de los envases durante el verano y determinar el nivel crítico para la sobrevivencia de adultos del gorgojo a altas temperaturas. Datos de campo demostraron que temperaturas dentro de los envases son capaces de exceder 35 °C y el tipo de tapa del envase influenció su temperatura interna. Además, no hubo diferencias de temperatura dentro de los envases. Ensayos en cámaras de crecimiento determinaron que el tiempo letal para matar el 50 y 90% de la población muestra (Tl_{50} y Tl_{90}) a 35 °C fue de 27.5 y 42.8 horas, respectivamente, mientras que a 40 °C, el Tl_{50} and Tl_{90} fue 15.0 y 25.0 horas, respectivamente. Como las temperaturas incrementaron hasta 50 °C, los valores de Tl_{50} and Tl_{90} fueron 5.0 y 11.0 minutos, respectivamente. Estos datos son evidencia que la mortalidad de *C. salviniae* ocurre más rápidamente como las temperaturas incrementan, especialmente a más de 45 °C, y que temperaturas extremas pueden ocurrir dentro de los envases de transporte.

Palabras Clave: *Cyrtobagous salviniae*; estrés por calor; *Salvinia molesta*; tolerancia termal

The effects of non-native invasive species on ecosystems include reduction of biodiversity, decline in native species (Gilbert & Levine 2013), changes in ecosystem function (Vilà et al. 2011), and negative economic impacts (Simberloff et al. 2005). Giant salvinia, *Salvinia molesta* Mitchell (Salviniaceae), is one of the world's worst invasive weeds (Koutika & Rainey 2015), and is responsible for nearly $7 million in damage to the state of Louisiana, USA (LSU 2015). The rapid growth rate of *S. molesta* can degrade habitats for other aquatic plants, fish, invertebrates, and wildlife (Barrett 1989; Madsen 2014), and can alter dynamics of the water column by preventing sunlight and oxygen from entering the waterbody (van Oosterhout 2006). Furthermore, mats of *S. molesta* can provide ideal breeding habitats for mosquitoes, which are vectors for human pathogens (Room et al. 1989; Lounibos et al. 1990).

Control tactics include physical and mechanical removal, lake drawdowns, aquatic herbicides, and biological control (Thomas & Room 1986; van Oosterhout 2006; Richardson 2008). While these tactics often provide short-term control of giant salvinia, biological control is more cost-effective and may provide sustainable long-term control. The salvinia weevil, *Cyrtobagous salviniae* Calder and Sands (Coleop-

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tera: Curculionidae), is a biological control agent of *S. molesta*, native to Brazil, and widely used throughout the world (Forno et al. 1983). The first release of *C. salviniae* in the US was conducted in Texas and Louisiana in 1999 (Tipping & Center 2005), and in 2001, mass releases of *C. salviniae* began throughout the adjoining states (Johnson et al. 2010), and continue throughout Texas and Louisiana.

To maximize its availability for field releases, *C. salviniae* is mass reared in outdoor earthen ponds or in temperature-controlled greenhouses (Sullivan et al. 2011; Knutson & Nachtrieb 2012; Wahl et al. 2016). In the US, rearing facilities harvest weevil-infested *S. molesta* when densities reach 30 to 50 adults per kg of fresh plant weight (Wahl et al. 2016), and plant material is transported to the field using plastic totes (Knutson & Nachtrieb 2012; Wahl et al. 2016). These totes are modified with 10 or more holes drilled in the bottom and sides to allow for drainage of excess water, covered with a fastened lid, and secured with zip ties (Wahl et al. 2016). The totes are transported in truck beds, utility trailers, and metal boats to field infestations up to 500 km from rearing operations. Despite the importance to a *S. molesta* biological control program, the heat tolerance of *C. salviniae* during transport conditions has not been studied.

