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Original Communication

In-transit temperature extremes could have negative effects on ladybird (*Coleomegilla maculata*) hatch rate

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ABSTRACT

The shipment of mass-produced natural enemies for augmentative release is a standard procedure used by the biological control industry. Yet there has been insufficient research on the effects of temperature change experienced during shipment, on the quality of the predators as they arrive at release sites. In this study, we monitored the in-transit environmental conditions inside polystyrene foam (Styrofoam) shipping containers and simulated the effect of low and high temperatures on egg hatch of the ladybird *Coleomegilla maculata*, a predator of small arthropods (e.g., aphids). We tested the prediction that a 24-h exposure to extreme temperatures reduces the rate of egg hatch. We measured the temperatures (and percent relative humidity) by placing data loggers inside containers, enclosed inside single-walled cardboard boxes, then shipping the boxes roundtrip from Mississippi to Georgia, Oregon, and California in August, October, December 2012, and March 2013. Our results indicate that the temperature fluctuated considerably in transit, reaching high and low values of 35 °C in October 2012 (California shipment) and 4 °C in March 2013 (Oregon shipment). Relative humidity sometimes correlated positively with temperature in the same containers. Relative humidity was lowest in the March 2013 shipments, averaging from 33% (Georgia shipment) to 43% (California shipment). In laboratory bioassays, a 24-h exposure to 36 °C and 6 °C significantly reduced *C. maculata* egg hatch when compared with the control, 25 °C. Egg hatch rate was 13% and 50% at 36 °C and 6 °C, respectively, in comparison to 64% at 25 °C. This suggests that brief exposure to high (rather than low) temperatures are more harmful to egg hatch. In summary, redesigning containers to limit temperature extremes (especially high temperatures) is necessary to facilitate shipment of fragile, developing stages (i.e., eggs) of predators intended as biocontrol agents.

KEYWORDS: biological control, Coccinellidae, development, mass rearing, pink-spotted lady beetle

INTRODUCTION

Coleomegilla maculata DeGeer (Coleoptera: Coccinellidae) is an important predator of insect and mite pests on plants in agricultural fields and natural landscapes in North, Central, and South America [1-4]. It also has the potential to reduce aphid population density in protected plant systems (greenhouses, glasshouses, and high tunnels) in North America [5, 6]. We have been developing and refining techniques for the efficient mass production of *C. maculata* with the vision of marketing it for augmentative releases to manage pests (e.g., aphid species) on crop plants grown in protected systems.

The effects of shipping conditions on the quality of mass-produced beneficial insects upon arrival at release sites is a major concern of the biocontrol industry [7-12]. Temperature extremes experienced by ladybird beetles (and other

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beneficial insects) during shipment from the production site to release sites could affect their reproductive health and performance [13]. Typically, extended exposure to temperatures below 8 °C for more than 24 h, can have negative effects on immature development in the two-spot ladybird *Adalia bipunctata* (L.) and *C. maculata* [14, 15]. Extended exposure to high temperatures can also affect immature development. For example, exposure of eggs of a ladybird, *Rodolia cardinalis* (Mulsant), to 34-37 °C for 72 h severely reduced egg hatch [16].

The biocontrol industry has utilized shipping containers made from various materials, but polystyrene foam (often called Styrofoam) containers, encased within a cardboard box appears to be the mainstay in recent years. Inside the foam containers, the insects are often held in plastic or paper tubes or Petri dishes, depending on the developmental stage of the insect being shipped. An ice pack or some other material (e.g., dry ice) has been used to moderate internal temperatures inside foam containers, especially during the warm seasons of the year. Only a few publications report the temperature inside shipping containers and the potential effects of temperature change on beneficial insects during transit (shipment) from the site of mass production to the site of augmentative release. Just one study provided evidence that containers made from polystyrene foam (Styrofoam) did not adequately maintain internal temperatures during shipment [17].

In this study, we investigated the internal temperature inside polystyrene foam containers and the possible effects of extreme temperatures on the development of *C. maculata* eggs. We tested the prediction that a 24-h exposure of *C. maculata* eggs to high temperatures, rather than low temperatures, would negatively affect hatch rate. Thus, the objectives of this research were to determine temperature variability inside containers during shipment by commercial carriers, and test the effect of temperature under simulated conditions in the laboratory, on embryonic development, i.e., egg hatch of *C. maculata*.

