Responses of *Delphastus catalinae* (Coleoptera: Coccinellidae), a Predator of Whiteflies (Hemiptera: Aleyrodidae), to Relative Humidity: Oviposition, Hatch, and Immature Survival

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ABSTRACT *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) is a predator of whiteflies. It is tropical in origin. Whiteflies cause problems in agriculture in both humid and arid environments. A study was conducted to determine any effects of relative humidity on oviposition, hatching, and survival of immature *D. catalinae*. Comparative tests were conducted among relative humidities of 25, 35, 50, and 85% and between 10 and 85% RH. All tests were conducted at 26°C; hence, vapor pressure deficits ranged from 5.04 to 30.25 mb. The study was conducted using the B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius), as the host insect, which was reared on collard, *Brassica oleracea* ssp. *acephala* de Condolle. Egg hatch and survival to the adult stage were reduced at the lower relative humidities. At 85% RH, 99% of the eggs hatched and ≈90% of the beginning cohort survived to the adult stage. Conversely, ≈85% hatched and ≈60% survived to the adult stage at 25% RH, whereas 50% survived to the adult stage at 10% RH. Eggs required more time to develop at 10% RH (5.3 d) compared with 85% RH (4.1 d); a test was not set up to compare the developmental times for the larval and pupal stages. Weights of male and female *D. catalinae* were significantly reduced with a reduction in humidity. The results also suggest that density of immature *D. catalinae*, limited food supply, or both may affect survival and size of the ensuing adults. These results help in the understanding of the ecology of *D. catalinae*, and they indicate that extremes in ambient moisture can have an impact on the population of this predator.

KEY WORDS *Bemisia tabaci*, *Delphastus catalinae*, biological control, predator, survival

Whiteflies are important worldwide insect pests in both humid and arid environments. They damage numerous plant species in greenhouse and field production systems by feeding and/or vectoring pathogens. The B-biotype sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (also reported as *B. argentifolii* Bellows & Perring) and other members of the *Bemisia* complex, are particularly a problem in crop production. Because of the continued concern by the agricultural community and general public concern about the prevalent use of pesticides, there is much interest in the use of alternative approaches, such as the use of beneficial organisms, to aid in pest management in the production of crops.

Several species of predators are associated with whiteflies, including *Delphastus catalinae* (Horn) (Coleoptera: Coccinellidae) (Muma 1955; Hoelmer et al. 1993a, 1993b; Legaspi et al. 1996; Nordlund and Legaspi 1996; Liu et al. 1997). *D. catalinae* is an obligate whitefly predator (Gordon 1994). Although this beetle is native to Colombia, South America, established populations can now be found in several tropical and subtropical locations, including southern California, and central and southern Florida (Gordon 1994, Pickett et al. 1997). Even though it probably initially arrived inadvertently on plant materials in Florida, intentional releases have been made in Florida, in the desert southwest of the United States, and in Hawaii (Pickett et al. 1997, Hoelmer and Pickett 2003). Furthermore, dissemination of *D. catalinae* has been made for research and biological control purposes to many international locations over the past several years (Hoelmer and Pickett 2003). Because of a similar appearance, *D. catalinae* had previously led researchers to report it as *Delphastus pusillus* (LeConte). The latter species occurs only in the eastern United States (Gordon 1994). Gordon (1994) provided a major revision of the genus *Delphastus*. He amended the original descriptions of several species, described new species and updated the species distribution. Although *D. catalinae* is marketed for whitefly control, there is uncertainty on the taxonomic status of the species of *Delphastus* in commercial colonies. Hoelmer and Pickett (2003) concluded that the species held in commercial insectaries are probably *D. catalinae* instead

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of *D. pusillus.* They further concluded that most of the published studies on the biology and behavior of *D. pusillis* on *Bemisia* probably pertains to *D. catalinae.* After observations that *D. catalinae* had invaded a research greenhouse infested with *B. tabaci* in Florida during the late 1980s (Hoelmer and Pickett 2003), several studies have demonstrated aspects of its potential for the control of *B. tabaci* (Hoelmer et al. 1993a, 1993b; Heinz and Parrella 1994; Liu and Stansly 1999).