Summer temperatures in Louisiana and Texas, USA, may reach the upper thresholds for weevil survival. The average high temperatures in spring and fall in Louisiana are 25 °C, while the summer mo have an average high of 33 °C (NCDC 2017); however, temperatures above 35 °C are common (National Weather Service Forecast Office 2019). *Cyrtobagous salviniae* is commonly released in the spring or fall to minimize the impact of extreme heat during the summer mo (Sanders et al. 2011). Although previous researchers claim cooler mo are the most efficient time to establish *C. salviniae* populations (Sullivan et al. 2011; Sullivan & Postle 2012), there are no temperature data from transport totes. Additional research is required to identify the impact of heat stress on *C. salviniae*-infested *S. molesta*, including evaluating the survivability of adults from Louisiana at upper lethal time/temperature intervals. Therefore, the objectives of this research were (1) to define summer transport temperatures inside totes, and (2) to determine the upper temperature threshold for adult weevil survival.

Materials and Methods

TEMPERATURE CONDITIONS INSIDE TRANSPORTATION TOTES

Data were collected to determine temperatures experienced by *S. molesta* and *C. salviniae* inside plastic totes during transportation. All 3 experiments were conducted during field harvests from rearing ponds in Houma (29.5600°N, 90.7700°E) or Lena (31.5200°N, 92.7300°E), Louisiana, USA, to various release sites from May through Sep of 2016. Although these mo are not recommended for weevil transport due to high air temperatures (Sanders et al. 2011), trials were conducted under unfavorable and worst-case conditions for *C. salviniae* transportation.

In the first study, internal tote temperature data were collected to determine temperature differences within a single tote, particularly at the top of the tote from sunlight, and at the bottom of the tote due to conductive heat from trucks, trailers, or boats. Data were collected on 14 and 22 May 2016 using 2 light-colored plastic totes (Rubbermaid® Roughneck Storage Box, Steel Gray, 68 L, 41.9 × 60.7 × 40.4 cm) and 6 HOBO Pendant® data loggers (Onset Computer Corp., Pocasset, Massachusetts, USA) set to record temperature every 30 min. Data loggers were placed within the tote, directly on the bottom and below the plant material, in the middle of the plant material, and directly on top of the plant material. Two types of lids were used to secure the totes, 1 with a conventional lid and 1 with a modified lid containing 10 holes (1.3 cm diam). Temperature collection occurred on both harvest days from 9:30 A.M. to 17:30 P.M. (n = 42 per lid type). Both types of lids were secured using zip ties, transported in a boat (towed by a truck) from the insect nursery, and released into a field site 10 h after initial harvest from the rearing site. Potential temperature differences due to placement of data loggers within the totes fastened with 2 types of lids in May 2016 were subjected to a 3-way analysis of variance (ANOVA) at $P \leq 0.05$ (SigmaPlot 11.0, Systat Software, Inc., San Jose, California, USA).

In a second study, loggers were used to assess the relationship between air and tote temperature using conventional and modified lids with holes. Data loggers were placed within the top 2.5 cm of plant material inside the tote to record internal tote temperature, and loggers were affixed directly on the lid of the corresponding tote to record air temperature. Initial data collected from the sunlight-exposed logger affixed on the top of the tote yielded abnormally high air temperatures. For instance, on 8 Jun 2016, the logger recorded an external temperature of 48.3 °C; however, the maximum recorded temperature for that d was 32.8 °C (NCEI 2017). As a result of these initial findings, both subsequent trials included HOBO loggers placed in 2 separate M-RSA Solar Radiation Shields (Onset Computer Corp., Pocasset, Massachusetts, USA) and mounted to transportation vehicles instead of directly mounted to the totes. The Solar Radiation Shield is a multi-plate plastic housing to protect the pendant device from direct sunlight, and functions as a thermal insulator for the most accurate measurements compared to several other methods (Ribeiro da Cunha 2015). Air temperature, tote temperature with conventional lids, and tote temperature with modified lids were subjected to a 1-way ANOVA, and post-hoc tests (Fisher's protected LSD) were used for pairwise comparisons ($P \leq 0.05$, n = 80) (SigmaPlot 11.0). Bivariate analysis also was used to define the linear relationship between the air temperature and 2 tote temperature variables ($y = \beta_0 + \beta_1 x_i$) (JMP®, Version 13, SAS Institute Inc., Cary, North Carolina, USA). The results of these analyses were used to determine temperatures and exposure periods for the laboratory mortality experiments.

