Effect of Humidity on Fecundity and Egg Incubation of Frankliniella bispinosa and Frankliniella occidentalis (Thysanoptera: Thripidae)

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Effect of humidity on fecundity and egg incubation of *Frankliniella bispinosa* and *Frankliniella occidentalis* (Thysanoptera: Thripidae)

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Abstract

Environmental factors are hypothesized to account for spatial and temporal differences in Florida in the abundance and distribution of the native thrips species *Frankliniella bispinosa* (Morgan) and the invasive *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae). Laboratory experiments were conducted at a constant temperature of 23 ± 1 °C to investigate the effects of humidity on the fecundity and egg incubation of *F. bispinosa* and *F. occidentalis*. Adult thrips were allowed to oviposit on green bean (*Phaseolus vulgaris* L.; Fabaceae) pods. Eggs were maintained at relative humidity treatment levels of 40 ± 5, 55 ± 5, 70 ± 5, and 80 ± 5%. Fecundity and time of egg hatch were determined every 12 h. Results showed that *F. bispinosa* had a higher fecundity and a shorter time to egg hatch compared with *F. occidentalis* at higher humidity levels. These results partially explained patterns of abundance and distribution of *F. bispinosa* and *F. occidentalis* in Florida. When relative humidity was high in summer and fall, populations of *F. bispinosa* were abundant and population levels of *F. occidentalis* were very low. Management strategies for *F. bispinosa* and *F. occidentalis* can be improved to accommodate the biological differences.

Key Words: thrips; fecundity; relative humidity; egg incubation

Resumen

Se postula que los factores ambientales dan cuenta para las diferencias espaciales y temporales de la abundancia y distribución de las especies de trips nativos *Frankliniella bispinosa* (Morgan) y el trips invasor *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) en la Florida. Se realizaron los experimentos de laboratorio a una temperatura constante de 23 ± 1 °C para investigar los efectos de la humedad sobre la fecundidad e incubación de huevos de *F. bispinosa* y *F. occidentalis*. Los adultos de trips se les permitió poner huevos sobre las vainas de las habichuelas (*Phaseolus vulgaris* L .; Fabaceae). Se mantuvieron los huevos a niveles de tratamiento de humedad relativa de 40 ± 5, 55 ± 5, 70 ± 5 y 80 ± 5%. Se determinó la fecundidad y el tiempo de eclosión de los huevos cada 12 y 24 horas, respectivamente. Los resultados mostraron que *F. bispinosa* tenía una fecundidad más alta y un menor tiempo de eclosión de los huevos en comparación con *F. occidentalis* en los niveles de humedad más altos. Estos resultados explican parcialmente el patrón de abundancia y distribución de *F. bispinosa* y *F. occidentalis* en la Florida. Cuando la humedad relativa es alta en verano y otoño, las poblaciones de *F. bispinosa* fueron abundantes y el nivel de población de *F. occidentalis* fue muy bajo. Se puede mejorar las estrategias de manejo para *F. bispinosa* y *F. occidentalis* para adaptar las diferencias biológicas.

Palabras Clave: trips; fecundidad; humedad relativa; incubación de los huevos

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is among the world's most important insect pests due to its widespread occurrence and its efficient ability to spread viruses including the *Tomato spotted wilt virus* (Bunyaviridae) (Wijkamp et al. 1993). *Frankliniella occidentalis* is native to western North America. Its extensive spread worldwide is attributed primarily to the movement of agricultural products such as cuttings, seeds, and whole plants (Kirk & Terry 2003). It was commonly referred to as a greenhouse pest, but it is now established outdoors nearly worldwide especially in areas with milder winters, including the entire USA, Australia, and southern Europe (Kirk & Terry 2003). The species was established in the southeastern USA in the mid 1980s (Reitz 2002).

There are many other economically important congener species in Florida. *Frankliniella bispinosa* (Morgan) is common and is found year round on a wide range of cultivated and uncultivated plants (Reitz 2002). This species is a pest of blueberry and citrus, where it injures floral parts leading to damage of the resulting fruits (Childers 1991; Childers & Bullock 1999; Arévalo-Rodriquez 2006; Rhodes & Liburd 2011). A related species, *Frankliniella tritici* (Fitch), is commonly found in the eastern part of the USA, including northern Florida (Funderburk et al. 2016). The species is not found in peninsular Florida south of Alachua County where *F. bispinosa* predominates. Biotic resistance limits the invasiveness of *F. occidentalis* in Florida, and the pest is primarily abundant in highly disturbed habitats such as crop fields where the intensive use of insecticides reduces competition from the congener species and mortality from predators (Funderburk et al. 2016).

