2003 BELTWIDE COTTON CONFERENCES, NASHVILLE, TN – JANUARY 6-10

REPRODUCTIVE DIAPAUSE IN TARNISHED PLANT BUGS AND ITS EFFECT ON NON-INSECTICIDAL CONTROL MEASURES

Gordon L. Snodgrass
Southern Insect Management Research Unit, USDA-ARS
Stoneville, MS

Abstract

Reproductive diapause in the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), was studied by dissection of field collected adults or adults reared from field collected nymphs in 1999-2001 near Stoneville in Washington County, MS. The critical photoperiod for diapause induction was about 12.5:11.5 (L:D) h, or 12 September. Overwintering adults collected from winter host plants in December 1999 and 2001 began breaking diapause in the second and third weeks of December at a day length near 10:14(L:D) h. Most of the overwintering females collected on winter host plants had mature eggs by the end of December in both winters. In the winters of 1998-1999 and 1999-2000, tarnished plant bug nymphs were found in January and produced an early new generation of adults by the second or third weeks of March. In these mild winters, host plants were not killed or stunted by cold weather. In the cold winter of 2000-2001, nymphs were not found until March with new generation adults produced in April. The effects of diapause on control of tarnished plant bugs by non-insecticidal methods which could be developed are discussed.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has long been known to overwinter as an adult in plant debris (Crosby and Leonard 1914). They described the overwintering adults as being in a state of hibernation, and noted that they were difficult to find in New York. Tarnished plant bug adults in the midsouth can be collected from wild hosts during every month of the year (Snodgrass et al. 1984). In the winter months of December, January, and February two species of wild host plants, shepherd's purse, *Capsella bursa-pastoris* (L.) Medicus, and henbit, *Lamium amplexicaule* L., are in bloom, especially in winters with average or above average temperatures. Henbit is very abundant, and fields can often appear purple because of henbit blooming in them. The presence of flower buds and blooms are important since tarnished plant bugs use them as a preferred feeding site and are seldom collected, even on their preferred wild hosts, until flower buds or blooms are present (Snodgrass et al. 1984).

Despite its importance in the adaptation of tarnished plant bugs to the many environments in which it is found, diapause in the tarnished plant bug has only been investigated in the laboratory by Bariola (1969). His study of diapause in tarnished plant bugs involved rearing them at several different temperatures and photoperiods in the laboratory. He found that nymphs were the life stage sensitive to a diapause-inducing stimulus, and that newly-hatched nymphs reared at 21 and 27° C in photoperiods of 12.5:11.5 (L:D) or less produced adults in diapause. Diapausing adults resulting from rearing nymphs at 12:12 (L:D) and 21° C became fully reproductive about two weeks after exposure to photoperiods of 13.5:10.5 (L:D) at 27° C. Newly-hatched nymphs reared at 21 and 27° C and 13:11 (L:D) produced a mixture of adults, some reproductive, others in diapause. Rearing newly-hatched nymphs at photoperiods of 13.5:10.5 (L:D) or longer prevented nymphs from developing into diapausing adults.

The present study was conducted to obtain information on reproductive diapause in the tarnished plant bug in the Mississippi River Delta of the midsouth. This information included determination of when diapause began and ended under field conditions. This information is needed, since diapause can affect several non-insecticidal control measures which could be developed and used for plant bugs.

Materials and Methods

All weather data used in the study was obtained from Stoneville Weather Station, Delta Branch Research and Experiment Station, Stoneville, MS. A weather data summary for Stoneville that presents average data for 1964-1993 is found in Boykin et al. (1995). References in the current study to thirty-year average weather data and day length were taken from this publication. Plant bugs were collected with a sweep net from wild host plants near Stoneville or other areas in Washington County, MS during the late summer, fall, winter, and spring. Collection of nymphs to be reared to obtain adults for dissection to determine reproductive status began in August of each year (1999-2001). As long as nymphs could be collected in the fall, only nymphs were kept and reared until they were adults. Adults reared from nymphs were dissected to determine their reproductive state when they were 7 d old or older. Allowing adults to reach this age ensured that the reproductive systems of the non-diapausing adults of both sexes were completely developed. Bariola (1969) found that mating occurred in reproductive adults between four and six days of age.