Bemisia proliferates in various climates around the globe, but only recent attention has been given to the effects of climatic conditions on the populations of *D. catalinae.* Temperature is an important limiting factor for both the *Bemisia* host and *D. catalinae.* We previously reported on short-term critical temperature limits for the survival of adult and pupal *D. catalinae* (Simmons and Legaspi 2004) and overwintering capacity in mild winters of coastal South Carolina and northern Florida (Simmons and Legaspi 2007). Temperatures below 5°C for 24 h had a considerable detrimental impact on survival of *D. catalinae* (Simmons and Legaspi 2004). Likewise, climate can dramatically affect the population and distribution of its *Bemisia* host (Gerling 1984, Simmons and Elsey 1995, Simmons 1998). Coastal South Carolina is the most northern range of eastern North America where feral populations of the B-biotype *B. tabaci* survive throughout the winter, although the population is much reduced compared with warmer months (Simmons and Elsey 1995). Severely depressed populations of *D. catalinae* can survive such mild winters where the low temperatures are commonly above 0°C (Simmons and Legaspi 2007). Humidity can affect developmental rate and survival of eggs and immatures of some arthropods, including species of Acari, Coleoptera, Diptera, Hemiptera, and Orthoptera (Chapman 1969, Ferro and Chapman 1979, Martinat and Allen 1987). However, there have been no published reports on the effect of relative humidity on populations of *D. catalinae.*

Knowledge of the ecology of whitefly predators will facilitate their role as a pest management component. The purpose of this study was to determine any influence of relative humidity on oviposition, hatch, egg developmental rate, and survival of immature *D. catalinae* on *B. tabaci* as a host. In addition, an experiment was conducted to determine any influence of photoperiod on oviposition by *D. catalinae.*

Materials and Methods

Oviposition and Photoperiod. Insects used in all experiments were from greenhouse colonies maintained at the USDA–ARS, U.S. Vegetable Laboratory, Charleston, SC. The whiteflies, B-biotype *B. tabaci,* had been reared on assorted vegetable crops since 1992 (Simmons 1994). Adult coccinellids (*D. catalinae*) were purchased commercially in 1999 and released to help suppress whiteflies in a research greenhouse, and they have persisted on *B. tabaci* since the original release. Voucher specimens of *D. catalinae* from the colony were deposited at the Division of Plant Industry, Gainesville, FL.

To assist with the methods for the humidity tests, an experiment was conducted to test for any influence of photoperiod on oviposition. Collard seedlings were grown in Jiffy starter pellets (Jiffy Products of America, Batavia, IL). Four collard seedlings, four- to six-leaf stage, were placed in a cage. The cage consisted of an 18-cm-depth by 20-cm-diameter clear plastic cylinder. The starter pellet and base of each plant was placed in an interlocking seal plastic bag. The bag was zipped sealed and cotton fiber was placed around the stem of the plant to complete the seal of the bag so that beetles could not crawl into the bag. Approximately 200–300 adult *B. tabaci* of undetermined sex were placed in each cage, and the cage was covered with fine mesh screening and secured with a pair of rubber bands around each cage. The cages were then held for 10–12 d at 25°C to allow the whiteflies to establish eggs and nymphs on the leaves. Wagner (1995) reported that the egg of B-biotype *B. tabaci* hatches in 7.1 d at 24°C on cotton. After the whitefly incubation period, each whitefly-infested plant was placed inside a 480-ml cylindrical paper carton with fine mesh screening secured to the top, and six adult female *D. catalinae* were added to each cage. Ten of these caged plants were placed into one of three photoperiod regimes (24:0, 12:12, and 0:24 [L:D] h) in environmental chambers at 26°C and 85% RH. Lighting in the chambers was by florescent bulbs oriented vertically along two opposite walls. After 24 h, the number of *C. catalinae* eggs deposited on the leaves was determined for each plant for each photoperiod regime. The experiment was repeated five times by using different chambers for each photoperiod test.

Oviposition and Humidity. An experiment was conducted to test for any influence of humidity on oviposition by *D. catalinae.* Two relative humidity extremes, 10 ± 6% and 85 ± 5% RH, were tested using environmental chambers (model I-36VL, Percival, Perry, IA) maintained at 26°C and a photoperiod of 16:8 (L:D) h. Vapor pressure deficit was 30.04 and 5.04 mb for the 10 and 85% RH treatments, respectively. Humidity for the 10% treatment was maintained with a built-in IAT-50 U in the chamber. Humidity for the 85% treatment was provided by an open container of water in the chamber. Collard plants were infested with whiteflies as described above. Six adult female beetles were then added to each 480-ml paper carton cage as described above. Ten cages were set up per treatment chamber. After 24 h, the number of eggs deposited were counted and recorded. The experiment was repeated eight times.

