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EFFECT OF TEMPERATURE AND RELATIVE HUMIDITY ON THE EGG AND LARVAL STAGES OF SOME CITRUS ROOT WEEVILS

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ABSTRACT

The effects of temperature and humidity on eggs and of temperature on larvae of *Artipus floridanus* Horn, *Pachnaeus litus* Germar, *Pachnaeus opalus* (Olivier), and *Pantomorus cervinus* (Boheman) were determined.

The low and high temperatures lethal to neonate larvae were determined by 12-hour exposure to temperatures ranging from –5 to 50°C.

The combined effects of temperature and RH on egg hatch were determined. Saturation vapor pressure deficit accounted for 59.4, 81.9, and 94.2% of the observed variation in percent egg hatch for *A. floridanus*, *P. opalus*, and *P. cervinus*, respectively. The thermal constant (K) and developmental threshold (ϕ) was calculated for egg development of each species.

A. floridanus was reared on artificial media at constant temperatures from 15 to 35°C. At 35°C, larvae died at ecdysis to the last instar. Between 20 and 30°C, 1412 degree days were required to complete development. Larvae held at 15°C had not pupated after 8 months (3600 degree days).

RESUMEN

Se determinaron los efectos de temperatura y humedad en los huevos, y de la temperatura en larvas de *Artipus floridanus* Horn, *Pachnaeus litus* Germar, *Pachnaeus opalus* (Olivier), y *Pantomorus cervinus (Boheman).*

Se determinaron las temperaturas letales bajas y altas de larvas neonatales exponiéndolas por 12 horas a temperaturas de –5 a 50°C.

Se determinaron los efectos combinados de temperatura y humedad relativa en la oclosión de los huevos. La deficiencia en la presión del vapor saturado fue responsable por el 59.4, 81.9, y el 94.2% de la variación observada en el porciento de oclosión de los huevos de *A. floridanus*, *P. opalus*, y *P. cervinus*, respectivamente. Se calculó la termal constante (K) y el umbral del desarrollo (ϕ) de los huevos de cada especie.

A. floridanus se crió en dieta artificial a temperaturas constantes de 15 a 35°C. A 35°C, las larvas murieron desde ecdysis hasta el primer estadío. Entre 20 y 30°C, se requirieron 1412 días grado para completar el desarrollo. Larvas mantenidas a 15°C no habían pupado después de 8 meses (3600 días grado).

Adult weevil *Pachnaeus opalus* (Olivier), *Pachnaeus litus* (Germar), *Pantomorus cervinus* (Boheman), and *Artipus floridanus* Horn feed on citrus leaves and lay eggs between leaves or (*P. cervinus*) in cracks on the tree. Recently, *P. cervinus* eggs have also been discovered on fruit. Larvae fall to the ground, burrow into the soil, and feed on roots (Woodruff 1981, Woodruff 1982, Woodruff & Bullock 1979). In many areas of Florida, they are considered minor pests of citrus (Griffiths & Thompson 1957, Simanton 1976). However, these species can severely damage small non-bearing trees. For example, four *P. litus* larvae significantly reduced the growth in height, trunk diameter, and root volume of young citrus trees after 3 months (Estrada-Ortiz et al. 1981). The combined feeding of larval and adult *A. floridanus* has been reported to stunt growth and kill 1-year-old-trees in coastal regions (Bullock 1984). The effect of weevil injury on overall vigor of a mature tree and subsequent its sensitivity to disease or freeze damage is unknown.

Although root weevils are endemic to Florida (Blatchley & Leng 1916), their geographical distribution and abundance varies broadly over the citrus growing regions. *A. floridanus* has been recorded along the east coast from Brevard County to the lower Florida Keys (Woodruff 1982). *P. opalus* appears to have a more northern distribution while *P. litus* is common in southern Florida (Woodruff 1981). Although their distribution suggests climatic effect, no temperature dependent studies have been reported for the different developmental stages of these species.

This paper reports the effect of temperature on egg and/or neonate larval survivorship for *A. floridanus*, *P. cervinus*, *P. opalus*, and *P. litus* as well as the combined effects of temperature and relative humidity on egg survivorship and development for *A. floridanus*, *P. cervinus*, and *P. opalus*. The developmental rate of *A. floridanus* reared on artificial diet vas also determined at different temperatures.