Collections of nymphs were made at intervals of about one week. When nymphs became difficult to collect (in November and December), adults were collected and dissected on the same or following day. Nymphs were reared outside in 1999 in a screened enclosure with a roof that provided protection from rain and partial shade during the day. Nymphs were placed in 4.6-liter cardboard containers whose tops and bottoms were organdy cloth. Each container held as many as 100 nymphs which were fed broccoli, *Brassica oleracea* L. var *botrytis* L. (cut up to remove big stems and provide mainly the flowers and buds for food). Nymphs of all sizes were reared together in the containers. New food was added every two d at which time old food was removed as needed and adults which had developed in the nymphal containers were removed. Adults were held in containers identical to those used for rearing nymphs using green bean pods, *Phaseolus vulgaris* L., as food. Rearing the plant bugs outside in 1999 created problems with high nymphal mortality which often exceeded 50% due to food spoilage caused by high humidity and heat. In 2000 and 2001, nymphs and the resulting adults were reared indoors using a room maintained at 25 ± 4^{o} C and relative humidity was not controlled. The rearing containers were held on a table in front of a south-facing window to receive natural daylight. The room was also illuminated with overhead fluorescent lights from 0700 to 1530 hours on weekdays.

The criteria used to determine diapause was the same used by Lees (1955), failure of the reproductive organs to enlarge and hypertrophy of the fat body. The female reproductive system of *L. lineolaris* is described in Davis (1955), and is very similar to that of *L. hesperus* (Strong et al. 1970). Although the male reproductive system of *L. lineolaris* has not been described, the male reproductive system of *L. hesperus* was described by Strong et al. (1970). In the current study, the male reproductive system of *L. lineolaris* was found to be very similar to that which they described for *L. hesperus.* Tarnished plant bugs in the process of changing from the diapause state to a reproductive state were also found. Such adults, both male and female, still had large to moderately sized fat bodies with reproductive organs in different states of enlargement. In a female, if the ovaries had one or more mature eggs (eggs in which the operculum was developed), or enlarged ovaries with developing oocytes, and/or she had been recently mated [the genital pouch was greatly enlarged (Strong et al. 1970)], she was considered to be reproductive even though the fat body was still enlarged. In males, it was often difficult to determine if the testis had begun to enlarge since they were frequently covered with a membrane with a white layer of what appeared to be fat. If white fluid was visible in the accessory glands and seminal vesicle, and the accessory glands had begun to enlarge, a male was considered to be reproductive. Adults to be dissected were killed in 70% alcohol and pinned ventrally through the thorax into a wax-bottom dissecting dish with the ventral side facing upward. The abdomen was opened up with sharp-pointed forceps to expose the internal organs which were examined under water at 10X magnification.

During January, February, March, and April of 1999-2002, winter and early spring wild host plants were sampled (as weather permitted) on a weekly basis with a sweep net to determine when nymphs were present and how long it took for new generation adults to be produced. Nymphs were determined as to instar using descriptions of the five nymphal instars found in Crosby and Leonard (1914).

Results

The critical photoperiod for diapause induction where 50% of the insects entered diapause (Tauber et al. 1986) was around 12 September (a day length of 12.5 h) in all three years (Table 1). Very few adults (8% or less) were found in diapause prior to 28 August in the study and most of the nymphs collected after 12 September produced adults in diapause (Table 1). A mixture of adults that were in diapause and reproductive were reared from nymphs collected between 28 August and the first two weeks in September. The percentage of nymphs collected after 12 September that produced adults in diapause reached 100% only one time for males (first week of November 1999) and five times (all five in 2000) for females.

Nymphs collected on 31 October through the third week of November 2001 produced adults that were increasingly reproductive each week (Table 1). The percentages of adults that were reproductive went from 20.9 and 5.9% for males and females, respectively, in the third week of October to 79.4 and 55.6% for males and females, respectively, in the third week of November. During this time period in the previous two years the percentages of males and females that were reproductive were 0 to 6% (10.1% for males in the third week of November 2000). Results from a control test conducted to check rearing conditions in the room in which nymphs were reared showed that the rearing conditions did not cause the increase in reproductive adults. First instar nymphs placed in the room on 18 November produced adults in which 91% of the males (n=111) and 100% of the females (n=87) were in diapause. In the fourth week of November, the percentages of the adults which were reproductive dropped to 27.9% for males and 3.5% for females. On or after the fourth week in November 2001 nymphs were not collected and reared, and the percentages in diapause shown are for adults collected from wild hosts and dissected.