The geographic distribution of thrips populations is influenced by a number of factors including temperature and humidity (Davidson & Andrewartha 1948a,b). Arévalo & Liburd (2007) recorded a clumped distribution of *F. bispinosa* in blueberry plantings in Florida, which they described as hot spots. The formation of these hot spots within fields was random, and the microclimate differences in temperature and humidity were believed to play a role in the development of these hot spots.

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We hypothesize that the native *F. bispinosa* is better adapted than *F. occidentalis* to conditions of high humidity, which may partially account for the distribution and abundance of these species in Florida (Chellemi et al. 1994; Funderburk et al. 2016). The objective of this research was to determine the effects of humidity on the fecundity and time of egg hatch on *F. bispinosa* and *F. occidentalis*.

Materials and Methods

COLONY ESTABLISHMENT

Frankliniella bispinosa adults were collected from unsprayed southern highbush blueberry (southern highbush (SHB), *Vaccinium corymbosum* L. × *V. darrowii* Camp; Ericaceae) in Marion County, Florida. Thrips were removed from flowers by using a vacuum extraction pump (IN26 Air Compressor and Vacuum, GAST Manufacturing Inc., Benton Harbor, Michigan) modified for small insects. Adult thrips from the colony were periodically slide mounted in CMC10 Medium (Master's Chemical Co., Elk Grove, Illinois) and examined under the compound microscope at 150 to 400× magnification to confirm species using the thrips identification key developed by Arévalo et al. (2009).

Organic green beans (*Phaseolus vulgaris* L.; Fabaceae) purchased at local supermarkets were provided as a food source and oviposition substrate. Beans were soaked in 3.5% Fit Fruit and Vegetable Wash (HealthPro Brands Inc, Cincinnati, Ohio) solution to remove any pesticide residues, and were thoroughly rinsed in deionized tap water and air dried before being put into the container that housed the colony. Thrips were placed in Ziploc containers (22 × 14 cm) (SC Johnson & Son, Inc., Racine, Wisconsin) in an environmental growth chamber (Percival I-36, Percival Scientific Inc., Nevada, Iowa) set at 23 ± 1 °C, 85 ± 10% relative humidity, and a photoperiod of 14:10 h L:D. Green beans were placed on filter paper (Fisherbrand Filter paper, Grade: P8, 15 cm diameter; Pittsburgh, Pennsylvania) inside the Ziploc containers. Separate weigh boats of honey and grounded bee pollen were included in each container to promote reproduction. Containers with thrips were serviced twice per week. Old green beans were replaced by fresh ones and old filter papers replaced by new ones, and fresh honey and bee pollen were provided as needed. *Frankliniella occidentalis* was obtained from an established colony (United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine [USDA APHIS PPQ] Center for Plant Health Science and Technology, Tallahassee, Florida) and reared in the same manner as described for *F. bispinosa*.

HUMIDITY EXPERIMENTS TO DETERMINE FECUNDITY AND EGG INCUBATION

Organic green beans were washed in distilled water and cut into 2-cm-length pieces. Each end was sealed with melted pure paraffin wax (Thermo Fisher Scientific, Waltham, Massachusetts) and allowed to cool and harden. A single piece was placed into each of 10 SOLO, P100 cups (SOLO Cup Company, Lake Forest, Illinois) to serve as the food source and oviposition substrate for thrips in the experiments. Two *F. bispinosa* or *F. occidentalis* adult females less than 48 h old were placed in each cup. Cups were covered with lids that had their centers removed and replaced with 0.1 mm mesh screen.

The experiment was a completely randomized design with 4 treatments and 10 replicates (each cup representing a replicate). Environmental chambers were maintained at a constant temperature of 23 ± 1 °C with a photoperiod of 14:10 h L:D, and relative humidity treatment levels of 40 ± 5, 55 ± 5, 70 ± 5, and 80 ± 5% were used in this experiment. Cups were established on different dates to ensure that each environmental chamber was used to collect data for replicates from each humidity treatment.