MATERIALS AND METHODS

1. Evidence for temperature change in polystyrene foam containers during shipment

We used Dickson™ data loggers to monitor temperature (as well as percent relative humidity) every six minutes inside the polystyrene foam containers of dimensions 30 cm (length) × 19 cm (width) × 12 cm (height) (Fig. 1). The ice pack held 16 ounces of a non-toxic, gel refrigerant; it was stored in a laboratory freezer (at -20 °C) until the data logger was placed in the foam container. The data logger and ice pack were secured in separate Ziploc® plastic bags. Then the foam container was placed inside a slightly larger,

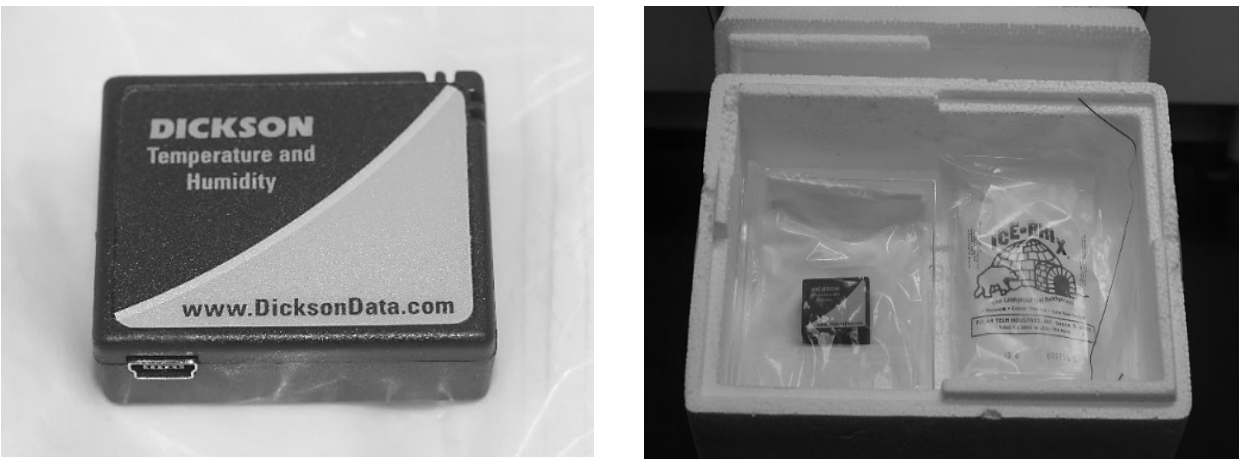

Fig. 1. Image of a data logger (left) and its position adjacent to an ice pack in a polystyrene foam container (right) prior to being enclosed inside a white single-walled cardboard box and shipped to designated locations in the USA.

single-walled cardboard box (ULINE™, 32 cm (length) × 26 cm (width) × 17 cm (height)), prior to pick-up by a commercial carrier, either United Parcel Service (UPS) or Federal Express (FedEx). Two replicate foam containers with an ice pack in each (housed inside separate cardboard boxes) were shipped roundtrip, from Stoneville, Mississippi (MS) to collaborators in three locations: Byron, Georgia (GA); Oxnard, California (CA); and Clackamas, Oregon (OR). The roundtrip shipments to these locations were from 13-16 August 2012, 15-18 October 2012, 3-7 December 2012, and 4-7 March 2013. Once the boxes reached their destinations, collaborators returned the boxes to us in Stoneville. Ice packs were not re-charged on the return trip to Stoneville. Upon receipt of the boxes, we removed the data loggers and transferred the data to Excel spreadsheets. Coded temperature (and humidity) data were transferred from the loggers, using Dickson data software, to Excel spreadsheets, and then decoded and plotted.

2. Simulated effects of temperature on *C. maculata* egg hatch rate

We determined the effects of short-term (24 h) exposure to a low and high temperature extremes on *C. maculata* eggs, simulating the low and high temperatures observed during shipment. The temperature treatments were 36 °C, 6 °C, and 25 °C (control), which were programmed into three replicate growth chambers of the same size, model, and approximate age. The relative humidity was set at 60% in the three replicate growth chambers during the experiment. No lighting was provided in the high (36 °C) and low (6 °C) temperature treatments, to closely mimic the lack of lighting inside a shipping container, while in transit. At the control temperature (25 °C), the photoperiod was 16 h light (L)/8 h dark (D).

The bioassays were done in plastic Petri dish (35 mm diam. × 10 mm height) arenas with lids. We used a completely randomized design with 10 replicate dish arenas (including one egg clutch per dish) per treatment in seven replicate trials. Each week, on the same day, at least 80 egg clutches (from at least five separate containers, housing 4-5 mating pairs) were harvested from our stock colony of *C. maculata,* which have been in rearing for more than 10 consecutive generations inside an environmental rearing room, set at 25 °C and 16 h light/8 h dark photoperiod. The *C. maculata* adult females were of the same approximate age and had the same feeding history, i.e., they were fed brine shrimp *Artemia franciscana* Kellogg (Anostraca: Artemiidae) eggs, i.e., decapsulated cysts, on the same day and at each harvest date per week. The age of the egg clutches was within one day of difference. Ten egg masses were randomly selected from the total and added to each replicate arena, not containing any water or food, for each treatment. We conducted seven trials of this experiment, mostly on consecutive weeks. The first trial began on 1st August 2013 and the final trial began on 18th September 2013. The number of eggs in each clutch was determined and recorded. Dishes were assigned randomly to treatment temperatures in separate plant growth chambers. After 24 h, all treatment dishes were removed and maintained at 25 °C and 16 h L/8 h D. Neither food nor water was added to the dishes at any time. Hatch rate was monitored until all eggs hatched or failed to hatch. Egg hatch typically began within 2-3 days and once neonates began moving on or away from hatched eggs (chorions) or unhatched eggs, we used a fine, camel-hair paint brush to remove them (then discard them) to reduce egg cannibalism. Hatch rate reflected the percentage of neonate larvae successfully emerging from the total number of eggs in a clutch.