Termination of diapause began in 1999 during the third week of December, and in 2001 during the second week in December (Table 1). Day length during these two weeks ranged from 9.9 to 10 h at Stoneville. Adults could not be collected after the first week in December in 2000 because of a colder than normal winter in 2000-2001 which stunted or killed the wild host plants. The

average high temperature in December was 7.2^0 C as compared to a thirty-year average for December at Stoneville of 12.2^o C. The average low temperature was -2.6^o C as compared to a thirty-year average of 2.3^o C. Temperatures in January and February of 2001 were normal, however, wild hosts did not get large enough to sample until mid-February. Over 90% of the adults collected from wild hosts were reproductive by the last week of December in 1999 and 2001.

In 1999 and 2000 henbit was the earliest host plant on which tarnished plant bug nymphs were found in the areas in Washington County that were sampled (Table 2). Fifth instar nymphs were present on henbit by 12 and 21 March in 1999 and 2000, respectively. This is not surprising since henbit is consistently the first winter host to bloom, usually by mid-December. The presence of fifth instar nymphs was used as an indicator that successful completion of a generation occurred on a host plant species. The presence of new adults was not used since new adults could have been produced on other host species and flown to where they were collected. The winter of 1999-2000 was not as warm as that of 1998-1999, but high and low temperatures were a little above the 30-year average in December 1999 and January-March 2000. During the cold winter of 2000-2001, henbit was severely stunted and was not utilized by tarnished plant bugs as a reproductive host. Shepherd's purse also blooms in Decembers with normal to above normal temperatures, but is not as abundant as henbit, and was not as preferred as a reproductive host. In 1999 and 2000, nymphs were first found on shepherd's purse in early March with fifth instar nymphs present by the third or fourth week of March. Sour dock can bloom in late-January, however nymphs were not found on it until the first week of March 1999 and the second week of March 2000. Fifth instar nymphs were found in 1999 in early-April and in the third week of March 2000. Nymphs were first found on buttercup in late-February to early-March with fifth instar nymphs present by the third or fourth week in March. The cold winter of 2000-2001 delayed the growth of most of the winter hosts normally utilized by tarnished plant bugs for food and reproduction. The first nymphs were found in the third or fourth weeks of March and the first fifth instar nymphs in the second and third weeks in April 2001 on daisy fleabane and butterweed. Thus, in 2001 new generation adults were not present on the wild hosts sampled until the second and third weeks of April. In the milder winters of 1998-1999 and 1999-2000, respectively, new generation adults were present on wild hosts sampled by the second and third weeks of March, about four weeks earlier than in 2000-2001.

Discussion

Rearing of nymphs collected from wild hosts was conducted under natural conditions of temperature and daylight outdoors in 1999, while this rearing was conducted indoors using fluorescent and natural light at a temperature of 25 $\pm$ 4^o C in 2000 and 2001. The laboratory rearing removed the effect that naturally occurring changes in temperatures each day might have had. The percentages of males and females entering diapause each week in 1999 and 2000 (Table 1) were very similar which showed that removal of these temperature changes had little effect on the induction of diapause. The percentages entering diapause in 2001 was very similar to those of 1999 and 2000 through 25 October. The large increase in numbers of reproductive adults produced from nymphs collected and reared in late October and November of 2001 was totally unexpected and its cause is unknown. On the fourth week of November (and thereafter) the percentages of reproductive adults declined since adults (not nymphs) were collected, and these were from an adult population that included adults produced previously in September and October.