Adult thrips were left inside the cups to oviposit on the beans. Adult thrips were removed after 24 h by using the vacuum suction pump. Eggs laid within the tissue of the bean were not easily visible under a dissecting microscope. Therefore, fecundity rather than egg output was measured (Munger 1942; Larentzaki et al. 2008). The green beans were maintained for at least 6 d at 23 ± 1 °C, 85 ± 10% relative humidity, and a photoperiod of 14:10 h L:D, and the number of larval thrips in each container was recorded at 12 h intervals. The number of larvae hatching over time in each cup measured fecundity and the time to egg hatch measured the egg incubation period.

STATISTICAL ANALYSES

Repeated measure analysis was initially done to determine the effect of humidity levels on fecundity of individual species over time, but the data were not significant for several time periods (SAS Institute 2012). Therefore, the effect of humidity on fecundity of individual species was determined at each 12 h interval by using analysis of variance (ANOVA) for a completely randomized design and subsequent Tukey's HSD test ($\alpha = 0.05$) (SAS Institute 2012). The effect of humidity on mean daily fecundity per female of *F. bispinosa* and *F. occidentalis* for data pooled across time intervals was determined using ANOVA for a completely randomized design and subsequent Tukey's HSD test ($\alpha = 0.05$). Time of egg hatch for different humidity treatments was compared using ANOVA and subsequent Tukey's HSD test ($\alpha = 0.05$).

Results

FECUNDITY

Humidity affected the fecundity of *F. bispinosa* and *F. occidentalis* (Figs. 1 and 2, respectively). Few larvae were observed for *F. bispinosa* and *F. occidentalis* until 60 and 72 h, respectively. The treatment effect of humidity on the mean number of larvae was significantly different for *F. bispinosa* at 60 h ($F = 15.46$; df = 3,36; $P < 0.0001$), 72 h ($F = 17.58$; df = 3,36; $P < 0.0001$), 84 h ($F = 19.33$; df = 3,36; $P < 0.0001$), and 96 h ($F = 12.3$; df = 3,36; $P < 0.0001$); and for *F. occidentalis* at 72 h ($F = 3.84$; df = 3,36; $P = 0.0174$), 84 h ($F = 3.93$; df = 3,36; $P = 0.0159$), 96 h ($F = 7.15$; df = 3,36; $P = 0.0007$), 108 h ($F = 8.48$; df = 3,36; $P = 0.0002$),

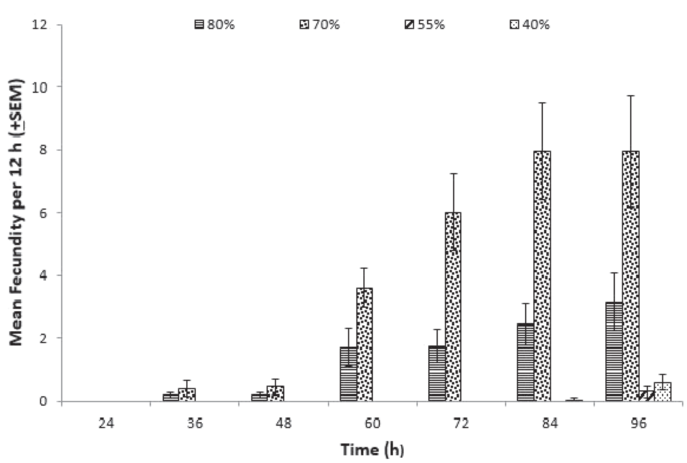

Fig. 1. Mean fecundity (± SE) per female of *Frankliniella bispinosa* at 12 h intervals in laboratory experiments at constant 23 °C and 4 relative humidity levels.

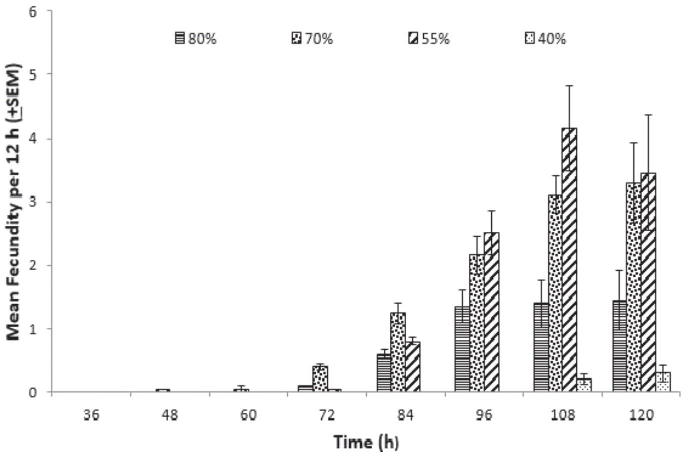

Fig. 2. Mean fecundity (± SE) per female of *Frankliniella occidentalis* at 12 h intervals in laboratory experiments at constant 23 °C and 4 relative humidity levels.