3. Statistical analysis

We plotted the roundtrip temperature data, then pooled the data and analyzed it following a complete randomized design. An analysis of variance (ANOVA) was used to compare the temperature inside individual shipping containers as they were shipped to the destination versus the return to the original location. The Student's *t*-test was used to compare the mean temperature and percent relative humidity amongst shipping containers for each site (location) per trip. A Pearson product-moment correlation analysis was used to test for a significant correlation between temperature and humidity readings in the same container during roundtrip. Percent relative humidity values were analyzed as proportional (rather than percentage) values, prior to the

Student's *t*-test or the Pearson correlation analysis. Following a randomized complete block design, we tested the simulated effects of temperature on percent egg hatch, in the laboratory by using an analysis of variance (ANOVA), with trial date as a blocking factor. Percent egg hatch data were arcsine square-root-transformed prior to analysis. The Tukey-Kramer HSD (honestly significant difference) method was used to separate means, following the analysis of variance (ANOVA), when necessary. Means were considered significantly different when $p < 0.05$. The following software assisted with data analysis: JMP 12 (SAS Institute Inc., Carey, NC, USA) and SigmaStat 3, interfaced through SigmaPlot 12 (Systat Software Inc., Richmond, CA, USA).

RESULTS

1. In-transit temperature change in Styrofoam containers

Considerable fluctuation in temperature occurred in polystyrene foam containers in transit between Stoneville MS and three locations, Byron GA, Clackamas OR, and Oxnard CA (Fig. 2).