MATERIALS AND METHODS

Adult *A. floridanus*, *P. cervinus*, and *P. litus* were collected in the vicinity of Ft. Pierce, FL and adult *P. opalus* from Ft. Green, FL. Adult weevils were maintained in the laboratory on citrus foliage in the manner described by Syvertsen & McCoy (1985). Eggs of *A. floridanus*, *P. opalus*, and *P. litus* were collected on double strips of wax paper as previously described (McCoy et al. 1985) while eggs of *P. cervinus* were collected on two attached strips of filter paper. Eggs were held in a moist chamber at 20° and neonate larvae vere collected as they dropped into a 30 dram vial.

The survivorship of neonate larvae was determined by exposing larvae collected within 24 hours of hatching to temperatures from –5 to 50°C for 12 hours in petri dishes lined with moist filter paper. Four cohorts of 10 neonate larvae were exposed to each temperature.

The effect of vapor pressure deficit on egg survivorship for *A. floridanus*, *P. cervinus*, and *P. opalus* was determined by transferring five egg masses (15-50 eggs/mass) < 24 hours old to Mason jars (ca. 1 liter) in which relative humidities (RH) of 40 to 100% were maintained using saturated salt solutions (50 ml): K_2CO_3 (40% RH); $NaNO_2$ (60% RH); KCl (80% RH); distilled water (100% RH). Saturation vapor pressure deficit was calculated for each combination of temperature and RH (Wieber et al. 1976). The jars were then held in darkness at 10, 20, 25, 30, and 35°C. *A. floridanus* eggs were also tested at 10, 15, and 40°C. Larval hatch was recorded daily for 60 days by counting the number of empty egg cases and unhatched eggs. Developmental times of eggs held at saturation (100% RH) were used to calculate the developmental threshold (ϕ) and thermal constant (K) for each species, using the linear model of Campbell et al. (1974) wherein I/developmental time = $y = a + bx$; x = temperature, $\phi = -a/b$; $K = 1/b$.

The developmental rates of *A. floridanus* larvae at 15 to 35°C were determined by rearing groups of 20 neonate *A. floridanus* in cups of artificial media (McCoy et al. 1985) in darkness. At 30-day intervals, larvae from 10 to 20 cups were removed from the media and each developmental stage was identified. The weight, body length, and head capsule width of each larva was recorded. In addition, the duration of the pupal period and time spent as an adult in the pupal cell at each temperature was determined by observing cups daily when the larvae reached the last instar. Developmental rates and thermal constant were calculated as above.

Results

The Effect of Temperature on Neonate Larvae

The low and high lethal temperatures for larvae after 12 hours exposure varied among weevil species (Table 1). Some larvae of *A. floridanus* and *P. cervinus* survived exposure to –5°C, and survival remained high between 5 and 40°C. *P. litus* was most sensitive to cold temperatures but survived at high rates between 10 and 40°C. *P. opalus* alone survived exposure to 50°C.

TABLE 1. Mean percent survival (± SD) of neonate larvae of *Artipus floridanus*, *Pantomorus cervinus*, *Pachnaeus litus*, and *Pachnaeus opalus* following 12-hour exposure to temperatures from –5 to +50°C.

Temp. (C°)	*A. floridanus*	*P. opalus*	*P. cervinus*	*P. litus*
–5	5 (6)	0 (0)	14 (10)	0 (0)
+5	65 (16)	100 (0)	95 (60)	0 (0)
10	100 (0)	100 (0)	100 (0)	75 (10)
15	100 (0)	100 (0)	99 (2)	90 (8)
20	97 (5)	100 (0)	100 (0)	100 (0)
30	100 (0)	100 (0)	95 (5)	97 (6)
35	97 (5)	100 (0)	97 (5)	100 (0)
40	100 (0)	100 (0)	92 (10)	90 (14)
50	0 (0)	100 (0)	0 (0)	0 (0)

Effect of Temperature and Relative Humidity on Eggs

As shown in Table 2, mean percentage egg hatch for *A. floridanus*, *P. opalus*, and *P. cervinus* declined at all temperatures as relative humidity was decreased. Linear regression analysis indicated that saturation vapor pressure deficit accounted for 59.4, 81.9, and 94.2% of the observed variation in percent egg hatch of each species, respectively.