Survival from Oviposition to Adult Emergence at Four Relative Humidities. A laboratory study was set up to test *D. catalinae* in four separate environmental chambers (Percival model I-36VL) maintained at 26 ± 1°C and a photoperiod of 16:8 (L:D) h. The chambers were maintained at 25, 35, 50, or 85% RH. A portable dehumidifier connected to a humidistat controller (model RHC, Green Air Products, Gresham, OR) in conjunction with an open container of water was used to maintain the humidity in each chamber. Collard seedlings were set up in interlocking seal plastic bags and infested with whitefly eggs and nymphs as described above. The bags were to help limit ambient

moisture and to prolong moisture on the pellets for the plants. After the whitefly incubation period, each bagged plant was transferred to a 480-ml cylindrical paper carton with fine mesh screening secured to the top. Six adult female *D. catalinae* were collected from the greenhouse colony and placed in each cage. Free water was not provided for the beetles. Cages with beetles were placed in each of the four aforementioned relative humidity chambers. Within 18–26 h, the adult beetles were removed from the cages. With the aid of a microscope, all eggs that were deposited on the leaves were circled with a red fine-point permanent marker (Sharpie, Series No. 30000, Stanford Corporation, Bellwood, IL) to aid in the relocation of the eggs. The number of eggs per plant was recorded. Four plants with beetle eggs were placed in each of the clear aforementioned 20-cm-diameter cylinder cages. During the experiment, water was added as needed within the plastic bag, and it was resealed. Three cages were set up per chamber for a total of 12 plants per trial. The cages were returned to the same relative humidity chamber in which oviposition had taken place. The plants were observed 8 d after the oviposition period, and the numbers of eggs that hatched were recorded for each plant. Liu (2005) reported that it takes 4 d for *D. catalinae* to hatch at 26°C and 55% RH; thus, any unhatched eggs found on day 8 were assumed not to be able to hatch. To augment the diet of immature whiteflies for the beetle larvae, ≈200–300 adult *B. tabaci* were added to each cage and the cages were returned to the humidity treatment from which they had been previously held. From 24–36 h after emergence, the gender and weight of each adult beetle were determined and recorded. The experiment was repeated eight times.

Survival from Oviposition to Adult Emergence at Two Relative Humidities. An experiment was conducted on survival from oviposition to the adult stage in a comparison between beetles held at 10 and 85% RH. The test was set up using the same materials and methods as for the previous test with four relative humidities, except that the number of eggs was limited. Six eggs remained on the leaves of each plant after all other eggs were removed within 24 h of oviposition. Data on hatch, survival, and weight were collected as in the aforementioned test. The experiment was repeated seven times. A total of 168 eggs were tested in each treatment of 10 and 85% RH.

An additional test was set up to test for any effect of humidity on egg developmental rate. Treatments of 10 and 85% RH under the above-described conditions were used. Eggs were obtained by allowing adult female *D. catalinae* to oviposit on collard plants set up in plastic bags as described above, and all plants were held at 85% RH to standardize the condition during the ovipositional period. Six beetle eggs were gently removed one at a time, by using a probe, and they were placed on the lower surface of a collard leaf infested with whitefly eggs and nymphs as described above. The six beetle eggs were placed within an area of ≈3 cm in diameter, which was marked with a red pen to facilitate the relocation of the eggs. One plant with eggs was placed in one of the 20-cm-diameter cylinder clear cages and laid on its side so that the leaf with eggs was turned upward. Four plants were set up for each treatment. Daily observations were made on egg hatch, and the number of days to hatch was recorded for each egg. Eggs that did not hatch were not included in the analyses. The experiment was repeated three times for each treatment.