ADULT HEAT MORTALITY UNDER LABORATORY CONDITIONS

Weevil populations tested in this study were from Natchitoches, Louisiana, USA (31.7700°N, 93.0600°E), established in 2013, and originally collected from Houma, Louisiana, USA (29.5600°N, 90.7700°E). Adult *C. salviniae* were extracted from *S. molesta* collected from the field site in the summer of 2016 using Berlese funnels (Boland & Room 1983), and held at laboratory temperatures (24 °C) for a maximum of 96 h at the Red River Waterway Commission facility in Lena, Louisiana, USA. Survival of adult *C. salviniae* was measured by placing groups of 10 adults on 2 growing tips (3–4 cm long) of fresh *S. molesta* (Croxdale 1978) inside sterile, plastic Petri dishes (100 × 15 mm, Carolina Biological Supply, Burlington, North Carolina, USA) containing moistened qualitative filter paper (9 cm diam, Ahlstrom-Munksjö, Helsinki, Finland). Filter paper was moistened with 1 mL of rainwater (pH 6.7) for treatment groups less than 24 h, and 2 mL of rainwater for exposures ≥ 24 h to prevent plant and weevil mortality as a result of dehydration and desiccation. Groups of 10 adults comprised a replicate, each treatment was replicated 4 times, and the entire trial was repeated within 1 mo. To understand a worst-case scenario and mimic field conditions, insects and plants were not provided an acclimation period, and adult *C. salviniae* were immediately exposed to temperatures of 35, 40, 45, or 50 °C in separate environmental growth chambers (Percival, I-36VLC8, Perry, Iowa, USA). Exposure temperatures were selected from temperatures experienced inside transportation totes (35–50 °C), and exposures were conducted in complete darkness to mimic transport conditions. Length of exposures for each temperature scenario are detailed in Table 1.

Table 1. Temperature and length of exposures to which a Natchitoches, Louisiana, USA, population (31.7700°N, 93.0600E°) of adult *Cyrtobagous salviniae* were exposed in environmental growth chambers to determine heat mortality.

Temperature (°C)	Length of exposure (units)
35	0, 5, 10, 15, 20, 24, 28, 32, 36, 40 (h)
40	0, 1, 5, 10, 20, 22, 24, 26 (h)
45	0, 20, 40, 60, 80, 100, 120, 140 (min)
50	0, 2, 5, 7, 10, 15 (min)

Immediately following exposure, groups were removed from the growth chambers and allowed to recover at laboratory temperatures (24 °C) for 1 h. Insect mortality was assessed by removing adult *C. salviniae* from plant material and returning the insects to the same Petri dishes with re-moistened filter paper (1 mL rainwater) for an additional h to record movement. Adults were placed in the dorsal position and considered deceased if they could not right themselves into a walking position (Mukherjee et al. 2014). Due to no significant differences between trials (P = 0.992), data were pooled (n = 80). Mortality data were analyzed using linear regression analysis ($y = \beta_0 + \beta_1 x_i$; ± 95% confidence intervals) with temperature as the independent variable and percent survival as the dependent variable for the coinciding temperatures (JMP®, Version 13, SAS Institute Inc., Cary, North Carolina, USA). Also, upper lethal time at which 50 and 90% mortality was experienced (ULt_{50} and ULt_{90}, respectively) was calculated, with 95% confidence intervals (Payton et al. 2003).

Results

TEMPERATURE INSIDE TRANSPORTATION TOTES

There were significant internal tote temperature differences between collection dates (May 2016) (P < 0.001) (Table 2). For example, on 15 and 22 May 2016, the recorded high temperature for both d was 29 °C (NCEI 2019), but differences in cloud cover could have been responsible for the differences between dates. Regardless of collection date, there was a significant difference in the lid type used (P = 0.048); the mean internal tote temperature for conventional lids was 2 °C higher than modified lids (Table 2). In addition, there were no differences in placement of the temperature logger throughout the tote (i.e., top vs. middle vs. bottom) (P = 0.729) (Table 2, Fig. 1); therefore, loggers were placed in the top 2.5 cm of plant material for future studies where air temperature was collected using solar radiation shields.