A. floridanus eggs exposed to 10°C for 22 days were dead (Table 2), as indicated by the absence of any sign of embryogenesis after 4 days at 30°C. Some development was observed in eggs held at 40°C, indicating tat te eggs had survived briefly at this temperature. The maximum and minimum temperatures tolerated by *P. cervinus* and *P. opalus* eggs could not be determined from the data (Table 2).

The developmental threshold (ϕ) and thermal constant (K) of eggs were as follows: *P. cervinus*, ϕ = 10.7°C, K = 266; *P. opalus*, ϕ = 11.2°C, K = 151; and *A. floridanus*, ϕ = 12.9; K = 94 (Table 3).

Effect of Temperature on the Developmental Biology of *Artipus floridanus*

A. floridanus reared on artifical media attained adulthood in 1412 degree days if held between 20 and 30°C. The developmental threshold was calculated to be 9.2°C (Table 3). Head capsule measurements and weights indicated that a last instar *A. floridanus* had head capsules 1.2 to 1.4 mm wide and weighed 40 to 50 mg.

At 15°C, more than half the larvae attained the last larval instar (as indicated by head capsule measurements) by 120 days. Mean larval weight at this time was 19.9 mg/larva. The mean larval weight increased to 40 mg by 180 days, but only one individual pupated prior to termination of the experiment at 240 days.

At 35°C, all larvae attained the last instar within 30 days but died at ecdysis to the pupal stage.

TABLE 2. MEAN PERCENTAGE HATCH (± SD) OF EGGS OF *ARTIPUS FLORIDANUS*, *PACHNAEUS OPALUS*, AND *PANTOMORUS CERVINUS* AT CONSTANT TEMPERATURES AND RELATIVE HUMIDITIES

RH (%)	Percentage hatch (± SD)						
	Temperature (°C)						
	10	15	20	25	30	35	40
A. floridanus							
100	0	70 (9)	100 (0)	100 (0)	100 (0)	0 (0)	0
80	0	—	81 (34)	100 (0)	100 (0)	0 (0)	0
60	0	—	89 (15)	94 (14)	79 (23)	0 (0)	0
40	0	—	56 (39)	63 (25)	54 (30)	2 (4)	0
P. cervinus							
100	—	—	94 (4)	98 (4)	76 (33)	6 (9)	—
80	—	—	75 (30)	70 (25)	35 (29)	2 (4)	—
60	—	—	77 (28)	43 (28)	15 (10)	12 (11)	—
40	—	—	21 (17)	31 (26)	6 (5)	10 (10)	—
P. opalus							
100	—	—	100 (0)	100 (0)	100 (0)	33 (18)	—
80	—	—	90 (7)	100 (0)	38 (32)	4 (4)	—
60	—	—	70 (23)	73 (20)	3 (4)	0 (0)	—
40	—	—	7 (10)	14 (21)	0 (0)	0 (0)	—

TABLE 3. LINEAR REGRESSION OF 1/DEVELOPMENT TIME VS. TEMPERATURE, DEVELOPMENTAL THRESHOLD, AND THERMAL CONSTANT (K) FOR EGGS AND IMMATURES OF *ARTIPUS FLORIDANUS* AND EGGS OF *PANTOMORUS CERVUNUS* AND *PACHNAEUS OPALUS*.

Development stage	Regression equation	r^2	Threshold	K
A. floridanus				
Eggs	Y = –0.1370 + 0.01060X	93.9	12.9	94
Immatures	Y = –0.0064 + 0.00078X	81.9	8.2	1282
P. cervinus				
Eggs	Y = –0.0404 + 0.00375X	96.0	10.8	267
P. opalus				
Eggs	Y = –0.0734 + 0.0066X	94.0	11.1	151

Between 20 and 30°C, larvae reached the last larval instar (as indicated by head capsule size and weight) in roughly one-half the degree days required for total development, then spent a prolonged period without change in size or weight before pupating (Table 4). No statistically significant differences in size or weight were noted between adults reared at 20, 25, or 30°C.