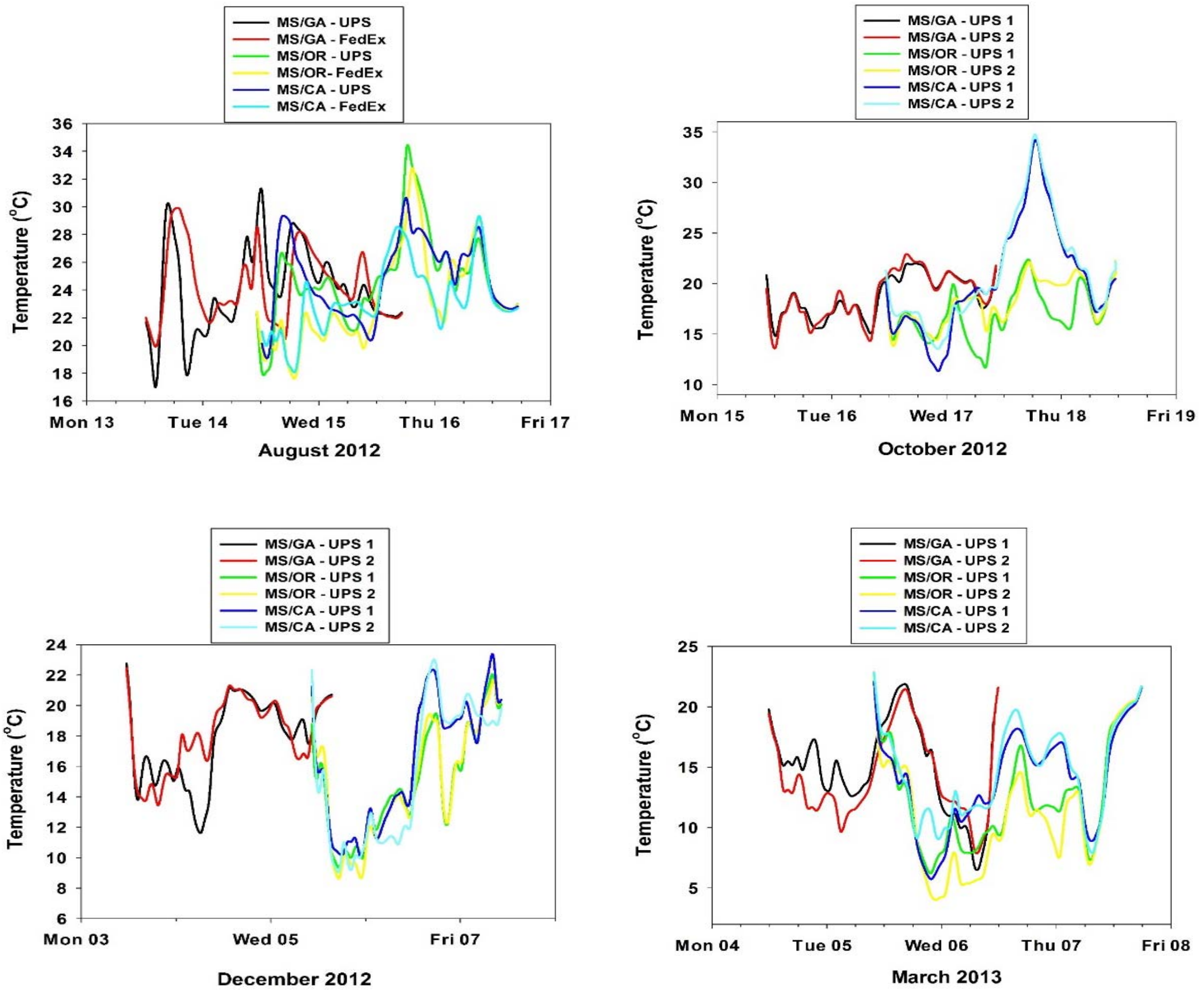

Fig. 2. Hourly temperature recordings in two polystyrene foam containers during roundtrip shipping (in August, October, and December 2012, and March 2013) between Stoneville MS (MS) and Byron GA (GA), Clackamas OR (OR), or Oxnard CA (CA). Refer to the Materials and Methods section for the exact shipping dates to each site and the number of observations per container per site.

In the August 2012 shipment, one container shipped to Georgia (MS/GA-UPS) registered a low of 17 °C and a high of 31 °C; one container shipped to Oregon (MS/OR-UPS) registered a high of 34 °C. The temperature inside individual polystyrene containers while being shipped to the three locations (in GA, OR, CA) often differed from that during the return trip (Table 1). Upon return to Mississippi (MS), all containers from Oregon (OR) and California (CA) registered warmer temperatures, ranging from 2 °C to 8 °C warmer, than during the shipment to these two locations on all dates. Two containers returning from Georgia (GA) in August 2012, and one container returning from the same location in March 2013, did not register significantly warmer

Table 1. Statistics for comparisons of temperature (in degrees Celsius) inside polystyrene containers during shipment from Stoneville MS to locations in Georgia (GA), Oregon (OR), and California (CA) and during return to Stoneville.

Month, destination, and carrier	Mean ± SD temperature during shipment	Mean ± SD temperature during return	*F*	df	*p*
August 2012					
GA-FedEx	24.32 ± 2.89	24.26 ± 2.2	0.006	1, 52	0.9384
GA-UPS	23.64 ± 3.75	24.46 ± 2.03	0.98	1, 52	0.3265
OR-FedEx	21.04 ± 1.75	25.76 ± 2.98	51.94	1, 53	< 0.0001
OR-UPS	23.29 ± 2.2	26.43 ± 3.25	17.73	1, 53	< 0.0001
CA-FedEx	22.01 ± 1.94	24.7 ± 2.34	21.34	1, 52	< 0.0001
CA-UPS	23.57 ± 2.75	26.22 ± 2.2	15.2	1, 52	< 0.0001
October 2012					
GA-UPS 1	17.21 ± 1.42	20.39 ± 1.21	71.24	1, 47	< 0.0001
GA-UPS 2	17.03 ± 1.67	20.68 ± 1.29	72.95	1, 47	< 0.0001
OR-UPS 1	15.63 ± 2.03	18.7 ± 2.15	26.77	1, 48	< 0.0001
OR-UPS 2	16.69 ± 1.53	19.81 ± 1.61	49.35	1, 48	< 0.0001
CA-UPS 1	16.54 ± 2.71	24.19 ± 4.8	46.71	1, 47	< 0.0001
CA-UPS 2	17.21 ± 1.97	24.75 ± 4.97	48.03	1, 47	< 0.0001
December 2012					
GA-UPS 1	15.66 ± 2.5	19.71 ± 1.1	60.51	1, 51	< 0.0001
GA-UPS 2	16.63 ± 2.27	19.48 ± 1.45	30.43	1, 51	< 0.0001
OR-UPS 1	12.6 ± 2.32	17.61 ± 2.65	49.81	1, 47	< 0.0001
OR-UPS 2	12.41 ± 3.01	17.45 ± 2.54	44.37	1, 47	< 0.0001
CA-UPS 1	12.87 ± 2.49	20.03 ± 1.66	136.18	1, 47	< 0.0001
CA-UPS 2	11.89 ± 2.75	19.57 ± 1.69	134.47	1, 47	< 0.0001
March 2013					
GA-UPS 1	15.2 ± 1.86	14.7 ± 5.08	0.2	1, 46	0.6548
GA-UPS 2	13.21 ± 2.28	15.25 ± 4.38	4.13	1, 47	0.0477
OR-UPS 1	10.98 ± 4.02	14.26 ± 4.07	9.36	1, 55	0.0034
OR-UPS 2	9.56 ± 4.82	13.38 ± 4.48	9.59	1, 55	0.0031
CA-UPS 1	12.14 ± 3.92	15.86 ± 3.36	14.78	1, 55	0.0003
CA-UPS 2	13.33 ± 3.47	16.21 ± 3.76	9.07	1, 55	0.0039

Mean temperature values during shipment and return of containers from MS to separate locations (GA, OR, or CA) are significantly different if $p < 0.05$, *F*-test (ANOVA). SEM, standard error of mean. Refer to Fig. 2 for all temperature data. *F*, statistic for ANOVA; df, degrees of freedom.

temperatures relative to the temperatures during shipment to this location. The mean temperature, roundtrip, in all containers was between 23-25 °C in the August 2012 shipment, with significant differences observed between containers shipped to Oregon and California (Table 2). In the October 2012 shipment, one container to Oregon (MS/OR-UPS 1) and one to California (MS/CA-UPS 1) registered a low of 12 °C and 11 °C, respectively. Containers shipped to California, MS/CA-UPS 1 and MS/CA-UPS 2 registered a high of 34 °C and 35 °C, respectively. The mean temperature, roundtrip, in all containers was between 17-21 °C in the October 2012 shipment, with significant differences observed between containers shipped to Oregon (Table 2). In the December 2012 shipment, containers to Oregon and California registered a low between 8-10 °C.

