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POPULATION DYNAMICS OF OVERWINTERING BOLL WEEVILS, *ANTHONOMUS GRANDIS GRANDIS* (BOHEMAN) IN THE LOWER RIO GRANDE VALLEY OF TEXAS.

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Abstract

Survival, morphological, physiological, and reproductive changes in overwintering boll weevils, *Anthonomus grandis grandis* Boheman, in the Lower Rio Grande Valley of Texas were studied. The number of boll weevils captured per trap declined significantly from the postharvest period to the beginning of the spring over two overwintering seasons: 2000-2001 and 2001-2002. The proportion of males and females trapped did not differ significantly. Ninety percent of captured weevils died within 47.6 days when fed, while 90% of unfed weevils died after 9.0 days. This trend was not affected by month of capture. Female weevil fat body ratings were significantly greater at the beginning of fall than in the spring, while most male weevils from September through March were rated as lean (82.5-100%). No differences in male reproductive parameters were observed in those captured during the cotton free-period compared with the middle of growing cotton season (June), except for testes size which was larger during the latter period. The percentage of females with oocytes in their ovarioles, and the percentage containing oocytes with yolk, were significantly lower in September than in June. During October-March, we did not observe any females with chorionated eggs, whereas in June 96% females contained them. We found that weevil females can oviposit eggs in the Lower Rio Grande Valley during the overwintering period after feeding 7-20 days on a reproductive diet of cotton squares. Females captured later in the winter fed at longer period before laying eggs.

Introduction

Newell and Paulson (1908) recognized that the most important step in the production of a cotton crop was to reduce the number of overwintering boll weevils. Diapause in the boll weevil, at least in temperate regions, is generally characterized by a period of extended dormancy in relatively distinct types of overwintering habitat (Bottrell et al. 1972). Previous research has shown a difference between boll weevil diapause in subtropical and tropical versus temperate environments. Basic research designed to address unanswered questions on the overwintering dynamics of boll weevils is vital to the successful expansion of eradication, containment, and management programs into subtropical and tropical environments.

In the subtropical Lower Rio Grande Valley (LRGV) of Texas, reproduction is halted in diapausing adults and metabolic activity is suppressed (Wolfenbarger et al. 1976; Graham et al. 1978, 1979; Guerra et al. 1982; Summy et al. 1993). In the subtropics and tropics, the purpose of diapause in the boll weevil is to facilitate survival during periods of food shortage while remaining active during extended periods of relatively mild climatic conditions (Guerra et al. 1984; Summy et al. 1988). The winter-time physiology of boll weevils has been extensively studied since the beginning of the 20-th century (Sanderson 1905, Brazzel and Newsom 1959, Carter and Phillips 1979), however, the manner in which *A. grandis* survives during the winter months has been difficult to elucidate. Diapause induction in subtropical adult boll weevils under selected feeding regimes, and the minimal effects of photoperiods and nighttime temperatures were reported by Spurgeon and Raulston (1997a, 1997b, 1998). Suppression of the overwintering boll weevil population is critical to its effective management in the LRGV of Texas. However, the controlling factors and population dynamics of overwintered boll weevil in the subtropics have not been extensively studied. Additional information on the mortality and reproductive potential after late season population suppression programs could reveal opportunities for improved effectiveness and economics of overwintering boll weevil control. The objectives of this study were to examine the survival, morphological, physiological, and reproductive changes in overwintering boll weevils in the subtropical south Texas environment.