The critical photoperiod for diapause induction was estimated to be 12.5 h (September 12), and was based on field collection and rearing of nymphs of different ages. Younger nymphs in each collection developed into adults at shorter day lengths than older nymphs collected on the same date. So, the percentage diapause determined for adults reared from nymphs collected on the same date is an average for all ages of nymphs present. Ideally, only first instar nymphs would have been collected and reared on each date, but this would be almost impossible to do in the field. Bariola (1969) concluded his laboratory studies by stating that the transitional day length separating the induction or prevention of diapause was 13 h, since newly- hatched nymphs reared at this day length produced a mixture of diapausing and reproductive adults. This day length occurred at 28 August at College Station, TX. Nymphs reared in his study at a photoperiod of 12.5:11.5 (L:D) h (12 September at College Station) produced diapausing adults. Therefore, he predicted that all adults in the field prior to 28 August should be reproductive, and all nymphs found after 12 September should enter diapause as adults. Nymphs completing development between 28 August and 12 September should be a mixture of reproductive and diapausing adults. His predictions were very similar to the findings in the present study. The day lengths on 28 August and 12 September are the same at Stoneville as they are at College Station. Most adults that developed from nymphs collected prior to 28 August were reproductive while nymphs collected after 12 September produced adults in diapause. A mixture of reproductive and diapausing adults developed from nymphs collected between 28 August and the first two weeks in September. Beards and Strong (1966) estimated the critical photoperiod for *L. hesperus* at Davis, CA, to be 13.5 h (August 20). *L. hesperus* began terminating diapause at Davis in November with about 50% of the population reproductive by the middle of November. They thought that nymphs found in the field in late October and in November did not enter diapause. This is similar to what was found with the tarnished plant bug in 2001 of the current study, where in late October and the first three weeks of November a high percentage of the adults reared were reproductive. However, this did not occur in the previous two years, and *L. hesperus* breaks diapause a month earlier at Davis than the tarnished plant bug does at Stoneville.

As seen in this study in the winter of 1998-1999 (Table 2), henbit allowed tarnished plant bugs to produce new generation adults by the second week in March. Sheperd's purse and buttercup can also be important as winter hosts but are not nearly as abundant as henbit. The cold winter of 2000-2001 prevented reproduction by tarnished plant bugs on their main winter hosts by stunting or killing these host plants. The first new generation adults were not produced until the second and third weeks of April, about three to four weeks later than in the previous two years.

Non-insecticidal control measures which could be developed for tarnished plant bugs that would be affected by diapause include destruction of wild host plants, use of winter trap crops, and mass release of sterile adults. Destruction of winter host plants by herbicides, mowing, or discing should begin by late-February in years with average or above average temperatures to prevent the development of new generation adults on the winter hosts by mid-March. This timing should also be used for winter trap crop crops for the same reason. Although winter trap crops for plant bugs are not grown in the midsouth, beginning in the mid-1990's growers in the midsouth began a farming practice where winter weeds were destroyed in their fields with herbicides mainly in February. This practice is widespread and saves growers money on weed control in their fields. Growers are essentially using winter weeds as a trap crop for plant bug control, although most of them are unaware of this benefit. Mass release of sterile plant bugs could be done in November and December at a time when no crops which are damaged by plant bugs are present. Diapausing plant bugs are unmated, and mate after they break diapause in December. Having sterile insects present to mate with native insects when they become reproductive in December could have its greatest impact on the native population at this time.

References Cited

Bariola, L. A. 1969. The biology of the tarnished plant bug, *Lygus lineolaris* (Beauvois), and its nature of damage and control on cotton. Ph.D. Dissertation, Texas A&M University, College Station.

Beards, G. W., and F. E. Strong. 1966. Photoperiod in relation to diapause in *Lygus hesperus* Knight. Hilgardia 37: 345-362.

Boykin, D. L., R. R. Carle, C. D. Ranney, and R. Shanklin. 1995. Weather data summary for 1964-1993 at Stoneville, Mississippi. Tech. Bull. 201. Mississippi Agr. and For. Expt. Sta., Mississippi State, MS. 49 pp.

Crosby, C. R., and M. D. Leonard. 1914. The tarnished plant bug. Cornell Agr. Expt. Sta. Bull. 346: 463-525.

Davis, N. T. 1955. Morphology of the female organs of reproduction in Miridae (Hemiptera). Ann. Entomol. Soc. Am. 48: 132-150.