Table 1. Mean number of eggs deposited when *D. catalinae* was held under three photoregimes for 24 h at 26°C

Photoregime	*n*	No. eggs/six females/24 h		
		Mean ± SEM	Range	Upper leaf surface
24:0	50	22.7 ± 2.8a	3–66	0.5 ± 0.16b
12:12	50	22.1 ± 2.0a	4–49	0.8 ± 0.18b
0:24	50	22.6 ± 2.6a	0–69	1.4 ± 0.30a

Means in columns followed by the same letters are not significantly different ($P > 0.05$) according to Student–Newman–Keuls test.

Data Analysis. Data were analyzed using SAS (SAS Institute 2002), and significance was determined at $P < 0.05$. Percentages were transformed using arcsine transformation before the analysis, but the results are presented on back-transformed data. Significantly different means were separated using the Student–Newman–Keuls test. Correlation relationships were tested between percentage of egg hatch and percentage of relative humidity.

Results and Discussion

Oviposition as Influenced by Photoperiod and Humidity. There were no significant differences ($F = 0.39, P > 0.68$) in the total number of *D. catalinae* eggs deposited at any of three photoperiods (Table 1). No significant chamber effect was detected on number of eggs deposited. An overall average of 23 eggs per six females and a range of 0–69 eggs were deposited within 24 h by sets of six females. Darkness increased the number of eggs that was deposited on the upper surface of the leaf, although only a few (4%) of the overall eggs were deposited on the upper surface (Table 1). In the other experiments herein, we similarly found most of the eggs on the lower leaf surface.

Humidity had an effect on the rate of oviposition for *D. catalinae* adult females held for 24 h at 10 and 85% RH. The latter resulted in a statistically greater number of eggs ($F = 4.01, P < 0.05$), but the magnitude was not large (Table 2). There were 0–67 eggs deposited

Table 2. Mean number of eggs deposited at two relative humidities for *D. catalinae* held in the laboratory at 26°C

% relative humidity	*n*	Mean ± SEM eggs	Min. no. eggs/six females	Max no. eggs/six females	% eggs deposited
10	70	16.8 ± 1.4b	0	54	43.7 ± 3.7b
85	70	20.6 ± 1.8a	0	67	56.3 ± 5.1a

Means in columns followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

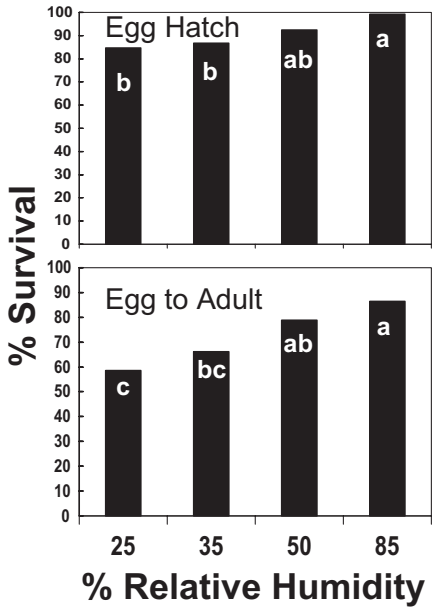

Fig. 1. Survival of *D. catalinae* at four relative humidity treatments when held in the laboratory from the time of oviposition until adult emergence on *B. tabaci*-infested collard plants (26°C); Means followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

for a given cage of six adult *D. catalinae* within 24 h. To avoid age bias, random female beetles from the colony population were used in the experiments. They represented samples of egg production by the colony, and their ages presumably ranged from recently emerged to old age. The preoviposition period for this species averages 5 d (Liu 2005), and viable eggs can be deposited for the remainder of their life (Simmons and Legaspi 2007). In research on a tropical leafhopper species, Alyokhin et al. (2004) concluded that there was no effect of humidity on oviposition when they studied field sites differing in annual precipitation (1,700–3,000 mm). Apparently, ambient moisture is a minor factor in affecting the oviposition behavior for *D. catalinae.*

Survival from Oviposition to Adult Emergence. Egg hatch by *D. catalinae* significantly decreased as relative humidity was changed from 85 to 25% (Fig. 1). Similarly, egg hatch was higher for the high humidity (85%) treatment when only two treatments (including 10% RH) were tested (Table 3). Egg hatch ranged from 84.7 to 99.3% in the experiment among the four relative humidity treatments (Fig. 1), and in the experiment between the two relative humidity treatments (Table 3). Egg hatch was moderately correlated ($r = 0.40$, $P < 0.001$) with relative humidity in the experiment with the four treatments (Fig. 1). Hatch performance (≈85%) was comparable for the lowest humidity treatments (10 and 25% RH) in two experiments, and hatch performance (≈100%) was comparable at the highest humidity treatments (85% RH) in the same two experiments (Fig. 1; Table 3).