Materials and Methods

The study was conducted during overwintering seasons in three commercial fields in Hidalgo County (2000-2001) and two experimental plots of the USDA-ARS Subtropical Agricultural Research Center in Weslaco (2001-2002). All study sites had been planted to cotton in previous years and had a history of boll weevil infestations. Boll weevils used in these experiments were collected from pheromone traps (Boll Weevil Eradication Foundation Traps). Traps were installed around the perimeter of each study site (15 traps for each commercial field and 8 traps for each experimental plot) at 1.2 m above the ground on sticks, with 50 m distance between traps. Each trap contained a grandlure dispenser (Hercon Boll Weevil Lures 10 mg), and dispensers were replaced weekly. Both traps and grandlure dispensers are available commercially (Great Lakes IPM Co., Vestaburg, MI). Trapping began in September, and traps were inspected once every 7 days. The weevils captured during the second week of the month from September through March for each of two overwintering seasons were taken to the laboratory, where the number of weevils per trap were recorded and the mean number of weevils per trap per week calculated. Weevils were sexed using the method of Sappington and Spurgeon (2000), and weighed on an analytical balance (Mettler Instrument Corp., Hightstown, NJ). Adult survival was determined monthly based on weevils trapped in the third week of the month from September through March. Samples of 200-300 weevils were divided into two groups, and one of group was fed daily for 10 days (2 squares per weevil, 7-9 mm in diameter at the widest part of the flower bud with intact bracteoles), while the other group was not fed. Mixed sex groups of 5 weevils were held in 15-cm diameter petri dishes (each dish was ventilated by a 4-cm diameter circular screened hole in the lid) in an environmental chamber at 25±1°C, 65% RH, and a photoperiod 11:13 (L:D) h. Temperature and humidity were monitored by a Fisher-brand Traceable Relative Humidity Meter with temperature readout (Fisher Cat. No. 11-661-12, Control Company, Friendswood, TX).

Body fat of monthly captured weevils (30 females and 36 males, September through March), presence of oocytes, oocytes with yolk, and chorionated eggs of monthly trapped of 28 weevil females (September through March, and June) , and testes rating, testes size, and seminal vesicle condition of 51 (17 monthly) trapped males (September, November, and March) were determined under dissecting microscope using method of Spurgeon et al. (2003).

Feeding and oviposition behavior was evaluated for overwintering boll weevil females. Trapped weevils (September through March) were sexed, and males were marked with red paint on the right elytron. Then mixed-sex groups of 10 weevils (5 males and 5 females) were held in 15-cm diameter ventilated petri dishes. Each dish contained a cotton wick saturated with water and was provided daily with 10 uninfested, greenhouse-grown squares with intact bracteoles. After 7 days, we took a random sample of 15 females. Each female was isolated in a 15-cm diameter ventilated petri dish and provided with 5 uninfested greenhouse grown squares, which were replaced daily the first 10 days after onset of oviposition, after which the experiment was terminated. Squares were removed daily and both feeding (open) and oviposition (sealed) punctures were counted under a dissecting microscope. The total number of punctures in each square (feeding + oviposition) was used as a measure of boll weevil puncturing activity according to the method of Everett and Earle (1964). The number of sealed punctures is a relative estimate of the number of eggs oviposited (Everett and Ray 1962). An egg-puncture ratio (sealed to total punctures), was used to characterize oviposition activity (Everett and Earle 1964). However, some authors have observed that boll weevil females may oviposit some eggs without sealing the puncture, or may oviposit some eggs on the external surface of the square (Cushman 1911, Mayer and Brazzel 1963). Recognizing these potentional limitations, we used the number of sealed punctures as a relative measure of egg production , because most reports of boll weevil oviposition are based on such counts, and dissection of eggs from the squares would have precluded subsequent estimation of fecundity and survival.

Statistical analyses were conducted using analysis of variance (ANOVA), and means were separated by Tukey's studentized range test (Wilkinson et al. 1992). The modeling of probability of death weevils or probability of dying weevils during the interval between observations in the treatment fed vs. none fed weevils during the cotton free period was done by non-linear least squares using PROC NLIN (SAS, 1999). An analysis of variance of the factorial arrangement (factor1 = treatment, factor2 = starting month) was done on the 50th, 90th, and 95th percentiles of the Weibull distribution (day at which a specified percent of the population has died) using PROC MIXED (SAS, 1979).