Table 2. Statistics for comparison of temperature (in degrees Celsius) inside polystyrene foam containers during roundtrip shipping between Stoneville MS and separate sites in Byron GA, Clackamas OR, or Oxnard CA.

Shipping month	Mean	SEM	SD	*n*	*t*	df	*P*
August 2012							
GA-UPS	24.29	0.35	2.54	54	0.45	106	0.65
GA-FedEx	24.05	0.41	3.01	54			
OR-UPS	24.83	0.43	3.16	55	2.36	108	0.02
OR-FedEx	23.36	0.46	3.39	55			
CA-UPS	23.35	0.34	2.52	54	2.99	106	0.003
CA-FedEx	24.89	0.38	2.80	54			
October 2012							
GA-UPS 1	18.77	0.29	2.07	49	0.11	96	0.91
GA-UPS 2	18.82	0.34	2.36	49			
OR-UPS 1	17.10	0.36	2.58	50	2.25	98	0.027
OR-UPS 2	18.18	0.31	2.21	50			
CA-UPS 1	20.44	0.78	5.47	49	0.59	97	0.56
CA-UPS 2	21.08	0.75	5.30	50			
December 2012							
GA-UPS 1	17.80	0.38	2.77	53	0.67	104	0.50
GA-UPS 2	18.13	0.32	2.35	53			
OR-UPS 1	14.95	0.50	3.52	49	0.06	96	0.96
OR-UPS 2	14.91	0.55	3.86	49			
CA-UPS 1	16.23	0.60	4.18	49	0.84	96	0.40
CA-UPS 2	15.49	0.64	4.50	49			
March 2013							
GA-UPS 1	14.95	0.55	3.79	48	0.92	95	0.36
GA-UPS 2	14.25	0.52	3.62	49			
OR-UPS 1	12.59	0.57	4.34	57	1.32	112	0.19
OR-UPS 2	11.44	0.66	5.00	57			
CA-UPS 1	13.97	0.54	4.08	57	1.04	112	0.30
CA-UPS 2	14.74	0.51	3.87	57			

Mean temperature values between containers shipped to separate locations (GA, OR, or CA) are significantly different if $p < 0.05$, Student's *t*-test. SEM, standard error of mean; SD, standard deviation. Refer to Fig. 2 for all temperature data. *n*, sample size; *t*, statistic for *t*-test; and df, degrees of freedom.

The temperature in no container exceeded 24 °C. The mean temperature, roundtrip, in all containers was between 15-18 °C, with no significant differences observed between containers shipped to any location (Table 2). In the March 2013 shipment, one container to Oregon (MS/OR-UPS 2) registered a low of 4 °C; none of the containers registered temperatures above 23 °C. The mean temperature, roundtrip, in all containers was between 11-15 °C in the March 2013 shipment, with no significant differences observed between containers (Table 2).

With the exception of the roundtrip shipment to Oregon in March 2013, percent relative humidity differed between containers destined to the same locations (Table 3). Mean percent relative

Table 3. Statistics for comparison of percent relative humidity inside polystyrene foam containers during roundtrip shipping between Stoneville MS and separate sites in Byron GA, Clackamas OR, or Oxnard CA.

Shipping month	**Mean**	**SEM**	**SD**	***n***	***t***	**df**	***P***
August 2012							
GA-UPS	60.64	0.75	5.48	54	9.27	106	< 0.001
GA-FedEx	52.44	0.47	3.48	54			
OR-UPS	56.57	0.27	2.04	55	3.26	108	0.001
OR-FedEx	58.66	0.58	4.29	55			
CA-UPS	56.10	0.39	2.85	54	27.10	106	< 0.001
CA-FedEx	73.63	0.52	3.80	54			
October 2012							
GA-UPS 1	50.35	0.16	1.14	49	21.66	96	< 0.001
GA-UPS 2	57.66	0.29	2.07	49			
OR-UPS 1	51.71	0.31	2.19	50	8.94	98	< 0.001
OR-UPS 2	57.00	0.50	3.57	50			
CA-UPS 1	56.64	0.41	2.91	49	31.15	97	< 0.001
CA-UPS 2	73.12	0.33	2.33	50			
December 2012							
GA-UPS 1	48.23	0.22	1.57	53	12.28	104	< 0.001
GA-UPS 2	44.65	0.19	1.42	53			
OR-UPS 1	48.77	0.17	1.22	49	13.98	96	< 0.001
OR-UPS 2	55.71	0.46	3.25	49			
CA-UPS 1	54.14	0.37	2.56	49	18.15	96	< 0.001
CA-UPS 2	64.44	0.43	3.03	49			
March 2013							
GA-UPS 1	32.81	0.43	2.98	48	4.32	95	< 0.001
GA-UPS 2	35.02	0.28	1.97	49			
OR-UPS 1	36.68	0.22	1.67	57	1.45	112	0.15
OR-UPS 2	37.13	0.21	1.61	57			
CA-UPS 1	38.72	0.27	2.07	57	9.65	112	< 0.001
CA-UPS 2	43.41	0.40	3.02	57			