and 120 h (F = 7.2 df = 3,36; P = 0.0007) but were not significant at 60 h (F =1.00; df = 3,36; P = 0.404). At each 12 h time interval, *F. bispinosa* and *F. occidentalis* produced very few larvae at the relative humidity of 40%, and *F. bispinosa* produced very few larvae at the relative humidity of 55%. Fecundity at each time interval was greatest for *F. bispinosa* at the relative humidity of 70% (Fig. 1), whereas fecundity at each time interval was greatest for *F. occidentalis* at the relative humidity levels of 55 and 70% (Fig. 2).

Mean fecundity of females of *F. occidentalis* and *F. bispinosa* at each humidity level were compared for data pooled across time intervals (Fig. 3). The effect of treatment was significant (F = 11.57; df = 7,72; P < 0.0001). Fecundity was greater for *F. bispinosa* at 70% relative humidity than at relative humidity levels of 40, 55, and 80% (Fig. 3). Fecundity of *F. bispinosa* at relative humidity levels of 40, 55, and 80% was not significantly different between the levels. Fecundity of *F. bispinosa* was greater at 70% relative humidity than the fecundity of *F. occidentalis* at all relative humidity levels. Fecundity of *F. occidentalis* at relative humidity levels of 55, 70, and 80% was not statistically different between the levels.

INCUBATION PERIOD OF EGGS

The effect of relative humidity on the time to egg hatch of *F. bispinosa* and *F. occidentalis* was significant (F = 37.4; df = 3,36; P < 0.000). Mean time to hatch of *F. bispinosa* eggs was significantly shorter at the relative humidity levels of 70 and 80% than at relative humidity levels of 40 and 55% (Fig. 4). Mean time to egg hatch of *F. bispinosa* at relative humidity levels of 70 and 80% was significantly shorter than mean time to egg hatch of *F. occidentalis* at all relative humidity levels (Fig. 4). Mean time to egg hatch of *F. occidentalis* was not statistically different from each other at relative humidity levels of 55, 70, and 80%. However, mean time to egg hatch of *F. occidentalis* was significantly longer at the relative humidity of 40% compared with the relative humidity levels of 55, 70, and 80% (Fig 4).

Discussion

Reitz (2008) reported a mean time of egg hatch of 3.0 d for *F. occidentalis* laid in green bean pods in the laboratory at constant 20 °C and 65% relative humidity. This compares favorably with the mean time of egg hatch at constant 23 °C and 70% relative humidity for *F. occidentalis* in this study (Fig. 4). Mean daily fecundity per *F. occidentalis* female at 70% relative humidity in this study was 9.1 (Fig. 3), which was over 4-fold greater than mean daily fecundity of 2.07 for *F. occidentalis* in Reitz's (2008) study. Pollen was added to the diet of females as an additional source of nutrition in this study, and pollen increases the fecundity of both *F. occidentalis* and *F. bispinosa* (Tsai et al. 1996; Hulshof & Vanninen 2002). Bi-song (2001) reported parameters of growth and reproduction of *F. bispinosa*, but the effects of humidity were not determined.

Lublinkhof & Foster (1977) reported that temperature affected the fecundity of *F. occidentalis*. Optimum fecundity and development occurred around 20 °C. Our results showed that fecundity and the period of egg incubation of *F. occidentalis* were not affected at relative humidity levels of 55% or higher. These results suggest that the species is adapted to a range of humidity conditions and would explain its ability to establish outdoors in geographic areas with different climates (Kirk & Terry 2003). Fecundity was lowest and the period of egg incubation was longest for *F. occidentalis* at the relative humidity of 40%. Flower thrips may not be adapted to very low relative humidity levels as the flower provides a suitable microhabitat for reproduction and development.