Results and Discussion

The number of captured boll weevils per trap decreased from September through March by 6.3-fold over the season of 2000-2001, and by 5.3-fold over the season of 2001-2002 (Table 1). The percentage of boll weevil reduction, from the postharvest period to the beginning of spring, reached 84.2% (2000-2001) and 81.0% (2001-2002) (Table 1). Graham et al. (1979) and Guerra et al. (1982) also observed that the number of boll weevils captured in traps in the LRGV peaked in September and then declined through the onset of spring. There was no difference in males and females captured (1.1:0.9 during 2000-2001 and 1.03:0.97 during 2001-2002) (Table 1). Guerra et al. (1982) reported that males were always more abundant than females in traps, and a slight variation in the sex ratio of trapped overwintering weevils in Arizona (1:1 to 1.2:1) was observed by Sivasupramaniam et al. (1995). Trapping data cannot be directly related to changes in boll weevil populations during the overwintering period, because the proportion of the population sampled is unknown. But our data showed a clear tendency of a reduction in numbers of trapped weevils over the winter in the LRGV, and that reduction is likely related to boll weevil mortality.

Weather conditions and food are the two main factors that can cause mortality of overwintering boll weevils. In the subtropics, boll weevil survival over extended periods is enhanced by mild climatic conditions (Table 2). At the study sites, the mean monthly air temperature (September-March) ranged from 13.4°C to 28.1°C, with a maximum of 19.3°C to 34.6°C, and a minimum of 7.4°C to 22.3°C. Some authors have demonstrated the relative cold tolerance of boll weevils (Slosser et al. 1994, Soreson et al 1996, Suh et al. 2002), more than 90% of nondiapausing weevils tolerated freezing temperatures of 0.0°C and -2.5°C for up to 8 hours (Slosser et al. 1994).

An extended feeding period in the fall allows the boll weevil to accumulate sufficient food reserves to survive the overwintering period. Boll weevils continue to feed at the time when their rate of metabolism and food requirements are considerably reduced. Boll weevil survival during the cotton-free period (overwintering season) may be prolonged by feeding on non- reproductive host plants, which in the overwintering period in the LRGV may include pollen and leaves of cultivated and weedy plants, but these sources are less preferred and are inadequate for high survival. Summy et al. (1993), and Bodden (1997) related the reduction numbers of trapped weevils over the overwintering period with their mortality and attributed the absence of cotton fruit as the main factor. Summy et al. (1993) noted that certain species of the genera *Cienfuegosia, Sphaeralcea, Thespesia,* and *Hibiscus* spp.are also known reproductive hosts of the boll weevils. But weevils deprived of cotton as a food source are unable to overwinter successfully in warm regions (Fye et al. 1970, Guerra et al. 1984, Summy et al. 1988).

Our study showed that only one treatment (fed vs. unfed) was found to be significant for the analysis of the estimated day when 50%, 90%, or 95% of the weevils had died (Figs. 1-2). The mean day when 50% of fed weevils died was 13.3 days and for unfed weevils it was 4.5 days. The mean day when 90% of fed weevils died was 47.6, and for unfed 9 days. The mean day when 95% of fed weevils died was 67.2 and for unfed 10.5 days. Survival was not affected by the month of capture or the start of observation. Fig. 1 depicts the effect of feeding on probability of death for each month when observation of a newly captured cohort of weevils was begun. Because only the differences between fed and unfed weevils were significant, Fig. 2 is a better representation of the survival of fed and unfed weevils regardless of month observation was began.

Female fat bodies declined significantly from the beginning of fall to until the spring (Table 3). 47.5% and 27.9% of those captured in September and October, respectively, were rated fat or intermediate. Only 22.6% of those captured in November were rated as intermediate, whereas during December - March 98.7-100.0% were rated as lean (Table 3). Most males captured from September through March were rated as lean (82.5-100.0%) (Table 3). Brazzel and Newsom (1959) showed that fat reserves are depleted very slowly in diapausing weevils during the winter in temperate zones. Rummel et al. (1999) reported that diapausing boll weevils with limited body fat had a lower percentage winter survival and emerged from winter habitat earlier in temperate zones than weevils with large fat reserves. Rankin et al. (1994) documented a direct relationship between the amount of fat body reserve and the propensity of adult boll weevils in the LRGV to engage in long-duration flight, but found no relationship between body fat reserve and ovarian development. Mitchell and Taft (1966) indicated that boll weevils needed very little fat reserve to survive the winter.