Our data confirmed a difference in lid type used, and there were significant differences in mean temperatures for all 3 components. For the collection periods in May 2016, the mean air, conventional lid, and the modified lid temperatures were 37.8, 38.3, and 35.5 °C, respectively, with an LSD of 1.3 ($P \leq 0.05$). The modified lid yielded temperature means that were 2.8 °C cooler than conventional lids, and 2.3 °C cooler than air temperature. For future use in management practices, a bivariate analysis was conducted to aid plant managers in estimating internal tote temperature based on air temperature (Fig. 2a, b). For example, when the average air temperature is 35 °C, the predicted temperatures with conventional and modified lids would be 36.1 and 33.2 °C, respectively (Fig. 2a, b). Alternately, if an air temperature reaches 40 °C, modified lids can be 3.9 °C cooler than conventional lids. Although, these internal tote temperature differences are minimal, *C. salviniae* may benefit from cooler conditions by modifying lid types, particularly at higher temperatures (≥ 40 °C) during transport. Consequently, natural resource managers should consider lid modifications in the future and release infested *S. molesta* when air temperatures would yield the lowest internal tote temperatures (i.e., early morning and earlier in the growing season).

ADULT HEAT MORTALITY UNDER LABORATORY CONDITIONS

Laboratory experiments yielded a strong relationship between length of exposure and air temperature. At 35 °C, the lethal time to kill 50 and 90% of the test population (ULt_{50} and ULt_{90}, respectively) was at 27.5 and 42.8 h of exposure, respectively (Table 3). At 40 °C, the ULt_{50} and ULt_{90} values were 14.8 and 24.8 h, respectively. Although lengthy exposures are unlikely, some plant managers travel 500 km from harvest to release and may take up to 10 h before weevils can be released. When *C. salviniae* are exposed to higher temperatures, mortality occurs more rapidly in shorter periods of time. In the laboratory studies when *C. salviniae* were exposed to 45 °C, the ULt_{50} and ULt_{90} values were 56.9 and 109.5 minutes, respectively (Table 3). At 50 °C, the calculated ULt_{50} and ULt_{90} values were 5 and 11 minutes, respectively (Table 3). While these air temperatures are unlikely in Louisiana and Texas, internal tote temperatures (with and without holes) reached 40 to 45 °C for short periods of time (< 30 min) during field trials (Fig. 1).

Using the bivariate formulas to predict internal tote temperatures based on air temperatures, plant managers then can use formulas derived from laboratory experiments to predict *C. salviniae* mortality based on length of exposure. If the predicted internal tote temperature is 35 °C and the travel time is 10 h, plant managers can

Table 2. Three-way analysis of variance ($P \leq 0.05$) for date of temperature collection, placement of temperature logger within transportation tote, and type of lid used on transportation tote.

Source of variation	Degrees of freedom	Sum of squares	Mean sum of squares	F	P
Date[a]	1	4,756,555	4,756,555	89.191	< 0.001
Placement of logger[b]	2	33,713	16,857	0.316	0.729
Lid Type[c]	1	211,201	211,201	3.960	0.048
Date × Placement	2	189,009	94,504	1.772	0.172
Date × Lid type	1	53,737	53,737	1.008	0.316
Placement × Lid type	2	21,301	10,651	0.200	0.819
Date × Placement × Lid type	2	8,348	4,174	0.078	0.925
Residual	240	12,799,190	53,330		
Total	251	18,073,054	72,004		

[a]15 May and 22 May 2016.
[b]Top, middle, and bottom of plant material within transportation tote.
[c]Conventional lids and lids modified with 10 (1.3 cm diam) holes.