Table 3. Mean percentages of hatch and survival to the adult stage for *D. catalinae* held at a high and a low relative humidity in the laboratory with eggs limited to six per plant (26°C)

% relative humidity	n^a	Mean ± SEM % hatch	Mean ± SEM % adult emergence
10	28	84.7 ± 1.95b	50.1 ± 3.4b
85	28	99.3 ± 0.37a	93.5 ± 2.0a

Means in columns followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

[a] For each treatment, 168 eggs were tested.

Similar to egg hatch, overall survival to the adult stage was reduced with a decrease in humidity (Fig. 1). In the experiment with four treatments, 86.5% of the immatures survived to the adult stage at 85% RH, whereas 58.5% survived to the adult stage at 25% RH (Fig. 1). Survival (93.5%; Table 3) to the adult stage tended to have been more favorable at 85% RH when egg density was limited to six *D. catalinae* per plant compared with when egg density was not limited. We suspect that beetle density may have had some influence on survival by limiting the food supply. Survivorships for immatures in the tests at 85% RH are similar to what was reported (88% survival from egg to adult at 26°C) by Liu (2005) whom studied the insects on leaf disks in petri dishes with ambient condition outside of the dishes at 55% RH. At 10% RH, survival from egg hatch to adult emergence was 50% (Table 3), which seems to be consistent with the adverse impact of humidity as was observed in the experiment with 25% RH (58.5% survival; Fig. 1).

Apparently, too little moisture may be deleterious. In the lepidopteran *Heterocampa guttivitta* (Walker), humidity affected survival but not developmental rate of the eggs (Martinat and Allen 1987). In our study, data on developmental rate for egg hatch indicate that more time (1.2 d) is needed for egg hatch at low humidity compared with high humidity (Table 4). Because the eggs were exposed to the separate treatments after the oviposition period, the full impact of low humidity on rate of egg development may be underdetermined herein. The 4-d duration of the egg stage under 85% RH is the same as reported by Liu (2005) for insects in petri dishes that were held at 55% RH and at the same temperature as in our study. In the experiment where survival was followed to the adult stage, unhatched eggs of different

Table 4. Mean number of days to develop from oviposition to eclosion for *D. catalinae* held at high and low relative humidity treatments (26°C)

% relative humidity	n^a	Mean ± SEM no. days to eclosion
10	33	5.3 ± 0.08b
85	52	4.1 ± 0.06a

Means followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

[a] Unhatched or lost eggs were not included in the analyses. The eggs were transferred from one collard leaf to a new leaf; 87 and 53% hatched at 85 and 10% RH, respectively.

Table 5. Mean weight of adult *D. catalinae* after the immature developed at four different relative humidities in the laboratory (26°C)

% relative humidity	*n*	Mean ± SEM wt (mg)		
		Males	Females	Average
25	318	0.336 ± 0.008b	0.407 ± 0.011b	0.369 ± 0.007c
35	336	0.380 ± 0.009a	0.426 ± 0.009ab	0.403 ± 0.006ab
50	440	0.375 ± 0.007a	0.411 ± 0.007ab	0.391 ± 0.005b
85	506	0.390 ± 0.008a	0.440 ± 0.008a	0.414 ± 0.006a

Means in columns followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

developmental stages were noticed. Once the eggs hatched, the larvae had access to moisture by feeding on the whitefly host and/or honeydew. Liu and Stansly (1999) observed that some *D. catalinae* larvae fed on honeydew and dew drops even when abundant whiteflies were present. Adults and larvae of this coccinellid species feed on a liquid diet. Based on where pupae were found, some larvae moved among leaves and plants within a cage. Similar to what we observed on plants in the greenhouse colony, in this study, some larvae pupated on the stem of the plant and in some cases on the container (i.e., plastic bag) holding the roots of the plants. However, pupae and pupa cases were not observed on the cages. The data suggest that at extremely low ambient moisture, the ability of *D. catalinae* larvae to replenish moisture in its body may have been hampered, leading to an increase in mortality. We observed that some died during the egg stage, larval stage, during eclosion, and shortly after exiting the pupal case.