No differences in male reproductive parameters were observed during the cotton-free period (September, November, and March) compared with males capture during mid-season cotton growth (June), except for testes size. Testes size was classified as normal for 60.0% and small for 40.0% of the males captured in September, and as small for 100% of the males captured in November and March. Tertes size was classified as about 92.0% normal for the males captured in June. Testes condition was classified as late reproductive for 70.8%, 87.1%, 90.1%, and 100% of the males captured in September, November, March, and June, respectively. Seminal vesicles contained sperm in 71.2%, 81.8%, 86.7%, and 100% of the males captured in September, November, March, and June, respectively.

The number of oocytes in the ovarioles, and the number of oocytes containing yolk in boll weevil females was significantly lower during the cotton-free period (September-March) than in the middle of the cotton growing season (June) (F = 9.2, df = 8, 237, P = 0.001 and F = 10, df = 8, 237, P = 0.001, respectively) (Fig. 3). A few females containing chorionated eggs were observed in September and March (4.8% and 5.1%, respectively), while none were found from October through February. In June, 96% of the sampled females contained chorionated eggs (Fig. 3).

Mean adult weight among trap-captured weevils in September was 1.5-fold higher than of the weevils captured in October, and averaged 1.8-fold higher than of the weevils captured from November through February (F = 58.8, df = 5, 732, P = 0.001) (Table 4). About 70.5% of weevils captured in September weighed 10.1-20.0 mg, while 77.3% weevils captured in October weighed 5.1-15.0 mg, and averaged 82.9% weevils captured from November through February weighed less than 10.0 mg (Table 4). Weight of adult weevils depend greatly on their conditions (water content, fat body content, egg compliment, feeding status, etc.). Of course, conditions and weight are variable, and we cannot standardize these data, but tendency of weight changing will be developed.

The reproductive potential of the boll weevil population is an important consideration in the success of any control strategy. During the cotton-free period in the LRGV, female boll weevils without access to cotton are in process of resorbing their unlaid eggs and entering reproductive diapause. However, when provisioned daily with greenhouse-grown cotton squares, commencement of oviposition began after 7, 15, and 20 days of feeding for boll weevils captured in September-October, November-December, and January, respectively. Fruit puncturing (feeding + sealed) and oviposition (egg [sealed] punctures/total [feeding + sealed] punctures) activities were significantly higher in September than in January and depended on the intensity of diapause. The number of eggs oviposited was the highest for weevils captured in September and lowest in February (Table 5). Thus, our data suggest that it may take longer for females to terminate diapause the longer they have been dormant. However, the relationship is not clear. Spurgeon and Suh (2003) found that the number of weeks of starvation (up to five) after diapause induction did not affect the proportion of females that terminated diapause after switching them to a reproductive diet. In our experiments, the rate of feeding by females was significantly less during most winter months than it was inthe fall (Table 5), and this may have effected the rate of diet-mediated termination of dormancy. The nature of the decline in feeding activity over the winter months is an intriguing phenomenon that will require further study.

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Table 1. Number of overwintering boll weevils trapped in LRGV, Texas.

Month	Boll weevils / trap					
	2000-2001[a]	% reduction	% trapped females[b]	2001-2002[c]	% redaction	% trapped females[d]
September	21.5±5.4a	0	50.0±0.0a	99.1±20.3a	0	49.9±0.5a
October	18.1±2.8ab	15.8	43.3±7.5a	51.0±6.9b	48.5	48.5±1.5a
November	12.1±1.1ab	43.7	50.0±0.0a	64.4±6.6ab	35.0	49.5±0.9a
December	8.4±2.5b	60.9	53.3±4.6a	22.3±2.3c	77.5	49.4±1.5a
January	5.5±0.3b	74.4	46.3±3.7a	32.1±5.9bc	67.6	49.9±0.9a
February	5.1±0.2b	76.2	49.0±0.2a	16.2±3.4c	83.6	48.9±2.4a
March	3.4±0.3b	84.2	47.8±1.4a	18.8±2.8c	81.0	49.9±1.8a

Means (±SE) within a column followed by the same letter are not significantly different (Tukey HSD, test).
[a]ANOVA $F = 7.5$; df = 6, 14 [7 treatments (months) and 3 replications (3 groups per 15 traps)]; $P<0.001$.
[b]ANOVA $F = 0.9$; df = 6, 14 [7 treatments (months) and 3 replications (3 groups per 15 traps)];$P=0.5$.
[c]ANOVA $F = 11.3$; df = 6, 105 [7 treatments (months) and 16 replications (traps per treatment); $P<0.001$.
[d]ANOVA $F = 0.05$; df =6, 105; $P=0.998$

Table 2. Mean daily temperatures (°C) during the overwintering period of boll weevils.