DISCUSSION

The sensitivity of *P. litus* larvae to low temperature appears to be consistent with its restricted distribution to south Florida (Woodruff 1981) and, conversely, the tolerance of *P. cervinus* to low temperature (–5°C) partly explains its wide geographical distribution in both temperate and subtropical regions (Chadwick 1965). Other weevil species survive a range of air and subsurface soil temperatures rarely exceeded under Florida conditions (Allen & McCoy 1979, Sites 1971).

The significant decline in egg hatch by all species of weevils at high vapor saturation deficits was due largely to desiccation. Desiccation could be a limiting factor during hot, dry weather that periodically occurs during spring and summer in Florida. However, eggs may withstand short periods of high vapor concentration deficit especially when oviposited between leaves.

Since *P. cervinus* may occasionally oviposit on fruit, regulatory agencies may require methods to eliminate eggs on fruit. The broad range of temperature survived by *P. cervinus* indicate that storage temperatures lethal to eggs would not be feasible as a control measure.

TABLE 4. DEVELOPMENT OF *ARTIPUS FLORIDANUS* REARED ON AN ARTIFICIAL MEDIA AT VARIOUS CONSTANT TEMPERATURES.

	Temperature (°C)		
	20	25	30
Mean no. days (± SD) required for			
Adult emergence	142 (11)	81 (11)	71 (1.2)
Adult in pupal chamber	—	3.6 (3.0)	2.3 (2.6)
Pupal development	—	14.0 (1.9)	10.8 (2.6)
Larval development	< 60	30 > x < 60	< 30

The slow development of *A. floridanus* larvae at 15 and 20°C has strong implications for the phenology of this weevil. In the field, there is probably little or no development between December and February when mean soil temperatures range between 15 and 20°C (Sites 1971). Larvae hatching in fall over a broad span of time would tend to emerge over a brief period of time after development resumes in spring time.

The fact that *A. floridanus* spent one-fourth of its development time as a mature larvae under these rearing conditions may be due to preferences for specific moisture concentrations for pupation as in *Diaprepes abbreviatus* (Schroeder 1987).

Since *A. floridanus* have never been successfully reared on a host plant, it is not possible to compare development on the diet with that on a host plant. The fact that larvae grow rapidly with survival in excess of 80% on this diet suggests that it is nutritionally adequate (McCoy et al. 1985).

Endnotes

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LARVAL PHOTOPERIOD AND METABOLIC RATE IN *DROSOPHILA MELANOGASTER*

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ABSTRACT

The metabolic rate of adult *Drosophila melanogaster* Meigen is partially determined by larval photoperiod. Generally, flies reared in a short-day environment have higher whole-animal and weight-specific metabolic rates than do flies reared under long-day conditions. The effect of photoperiod on metabolic rate may help explain why short-day flies develop faster and have higher Malthusian fitness than do long-day flies. The photoperiod effect may also facilitate the maintenance of viable populations in seasonally variable environments.

RESUMEN

El grado de metabolismo del adulto de *Drosophila melanogaster* Meigen es parcialmente determinado por el fotoperíodo de las larvas. Generalmente, las moscas criadas en un medio ambiental de dias cortos, tienen el metabolismo total y del peso específico más alto que las moscas criadas bajo condiciones de dias largos. El efecto del fotoperíodo en el grado de metabolismo pudiera ayudar a explicar el por qué moscas expuestas a dias cortos se desarrollan más rápido y tienen adaptación Maltusiana más alta que moscas expuestas a dias largos. El efecto del fotoperíodo también pudiera facilitar el mantenimiento viable de las poblaciones en medios ambientales estacionales variables.

In *Drosophila melanogaster* Meigen, contrasts in life history characteristics observed among different experimental treatments may be partially explained by differences in rate of metabolism, whether genetically encoded or environmentally induced. For example, flies kept in short-day (8-h light:16-h dark) environments have higher instantaneous birth rates and peak fecundities than do flies kept in long-day (12-h light:12-h dark) environments at the same temperature (Giesel 1986). Also, short-day flies and their offspring develop to maturity more rapidly than do long-day flies and their offspring (Giesel 1986, 1988). These observations suggest the possibility that metabolic rate in *D. melanogaster* is negatively correlated with photoperiod experienced during the development of an individual. Here we test this hypothesis by investigating the effect of larval photoperiod on metabolic rate in this species.