Adult Weight. In every test within a given humidity treatment, females weighed significantly more than males. Overall weights of both males and females were generally reduced with an increase in percentage of relative humidity (Tables 5 and 6). In the test with four humidity treatments, weights for the highest (0.414 mg) and the lowest (0.369 mg) percentage of relative humidity differed significantly ($F = 9.16, P < 0.05$), whereas the weights of adults that developed at 35 and 50% were statistically the same (Table 5). Using a sample of 100 beetles of each sex from the greenhouse colony, adult *D. catalinae* males and females had mean weights of 0.500 ± 0.015 and 0.564 ± 0.021 mg, respectively. These sizes were similar to the experiment with limited *D. catalinae* egg density at 85% RH. In that experiment, the average weight for males and females were 0.490 and 0.567 mg, respectively (Table 6). In a study under 24°C and 70% RH with the greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), as a host, Lucas et al. (2004) reported an average weight of 0.53 mg for *D. catalinae.* They did not provide data on the weight by sex. Weight can be used as an index of size, and size may be an indication of fitness of an organism. However, no data were collected on the reproductive and survival of the adults in response to different relative humidities. The data do not define the specific optimal relative humidity for *D. catalinae*, but they indicate that it would be >50% RH. Nevertheless, very high humidity may provide adverse pathological conditions. At 85%, there was a high incidence of mold and mildew on the leaves, which was not noticeable at low relative humidity. Although there were adult whiteflies that continued to oviposit on the leaves throughout the experiment, we suspect that food may have been limited at times in part due to the presence of the mold and mildew. Moreover, because of the relatively high number of *D. catalinae* larvae on leaves (a maximum of 42, but generally between 15 and 20 eggs per plant) in the experiment with multiple humidities, insufficient diet may have been related to the general small size compared with the experiment with six eggs per plant. In other laboratory observations, we have rarely observed cannibalism by the larvae. We do not know whether cannibalism may have been a factor on survival in either experiment, but such behavior was not observed in this study. Nevertheless, in the experiment with low density, the beginning cohorts were equal. The larvae tend to complete feeding on a host once they start feeding, but prey handling time tends to increase under food deprivation (Liu and Stansly 1999).

Table 6. Mean weight of adult *D. catalinae* after the immature developed at a high and a low relative humidity with eggs limited to six per plant in the laboratory (26°C)

% relative humidity	*n*	Mean wt (mg) ± SEM		
		Males	Females	Average
10	339	0.440 ± 0.008b	0.489 ± 0.007b	0.467 ± 0.006b
85	603	0.490 ± 0.005a	0.567 ± 0.006a	0.529 ± 0.004a

Means in columns followed by different letters are significantly different ($P < 0.05$) according to Student–Newman–Keuls test.

In conclusion, results from this study indicate that populations of *D. catalinae* can be affected by the amount of humidity in the environment. Vapor pressure deficit, which expresses moisture status independent of temperature, is biologically more meaningful than relative humidity (Ferro and Chapman 1979). Ambient moisture was not constant in the study herein because temperature was held constant. Vapor pressure deficits in this study ranged from 30.25 mb (10% RH) to 5.04 mb (85% RH). A failure of *D. catalinae* to control *B. tabaci* in the field on cotton, *Gossypium hirsutum* L. (Heinz and Parrella 1994) may be only partly related to the effect of humidity on the population. The role of temperature is very important on the biology and ecology of *D. catalinae* (Simmons and Legaspi 2004, Simmons and Legaspi 2007, Legaspi et al. 2008). As such, increased or decreased temperatures can affect biological parameters of this predator, but there has not been a report on a study of any interaction of temperature and humidity. The intensity and duration of exposure to adverse factors can limit the survival of insects. During a typical day within either an arid environment such as the U.S. southwest desert or a humid environment such as the southeastern United States, relative humidity may not vary excessively between the low and high extremes. Similarly, vapor pressure deficit within a closed greenhouse system generally does not vary excessively, and it is usually under humidity conditions that are favorable to the population of *D. catalinae.* The results of this study support that although *D. catalinae* can exist in a wide

range of humidities, low ambient moisture can have a negative impact on its population dynamics.

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