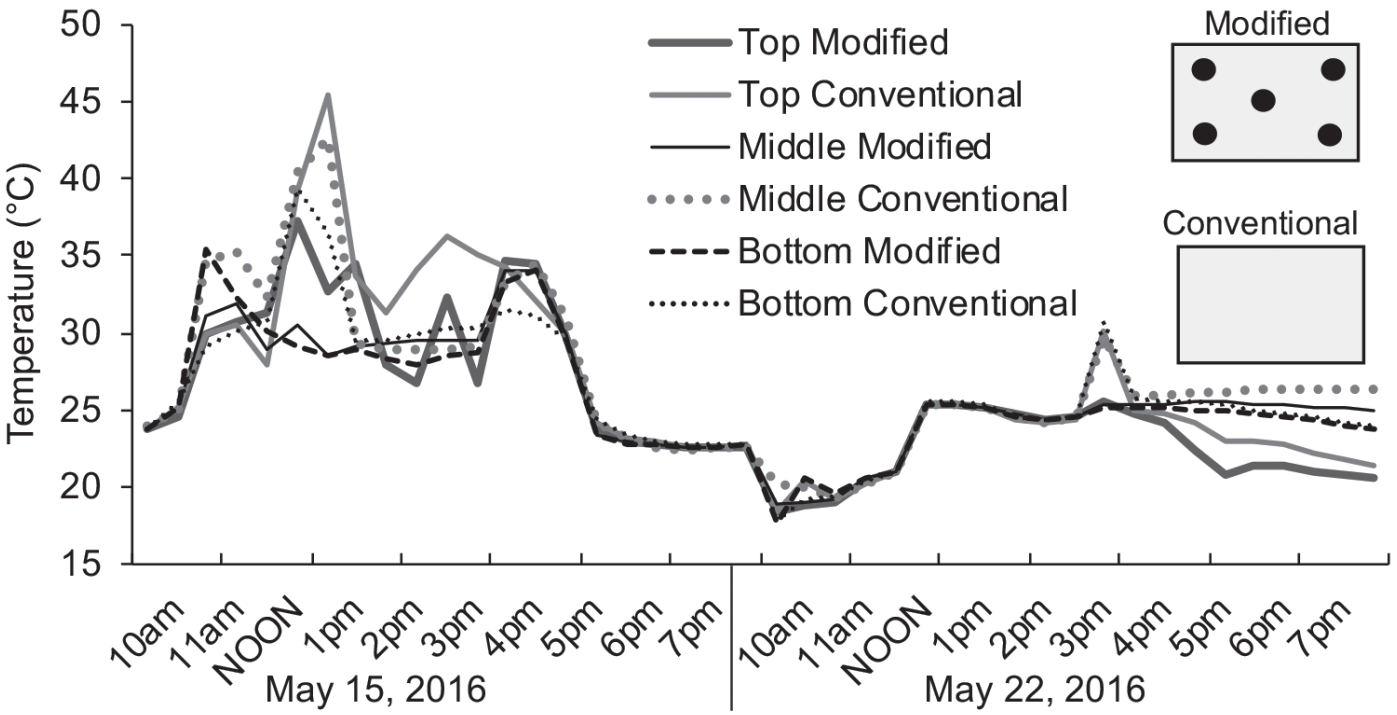

Fig. 1. Temperature (°C) in transportation totes with conventional and modified (1.3 cm holes throughout) lids. Placement of data loggers were at the top, middle, and bottom of plant material within the tote during 2 harvest d in May 2016.

expect an insect mortality of 4.2% (Fig. 3a). As expected, warmer temperatures within the totes resulted in increased mortality. At 40 °C for 10 h of exposure (transport), plant managers can expect 31% mortality (Fig. 3b), and should estimate lower insect densities at the time of release to reflect this loss. Although the data presented in this research is a worst-case scenario where *C. salviniae* would be exposed to a specific temperature (i.e., 40 °C) continuously for several h, the totes will have an opportunity to cool down if abiotic factors such as cloud cover and rain occur, which likely will aid in reducing weevil mortality.