Mean relative humidity values between containers shipped to separate locations (GA, OR, or CA) are significantly different if $p < 0.05$, Student's *t*-test. SEM, standard error of mean; SD, standard deviation. Refer to Fig. 3 for all humidity data. *n*, sample size; *t*, statistic for *t*-test; and df, degrees of freedom.

humidity exceeded 70% in the shipments to/from California in August and October 2012; it was less than 40% in the shipments to/from Georgia and Oregon in March 2013 (Table 3). Interestingly, there was a weak, positive correlation between temperature and percent relative humidity in the containers to/from Oregon in August and October, to/from California in October, December and March, and to/from Georgia in December (Fig. 3, Table 4).

2. Simulated effects of temperature on *C. maculata* egg hatch rate

One-day (24 h) exposure to one low and one high temperature, designed to simulate the predicted low and high temperatures observed in shipping containers (see section 1), affected hatch rate of *C. maculata* eggs in bioassays in environmental growth chambers (Table 5). Significantly fewer eggs hatched from clutches held for 24 h at 36 °C than at 6 °C or 25 °C

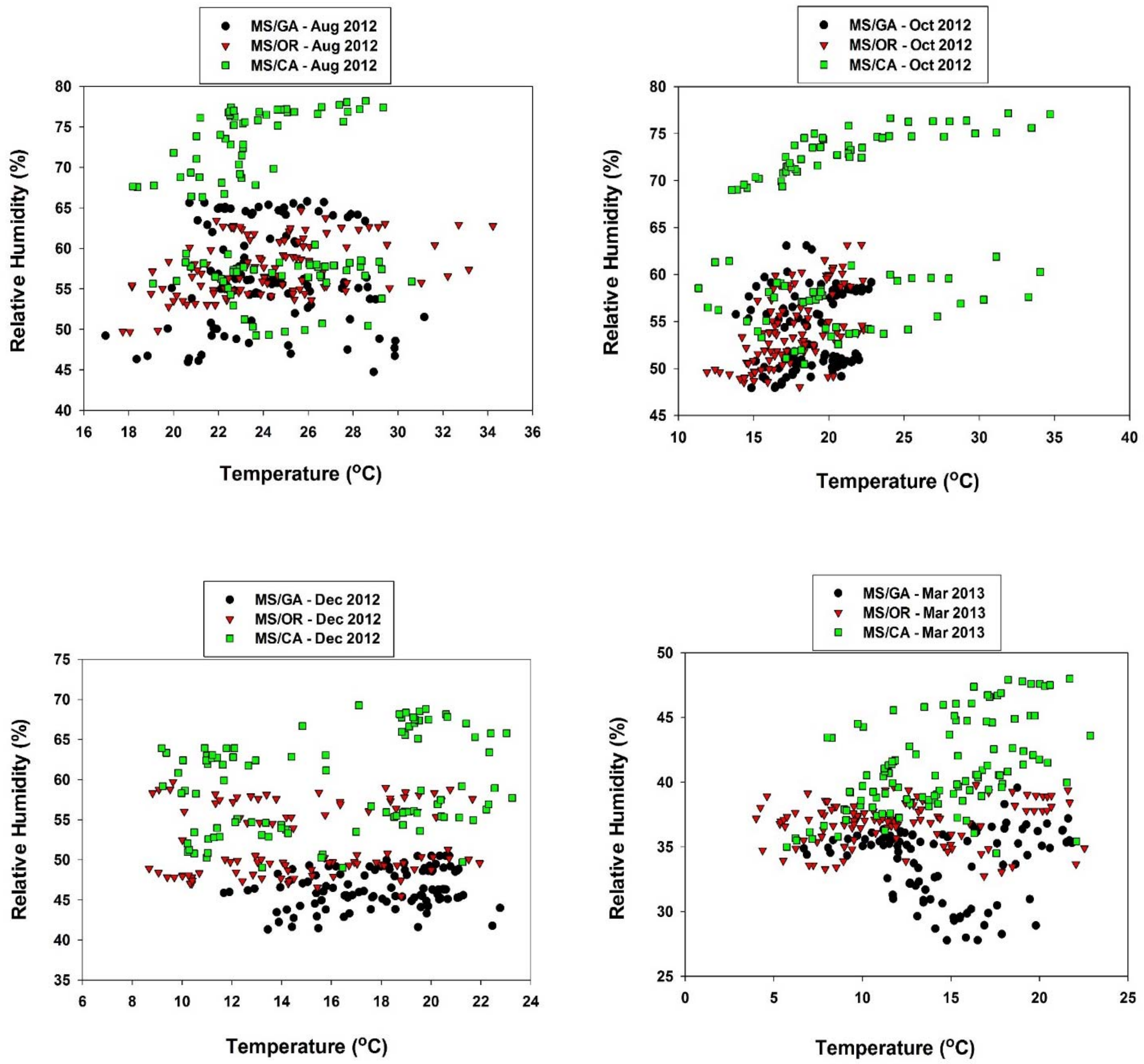

Fig. 3. Scatterplots of hourly temperature versus relative humidity recordings in two polystyrene foam containers during roundtrip shipping (in August, October, December 2012, and March 2013) between Stoneville MS (MS) and Byron GA (GA), Clackamas OR (OR), or Oxnard CA (CA).