Lees, A. D. 1955. The physiology of diapause in arthropods. Cambridge Univ. Press, Cambridge, England. 151 pp.

Snodgrass, G. L., W. P. Scott, and J. W. Smith. 1984. Host plants and seasonal distribution of the tarnished plant bug (Hemiptera: Miridae) in the delta of Arkansas, Louisiana, and Mississippi. Environ. Entomol. 13: 110-116.

Strong, F. E., J. A. Sheldahl, P. R. Hughes, and E. M. K. Hussein. 1970. Reproductive biology of *Lygus hesperus* Knight. Hilgardia 40: 105-147.

Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. Seasonal adaptations of insects. New York: Oxford Univ. Press, 411 pp.

Table 1. Occurrence of reproductive diapause in field-collected tarnished plant bugs. Nymphs were reared outdoors or indoors near natural light and dissected as adults seven days or more of age.

	1999				2000				2001			
	M		F		M		F		M		F	
Date collected[a,b,c]	n	%diap.	n	%diap.	n	%diap.	n	%diap.	n	%diap .	n	%diap.
Aug. 1-7	10	0	10	0								
8-14	20	0	14	0								
15-21	10	0	14	7.1	204	2.0	195	1.0				
22-29	15	0	20	0	213	8.0	207	4.8	87	2.2	106	3.8
Sept. 1-7	16	6.3	13	15.4	106	28.3	135	23.7	59	42.4	56	14.3
8-14	24	40.5	31	48.4	105	51.4	125	34.4	86	63.9	80	48.8
15-21	15	86.7	30	86.7	138	84.8	96	85.4	93	87.1	98	73.5
22-29	50	98.0	52	98.1	114	98.2	109	100.0	129	79.1	135	94.1
Oct. 1-7	39	92.3	50	90.0	68	95.6	80	95.0	122	84.4	100	87.0
8-14	58	96.6	53	92.5	80	90.0	90	93.3	87	80.5	126	86.5
15-21	48	91.6	41	92.7	120	96.7	103	99.0	72	77.8	102	82.4
22-29	50	98.0	52	98.1	114	98.2	109	100.0	129	79.1	135	94.1
31					76	92.1	68	100.0	144	54.9	175	67.4
Nov. 1-7	96	100.0	114	95.3								
8-14	54	94.4	50	98.0	251	92.4	248	97.2	134	50.0	146	56.2
15-21	50	96.0	50	94.0	99	89.9	101	100.0	180	20.6	198	44.4
22-29	54	92.6	59	93.2	157	85.4	106	100.0	122	72.1	113	96.5
30									120	80.0	88	98.9
Dec. 1-7					120	87.5	90	97.8	119	85.7	84	94.1
8-14	55	89.1	52	88.5					138	36.2	75	54.7
15-21	17	76.5	29	58.6					67	3.0	31	12.9
22-29	70	8.6	45	8.9					15	0.0	13	7.7
Jan. 8-14					46	2.2	26	7.7				

[a] Nymphs were reared in an outside enclosure in natural daylight and temperatures in 1999. In 2000 and 2001, they were reared indoors with natural daylight at 25 $\pm$ 4° C.

[b] Data shown for December 1999 and January 2000, and on or after 15 November 2000 and 22 November 2001, are from dissection of plant bugs collected as adults.

[c] No adults were collected after the first week in December 2000 because of cold temperatures which killed or stunted winter host plants.

Table 2. Winter and early-spring host plants of the tarnished plant bug in Washington County, MS.

	1999		2000		2001	
Plant host	First nymphs	5th instar	First nymphs	5th instar	First nymphs	5th instar
Sheperd's purse	9 Mar.	18 Mar.	7 Mar.	22 Mar.		
Buttercup	2 Mar.	23 Mar.	29 Feb.	21 Mar.		
Sour dock	4 Mar.	6 Apr.	14 Mar.	21 Mar.		
Henbit	25 Jan.	12 Ma.	18 Jan.	21 Mar.		
Daisy fleabane					22 March	10 April
Butterweed					23 March	19 April

[a] The host plants were sampled weekly with a sweep net to determine when the first nymphs were present and when nymphs became fifth instar.