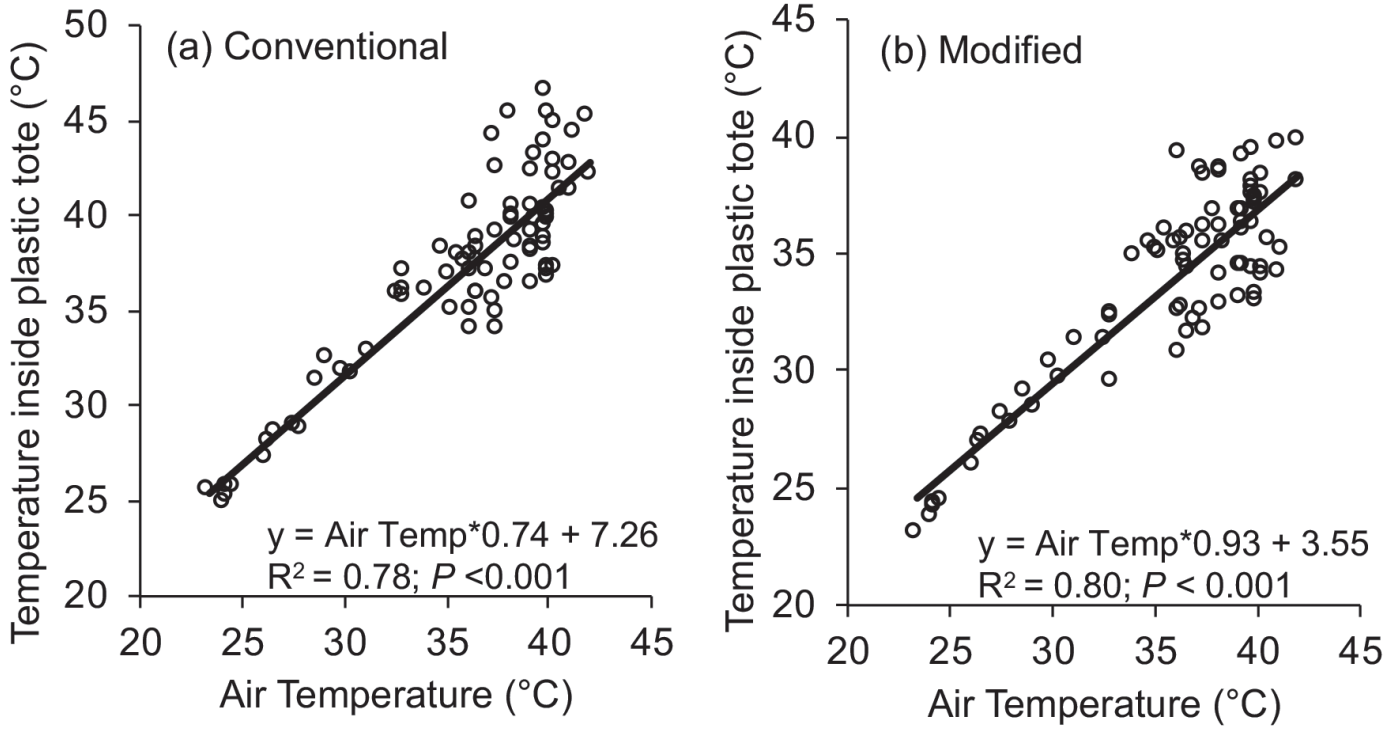

Fig. 2. Bivariate analysis of air temperature and temperature experienced inside transportation totes for *Cyrtobagous salviniae* (a) with 1.3 cm circulation holes in modified fitted lids (n = 80), and (b) conventional lids without circulation holes ($P \leq 0.05$; n = 80) in May 2016.

Table 3. ULt_{50} and ULt_{90} of a Natchitoches, Louisiana, USA, population of *Cyrtobagous salviniae* exposed to 4 temperature regimes in 2016.