(F = 86.0, df = 2, 201; p < 0.001). Trial date had no effect on hatch rate (F = 0.96, df = 6, 201; p = 0.45). Egg hatch was also reduced at 6 °C in comparison to the control (25 °C). Note that mean clutch size, i.e., mean number of eggs in each clutch, prior to placing clutches in temperature treatments was 15.5, 14.8, and 14.2 eggs in the 36 °C, 6 °C, and 25 °C treatments, respectively. Clutch size did not differ significantly amongst the treatments (statistics not shown).

Table 4. Statistics for correlation analysis between hourly temperature and percent relative humidity inside the polystyrene foam containers during roundtrip shipping between Stoneville MS and separate sites in Byron GA, Clackamas OR, or Oxnard CA.

Shipping month	*R*	*p*	*n*
August 2012			
MS/GA	0.026	0.79	108
MS/OR	0.42	< 0.001	110
MS/CA	-0.14	0.16	108
October 2012			
MS/GA	0.15	0.14	98
MS/OR	0.60	< 0.001	100
MS/CA	0.20	0.049	99
December 2012			
MS/GA	0.24	0.01	106
MS/OR	0.11	0.30	98
MS/CA	0.26	0.009	98
March 2013			
MS/GA	0.01	0.92	97
MS/OR	0.12	0.19	114
MS/CA	0.52	< 0.001	114

Temperature and relative humidity values are significantly correlated if p < 0.05, Pearson product moment correlation. R, statistic for correlation analysis; n, sample size.

Table 5. Effect of temperature during simulated overnight shipment on mean percent hatch rate of *C. maculata* eggs.

Temperature	Trials	Clutches (n)	Mean	SEM	SD	Lower 95%	Upper 95%
25 °C	7	70	63.83 a	2.98	24.93	57.88	69.78
6 °C	7	70	49.99 b	3.48	29.11	43.05	56.94
36 °C	7	70	13.45 c	3.03	25.33	7.41	19.49

Egg clutches were kept in complete darkness in the low (6 °C) and high (36 °C) temperature treatments. Relative humidity was maintained at 60% in all treatments. Total number of clutches (n) was 210. The mean values followed by different alphabets are significantly different (p < 0.05), as obtained using the Tukey-Kramer HSD method. SEM, standard error of mean; SD, standard deviation.

DISCUSSION

Temperatures inside the polystyrene foam containers fluctuated considerably during shipment, reaching a high of 35 °C in one container sent to California (October 2012) and a low of 4 °C in one container sent to Oregon (March 2013), which suggests the necessity of redesigning shipping containers to limit extremes in temperature during shipment of natural enemies to release sites for augmentative biological control of plant pests. Another study reached a similar conclusion [17]. Note that temperatures were always warmer during the return from, rather than during the shipment to, Oregon and California. This incongruity was most likely attributed to the ice packs. Ice packs were not recharged (i.e., not re-frozen or replaced with a new, frozen ice pack) prior to the return trip. As a consequence, extreme high temperatures were experienced mostly during the return trips. This indicates that cooling packs do provide some temperature buffering inside shipping containers during spring and summer. However, cooling packs often resulted in lower temperature extremes in shipments during the fall and winter months. To our knowledge, no other published research has monitored temperature change in shipping containers made of polystyrene foam.

Cool packs reduce high temperature extremes during summer, but could cause detrimental low temperature extremes in shipments during cooler months. For this reason, we consider the improvement of thermal insulation the most important factor to reduce the impact of temperature extremes on shipped natural enemies. New insulating materials need to be tested for use in shipping containers. Package design could also be an important way to minimize the negative effect of cooling packs when ambient temperatures do not reach temperatures sufficiently high to buffer their cooling effect. Warming elements based on exothermic chemical reactions, which are commonly used in hand warmers, could be used to maintain warm temperatures in shipping containers during winter or sent to northern geographical destinations, similarly as cooling packs are used to reduce the impact of high temperature extremes. It is difficult to predict what type of extreme temperature conditions a particular package will experience during shipment, in most cases. Another possibility could be the use of a container with double walls separated by vacuum or filled with inert gases, such as nitrogen or CO_2. Also, it would be helpful if the container included some type of disposable sensor, which could warn the recipient that the shipment was being exposed to extreme temperatures while in route.