Frankliniella bispinosa is adapted to the high humidity conditions of Florida. In southern and northern regions, average percentage of relative humidity in summer and fall months is in the 80's in the morn-

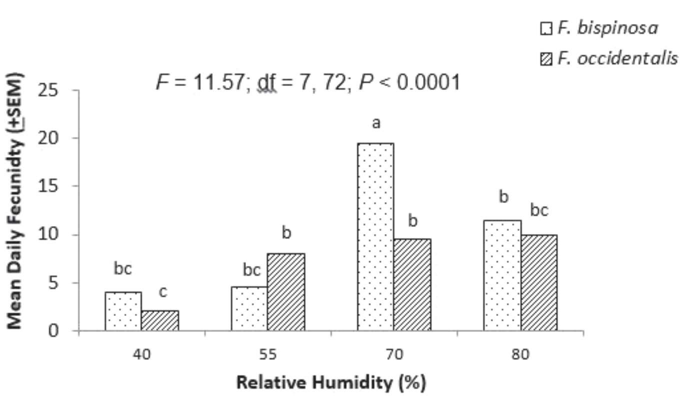

Fig. 3. Mean daily fecundity (± SE) of *Frankliniella bispinosa* and *F. occidentalis* for data pooled across time intervals in laboratory experiments at constant 23 °C and 4 relative humidity levels. Means with the same letter are not significantly different according to ANOVA and subsequent Tukey's HSD test (α = 0.05).

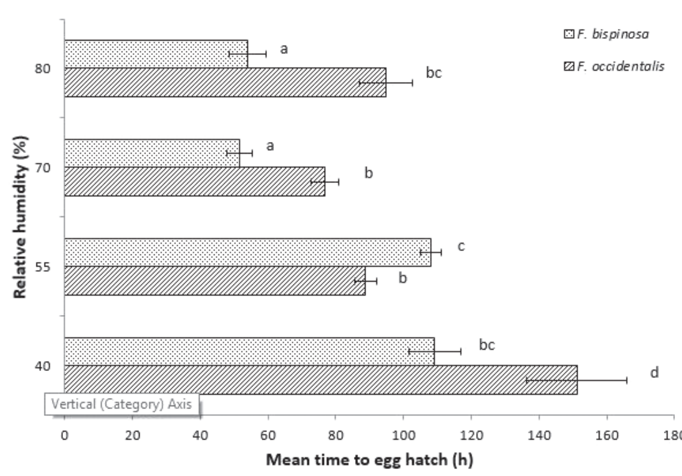

Fig. 4. Mean time of egg hatch (± SE) for *Frankliniella bispinosa* and *F. occidentalis* in laboratory experiments at constant 23 °C and 4 relative humidity levels. Means with the same letter are not significantly different according to ANOVA and subsequent Tukey's HSD test (α = 0.05).

ing and in the 60's in the afternoon (COAPS 2015). Fecundity of *F. bispinosa* in this study was greatest at 70% relative humidity, and the period of egg incubation was longest at 70 and 80% relative humidity. The species is better adapted than *F. occidentalis* to high humidity conditions. Fecundity of *F. bispinosa* was greater and the period of egg incubation was shorter than those of *F. occidentalis* in the high relative humidity treatments of 70 and 85% included in this study.

Northfield et al. (2011) evaluated in the laboratory the effects of competition on reproduction of *F. occidentalis* and *F. bispinosa* in the flowers of *Capsicum annuum* L. (Solanaceae). *Frankliniella occidentalis* showed superior competitive ability, being better able to reproduce in dense interspecific populations. The effect of humidity was not controlled in Northfield et al.'s (2011) experiments. The laboratory results did not support observations that *F. bispinosa* outcompetes *F. occidentalis* under field conditions, and the mechanism remained unexplained. Our results in this study provided evidence that *F. bispinosa* has a reproductive and development rate advantage over *F. occidentalis* under conditions of high humidity.

Frankliniella occidentalis is the predominate thrips in Florida habitats disturbed by insecticides that exclude interspecific competition and natural enemies and by fertilizers that increase its preference and performance compared with *F. bispinosa*. This increase in *F. occidentalis* resulted in widespread crop losses. Demirozer et al. (2012) developed integrated pest management programs that were effective, economical, environmentally sound, and sustainable. One component of the program involved increasing competition from congeners including *F. bispinosa*. Our results provided information of the abiotic conditions that enhance interspecific competition of *F. occidentalis* by *F. bispinosa*. This information should be considered in future management efforts.

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