Month	2000-2001[1]			2001-2002[2]		
	Mean	Max	Min	Mean	Max	Min
September	28.1	34.6	21.5	27.6	33.0	22.3
October	23.4	28.8	17.9	24.4	31.0	17.8
November	19.7	25.5	13.8	21.1	27.0	15.1
December	13.4	19.3	7.4	17.7	23.7	11.8
January	14.7	20.4	8.9	16.5	23.2	9.7
February	19.4	24.4	14.4	15.2	21.3	9.2
March	19.2	25.0	13.5	20.7	26.4	14.9

[1] - Hidalgo, LRGV weather data,
[2] - Weslaco, LRGV weather data

Table 3. Percentage distribution of overwintering boll weevil fat body (females/males).

Month	Fat	Intermediate	Lean	Mean fat body rating[a]
September	21.7 / 0	26.8 / 5.0	52.5 / 95.0	1.7±0.4 / 1.1±0.05
October	8.2 / 0	19.7 / 17.5	72.1 / 82.5	1.4±0.2 / 1.2±0.02
November	0 / 0	22.6 / 0	87.4 / 100	1.2±0.03 / 1.0
December	0 / 0	9.1 / 0	90.9 / 100	1.1±0.01 / 1.0
January	0 / 0	0 / 0	100 / 100	1.0 / 1.0
February	0 / 0	0 / 0	100 / 100	1.0 / 1.0
March	0 / 0	1.3 / 2.5	98.7 / 97.5	1.01±0.01 / 1.1±0.02

[a] Fat=3, Intermediate=2, Lean=1.

Table 4. Percentage distribution of overwintering boll weevil weight (mg) among those captured by trap in LRGV, 2000-2001.

Month/adult wt	<5.0	5.1-10.0	10.1-15.0	15.1-20.0	>20.0	Mean adult wt, mg[a]
September	2.7	21.5	44.0	26.5	5.3	13.3±4.9a
October	14.6	57.3	20.0	8.1	0	9.0±3.6b
November	21.9	58.9	13.7	5.5	0	7.8±3.4c
December	28.3	56.5	11.1	4.1	0	7.1±3.2c
January	32.0	54.0	14.0	0	0	6.7±2.9c
February	36.0	44.0	15.0	5.0	0	7.2±3.7c

Means (±SE) within a column followed by the same letter are not significantly different (Tukey HSD, test).
[a]ANOVA F = 58.8, df = 5, 732, P < 0.001)

Table 5. Punctures in cotton squares by trapped overwintering boll weevil females.

Month	Punctures / female / day			Puncture ratio: eggs / feeding+eggs[d]
	Feeding[a]	Egg[b]	Feeding+Eggs[c]	
September	10.2 ± 0.7a	9.6 ± 0.7a	19.8 ± 1.2a	0.403 ± 0.02a
October	12.4 ± 1.2a	7.1 ± 0.6b	19.5 ± 1.4a	0.385 ± 0.03b
November	4.8 ± 0.7b	4.0 ± 0.7cd	8.8 ± 1.2bc	0.353 ± 0.05b
December	9.8 ± 0.7a	2.8 ± 0.3de	12.6 ± 0.9b	0.188 ± 0.02c
January	5.5 ± 0.4b	1.5 ± 0.2ef	7.0 ± 0.5c	0.168 ± 0.02c
February	4.1 ± 0.4b	0.4 ± 0.1f	4.5 ± 0.4c	0.079 ± 0.02d

Means (±SE) within a column followed by the same letter are not significantly different (Tukey HSD, test).
[a]ANOVA $F = 18.2$; df = 5, 598; $P<0.001$.
[b]ANOVA $F = 52.4$; df = 5, 598; $P<0.001$.
[c]ANOVA $F = 39.4$; df = 5, 598; $P<0.001$.
[d]ANOVA $F = 28.4$; df =5, 598; $P<0.001$.