The observation that relative humidity correlated positively with temperature in some containers is not unexpected. Moderate short-term fluctuations in relative humidity during shipment would not be expected to cause significant harm to developing predators. Even a high relative humidity (70% or above) could be beneficial to certain predators such as predatory mites [18] and ladybird beetles [7, 19]. In contrast, low relative humidity (40% or lower) can be harmful, causing dehydration resulting in a reduction in survival rate [18]. Remedies to curb low humidity in containers would become necessary to limit disruption in immature development of predators, and subsequent quality of these predators upon arrival at the release site. One possible way to maintain high humidity inside the shipping containers is by including small bags filled with water-saturated polyacrylamide crystals. At the right level of saturation, polyacrylamide crystals do not leak, and provide a source of drinking water for the shipped arthropods (larval, nymphal, or adult stages).

Our simulated shipment bioassay indicates that 24-h exposure to extreme temperatures (36 °C and 6 °C) can negatively affect *C. maculata* egg hatch, especially at the high temperature. Due to the evaporation of water, the egg chorion could become desiccated. As a consequence, first instars would have more difficulty penetrating the chorion as they attempt to hatch. The effect of cold temperature was less disruptive to *C. maculata* hatch rate in the study. Likely, the cold temperature slightly decreased embryonic development and potentially caused minor damage to tissues in some embryos.

The first instar *C. maculata* larvae hatching from their egg shells, after exposure to low or high temperature during the embryonic stage, were mobile and appeared healthy. However, we did not determine if larval and pupal development were altered as a repercussion of the temperature treatments in this study. Note that exposure of the eggs of the multicolored Asian ladybird beetle *Harmonia axyridis* (Pallas) to 39 °C, for just 1 h, had reduced the survival rate of emergent larvae and pupae in laboratory bioassays [20].

CONCLUSION

In conclusion, we have shown that the temperature fluctuated considerably in transit, reaching high and low temperatures of 35 °C in October 2012 (California shipment) and 4 °C in March 2013 (Oregon shipment). In the laboratory bioassays, a 24-h exposure to 36 °C and 6 °C significantly reduced *C. maculata* egg hatch when compared with the control, 25 °C. Redesigning containers to limit temperature extremes (especially high temperatures) is necessary to facilitate shipment of fragile, developing stages of predators, i.e., eggs, destined for augmentative release.

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CONFLICT OF INTEREST STATEMENT

Both authors, Eric W. Riddick and Juan A. Morales-Ramos declare no conflicts of interest, including affiliations, relationships or financial interests relevant to the content of this manuscript.

REFERENCES

1. Gordon, R. D. 1985, Journal of the New York Entomological Society, 93, 1-912.
2. Munyaneza, J. and Obrycki, J. J. 1998, Environmental Entomology, 27, 117-122.
3. Krafsur, E. S. and Obrycki, J. J. 2000, Annals of the Entomological Society of America, 93, 1156-1163.
4. Hodek, I. and Evans, E. W. 2012, Ecology and Behaviour of Ladybird Beetles (Coccinellidae), I. Hodek, H. F. van Emden and A. Honěk (Eds.), Blackwell Publishing Ltd., United Kingdom, 141-274.
5. Gurney, B. and Hussey, N. W. 1970, Annals of Applied Biology, 65, 451-458.
6. Rondon, S. I., Cantliffe, D. J. and Price, J. F. 2005, Florida Entomologist, 88, 152-158.
7. Bartlett, B. R. 1962, Annals of the Entomological Society of America, 55, 448-455.
8. Steward, V. B., Kintz, J. L. and Horner, T. A. 1996, HortTechnology, 6, 233-237.
9. O'Neil, R. J., Giles, K. L., Obrycki, J. J., Mahr, D. L., Legaspi, J. C. and Katovich, K. 1998, Biological Control, 11, 1-8.
10. Heimpel, G. E. and Lundgren, J. G. 2000, Biological Control, 19, 77-93.
11. Bjørnson, S. 2008, Biocontrol Science and Technology, 18, 633-637.
12. Bueno, V. H. P., Carvalho, L. M. and van Lenteren, J. C. 2014, Bulletin of Insectology, 67, 175-183.
13. Simmons, A. M. and Legaspi, J. C. 2004, Environmental Entomology, 33, 839-843.
14. Frazier, B. D. and McGregor, R. R. 1992, Canadian Entomologist, 124, 305-312.
15. Obrycki, J. J. and Tauber, M. J. 1978, Canadian Entomologist, 110, 407-412.
16. Grafton-Cardwell, E. E., Gu, P. and Montez, G. H. 2005, Biological Control, 32, 473-478.
17. Bjørnson, S. and Raworth, D. A. 2005, Biocontrol Science and Technology, 15, 755-760.
18. Ghazy, N. A. and Amano, H. 2016, Experimental and Applied Acarology, 69, 277-287.
19. Simmons, A. M., Legaspi, J. C. and Legaspi, B. C. 2008, Annals of the Entomological Society of America, 101, 378-382.
20. Zhang, S., Cao, Z, Wang, Q., Zhang, F. and Liu, T.-X. 2014, Journal of Thermal Biology, 39, 40-44.