Temperature (°C)	*n*	Slope ± SE	Lt_{50} [a] (95% CI)[b]	Lt_{90} (95% CI)	r^2
35	400	−21.90 (4.7)	27.5 (22.8–32.2) h[c]	42.8 (38.1–47.5) h	0.83
40	320	−9.03 (2.6)	14.8 (12.2–17.4) h	24.8 (22.2–27.4) h	0.96
45	320	6.77 (4.3)	56.9 (52.6–61.2) m[c]	109.5 (105.2–113.8) m	0.88
50	240	17.7 (4.6)	5.0 (0.4–9.6) m	11.1 (6.5–15.7) m	0.86

[a]ULt_{50} and ULt_{90} values represent the time in hours to kill 50 and 90% of the populations, respectively.
[b]Non- overlapping CI (confidence intervals) indicate significant differences.
[c]Abbreviations: h = hours of exposure, m = minutes of exposure.

Discussion

The temperatures evaluated in this study are outside of optimum range for *C. salviniae* development (19–30 °C) and for *S. molesta* growth (13–33 °C) (Room et al. 1984), and therefore are valuable in understanding weevil and plant survival under these extreme conditions. Establishment, reproduction, and persistence of *C. salviniae* are vital to the efficacy of this biological control agent (Allen et al. 2014), and while little is known about heat stress on other life stages (e.g., egg, larva, and pupa), these extreme temperatures, even in short cycles, may likely affect future generations of *C. salviniae*.

Until recently, low *C. salviniae* densities after release were assumed to be due to insect dispersal; however, the findings of this study offer alternate insight to low densities as a result of unfavorable heat exposures dur-