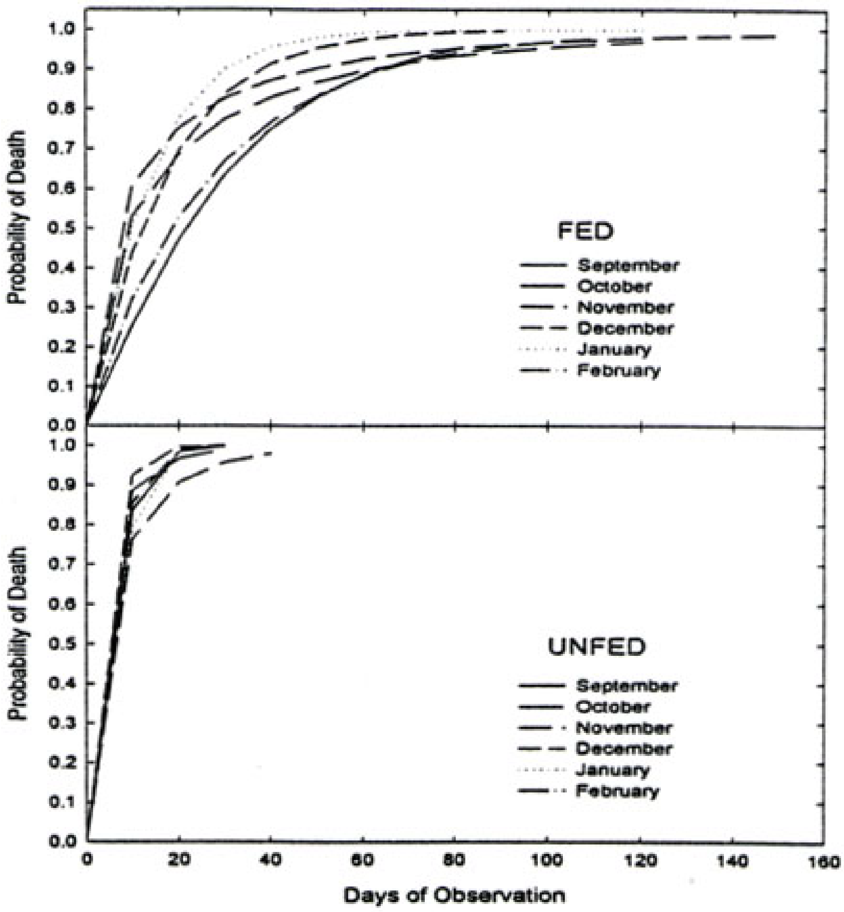

Figure 1. Probability of death by month observation began for fed and unfed weevils.

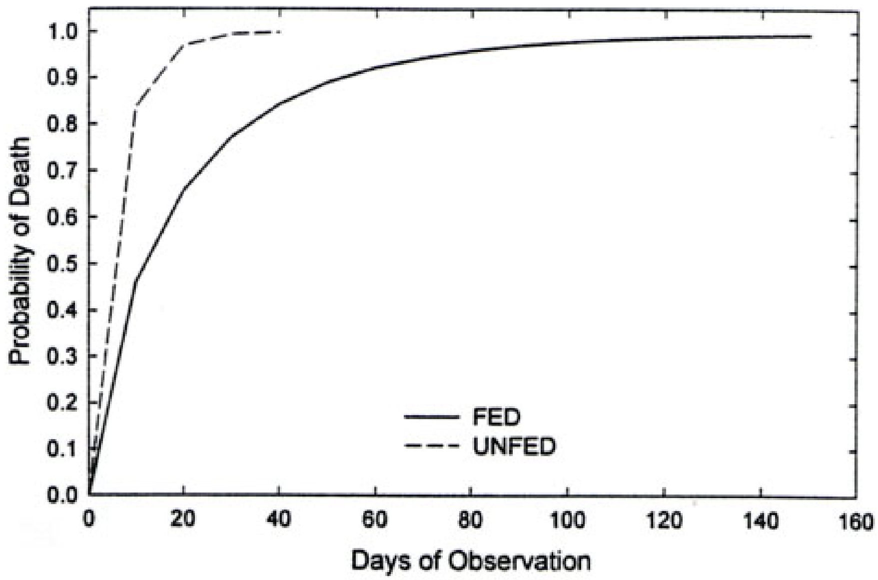

Figure 2. Probability of death for fed and unfed weevils pooled over months when observation began.