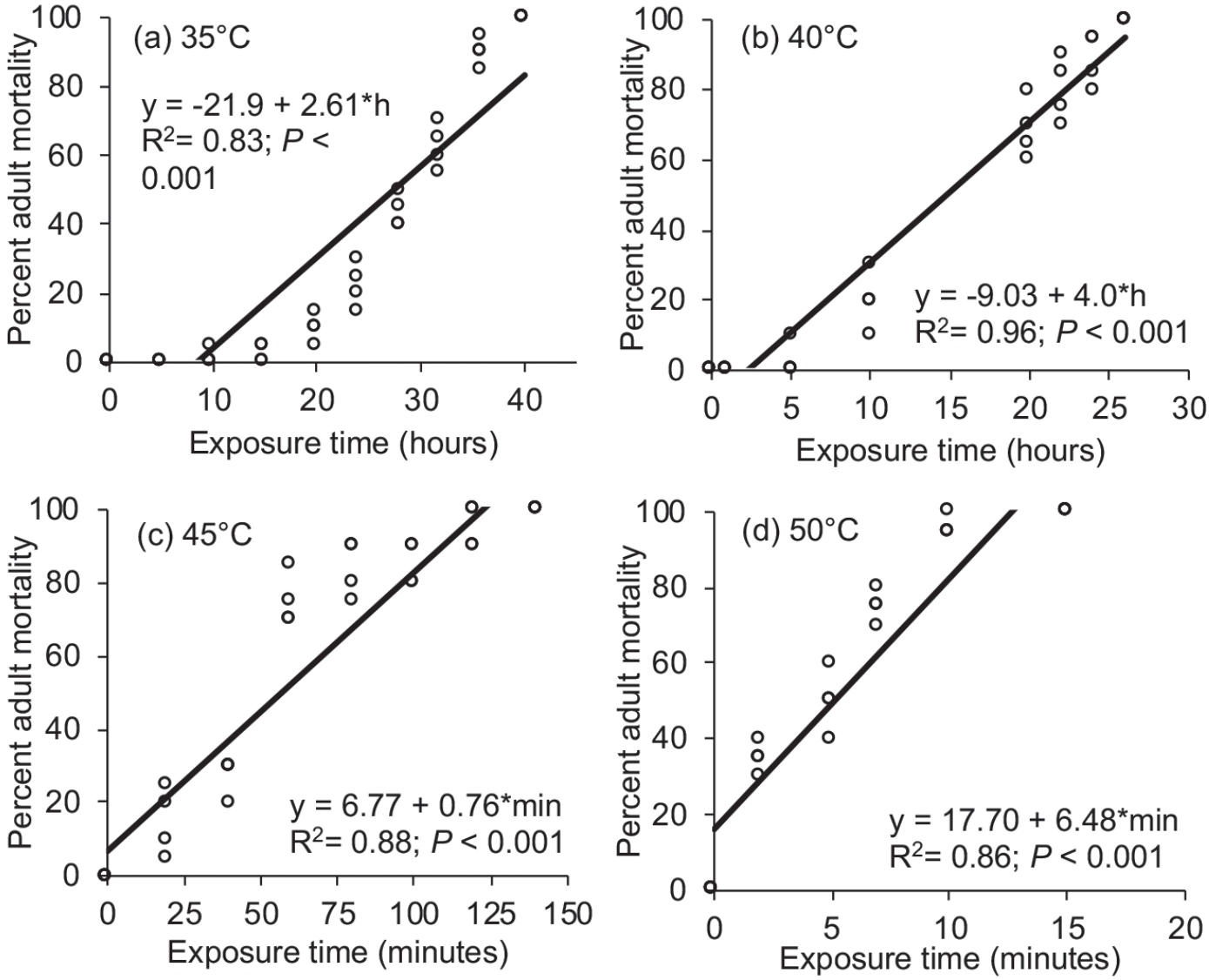

Fig. 3. Percent mortality of *Cyrtobagous salviniae* exposed to 4 temperatures in environmental growth chambers: (a) 35 °C, (b) 40 °C, (c) 45 °C, and (d) 50 °C.

ing transportation. This research will provide plant managers with information to adjust insect release estimates depending on air temperature and length of travel, and possibly explain lower insect densities in future sampling events. Furthermore, plant managers can confidently release *C. salviniae* during summer mo if the anticipated air temperatures are lower than data presented here.

Weather conditions and heat events, particularly during the hottest part of the d, directly affect the behavior and development of insects (Cui et al. 2011). The impacts of heat stress on different life stages have been investigated on a limited number of species (Zani et al. 2005). This research supports previous findings when *C. salviniae* were exposed to lethal temperatures for 1 h and a ULt_{50} of 43.7 °C was estimated (Allen et al. 2014). In the present research, a 1 h exposure at 45 °C yielded 52% *C. salviniae* mortality. Similarly, *S. molesta* heat mortality was investigated in a laboratory setting, and results by Whitman & Room (1991) indicated *S. molesta* buds were killed at temperatures > 43 °C for exposures of 2 to 3 h. Although the current study focused on *C. salviniae* mortality, temperatures were greater for shorter periods of time, and likely would have impacted plant survival under these extreme conditions.

Since resource agencies release *S. molesta* along with all life stages of the weevil, future research should investigate mortality of eggs, pupae, and larvae, and use lids with holes for optimum transportation and survival. Although previous recommendations suggested to release in *C. salviniae* in the spring or fall when temperatures are cooler (Sanders et al. 2011; Sullivan et al. 2011; Sullivan & Postle 2012), our data defined the temperature conditions and associated mortality so that releases can be made throughout the *S. molesta* growing season. For instance, when *C. salviniae* in rearing facilities reach optimum densities, particularly outside of the spring or fall, these data provide suggestions to alleviate extreme temperatures within transportation totes, as well as methods to estimate *C. salviniae* mortality at specific air temperatures.

Acknowledgments

This research was supported by the Red River Waterway Commission and Louisiana State University Agricultural Center. Appreciation is extended to Nathan Vercher for technical assistance, as well as the Louisiana Department of Wildlife and Fisheries, and the U.S. Army Engineer Research and Development Center Aquatic Plant Control Research Program for project support. The manuscript was improved by comments of 2 anonymous reviewers. Permission was granted by the Chief of Engineers to publish this information. Citation of trade names does not constitute endorsement or approval of the use of such commercial products.

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