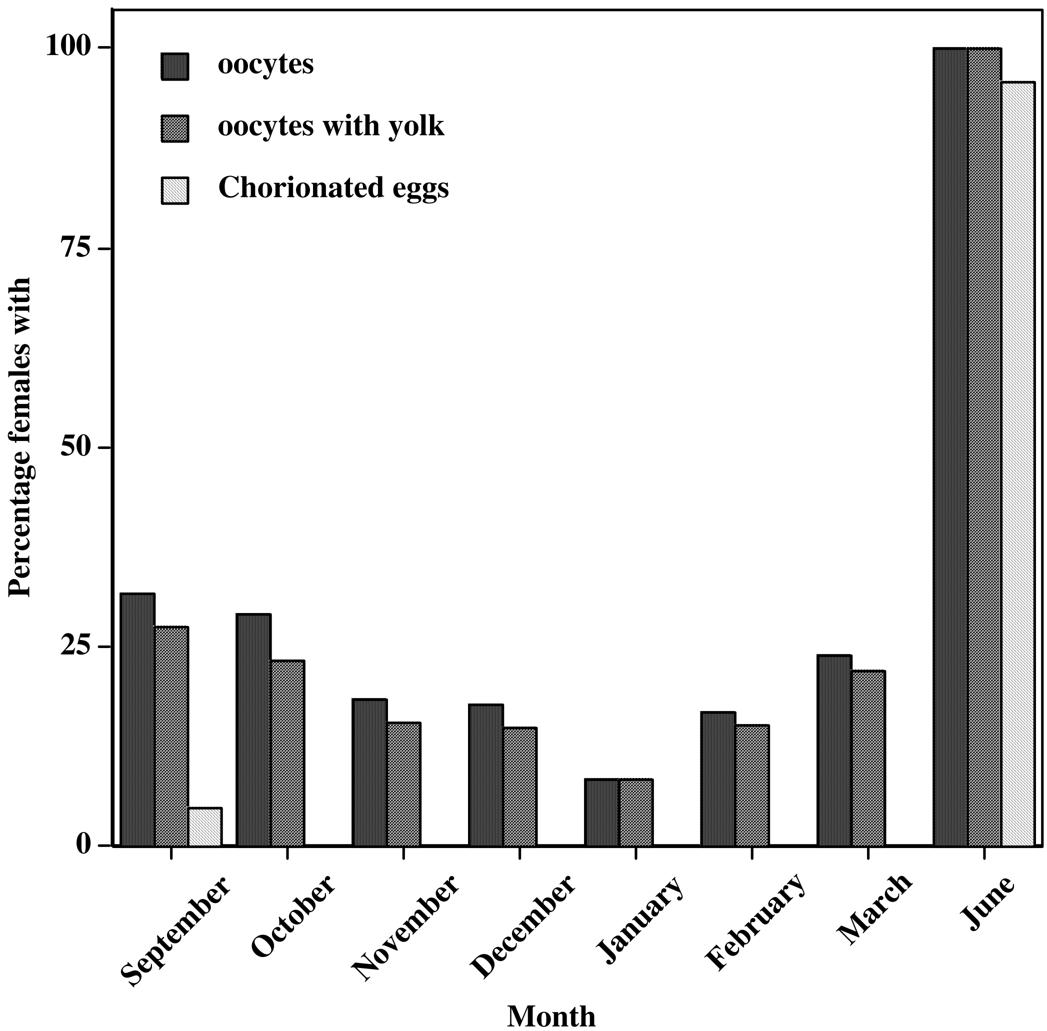

Figure 3. Number of oocytes in ovarioles in boll weevil females.