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Joel D. McMillin
Iowa State University

Michael J. Anderson
Iowa State University

Elizabeth E. Butin
Iowa State University

Elwood R. Hart
Iowa State University

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PHENOLOGY AND INFESTATION PATTERNS OF THE COTTONWOOD TWIG BORER (LEPIDOPTERA: TORTRICIDAE) IN IOWA

Joel D. McMillin[1,3], Michael J. Anderson[2], Elizabeth E. Butin[2], and Elwood R. Hart[1, 2]

ABSTRACT

Cottonwood twig borer, *Gypsonoma haimbachiana* (Lepidoptera: Totricidae), phenology and infestation patterns on *Populus* spp. were examined over a 2-year period in Iowa. Weekly sampling of infested shoots during the host growing season verified the existence of five instars. Head capsule size increased nonlinearly from the first to the fifth instar and corresponded to a concomitant geometric increase in the volume of larval feeding galleries. The sampling indicated that the cottonwood twig borer had two generations per year in Iowa. Corresponding with the two generations, two peaks of larval abundance were observed; one in the second week of June and the other in the first week of August. Greater volume of feeding galleries occurred in the early season generation compared with the late season generation. Sampling of infested shoots revealed that more than 80% of infested terminals contained only one active attack (freshly bored hole in tree terminal with frass present); more than 88% of feeding galleries contained only one larva; and more than 80% of the larvae were found in the first active attack nearest the terminal apex. These data were compared with results published on the phenology and attack patterns of the cottonwood twig borer in the southern United States.

The cottonwood twig borer, *Gypsonoma haimbachiana* (Kearfott) (Lepidoptera: Tortricidae), infests *Populus* species and hybrids throughout the range of its hosts (Morris et al. 1975). Feeding by larvae causes a shortening of internodal distance and increased forking, breakage, and mortality of terminal and lateral shoots (Solomon 1995). This feeding damage can threaten the economic viability of short-rotation *Populus* plantations (Morris 1967, Payne et al. 1972). Although the phenology and infestation patterns of the cottonwood twig borer have been reported for short-rotation cottonwood plantations in the southern United States (Morris 1967, Stewart and Payne 1975), similar studies have not been conducted in other regions of its range. Because insect phenology and morphology can vary by geographic location (references cited in Goettel and Philogène 1979), more information is needed to understand the type and timing of damage caused by the cottonwood twig borer before economic injury levels can be determined or management programs can be developed.

[1] Department of Forestry, Iowa State University, Ames, IA, 50011.

[2] Department of Entomology, Iowa State University, Ames, IA, 50011.

[3] Current address: USDA Forest Service, Pactola Ranger District, Rapid City, SD 57702

The life cycle and phenology of the cottonwood twig borer has been documented in the southern United States (Morris 1967). Females oviposit small clusters of eggs on the upper surface of leaves. The first instars mine major leaf veins until the first molt and then tunnel into tender shoots at the base of developing leaves. Instars 2 through 5 feed on actively growing tissues in shoots for approximately 21 days. Pupation occurs in bark crevices or in the litter beneath trees. Larvae overwinter in hibernacula on the bark or leaf scars of cottonwood trees (Stewart and Payne 1976). Four to five generations occur per year, with a summer generation developing in 40–45 days in Mississippi.

The population and attack density of cottonwood twig borer can differ significantly by year and location (Payne et al. 1972, Stewart and Payne 1975). Lower levels of attack were found in natural stands compared with plantations, and plantations themselves varied in attack density (Woessner and Payne 1971, Payne et al. 1972). In Texas, nearly 100% of the upper terminal shoots and 50% of the lateral shoots were killed by late August or September (Stewart and Payne 1975). Fifteen-inch terminal shoots in Mississippi were observed to contain up to 25 larvae and three different instars during late summer (Morris 1967).

In this paper, we describe the phenology and infestation patterns of the cottonwood twig borer in Iowa, using weekly sampling during the host growing season over a 2-year period. Through measurements of head capsule width, we established the width range by instar, the volume of larval feeding gallery by instar, and followed the population of each instar through time. Classification of active attacks (freshly bored entrance holes in terminal shoots) were made for evaluating the type of active attack most frequently containing larvae.

MATERIALS AND METHODS

Research sites. Observations and measurements of cottonwood twig borer phenology and infestation patterns were conducted during the 1995 growing season (25 May – 19 August) in an agroforestry planting at the Ames Municipal Water Pollution Control Facility near Ames, Iowa. The planting design was alternating 16-m-wide and 365-m-long strips of herbaceous crops (*Panicus virgatum*) and of hybrid poplar (*Populus deltoides x P. nigra* var. 'Eugenei') (Colletti et al. 1994). The hybrid poplars were planted with a 1.3 m x 2.6 m spacing in May 1992. Three plots were selected at random within the plantation and each plot was considered one replication, for a total of three replications. Fifteen trees were designated per replication with five trees selected from each of the three pairs of rows in each replication.

The phenology studies in 1996 (31 May–30 August) were performed at the Institute for Physical Research and Technology (IPERT) near Ames, IA, because the cottonwood twig borer population was too low at the Ames Water Pollution Control Facility site. Ten trees were selected at random in each of four plots within *Populus* trial plantings that contained 2-year old 'Eugenei' and other *Populus* clones.

Phenology of cottonwood twig borer. Sampling consisted of removing 15 (1995 season) or 10 (1996 season) infested shoots of actively-growing lateral branches per plot on a weekly basis. During weeks 7 and 8 of 1995 and 5 and 6 of 1996, shoots were not collected because few active attacks could be found. Infested shoots were taken to the laboratory and dissected to collect larvae. Larvae were placed into glass vials of 70% ethyl alcohol to preserve them before measuring head capsule width to determine the range

within each instar. Head capsule width was measured to the nearest 0.01 millimeter at the widest area of the head, using a microscope with an ocular micrometer. Head capsule width data were analyzed using a two-way (sampling date, replicate) ANOVA.

To determine the instar and place where larvae overwinter on terminal shoots, 30 shoots were sampled from each of two sites (IPERT and Hinds Research Farm near Ames, IA) in February 1998. Twenty-five-cm-long terminal and lateral shoots were collected from trees that had sustained cottonwood twig borer attacks in the 1997 growing season. The shoots were observed in a laboratory for the presence of hibernacula and dormant buds were dissected to find larvae.

Volume of larval feeding galleries was measured in 1996 using the same shoots as in the larval phenology study. Only galleries that had one larva were used. Gallery widths and lengths were measured to the nearest 0.01 mm, and derived values were entered into the formula for the volume of a cylinder ($\pi r^2 h$). Mean volumes of previous instars were subtracted from those of succeeding instars to determine the mean volumes of instars 3 through 5. Volume of feeding gallery data were analyzed using a two-way (sampling date, replicate) ANOVA.

Infestation patterns of cottonwood twig borer. The seasonal abundance of cottonwood twig borer larvae was determined by counting the number of active attacks per tree and per lateral shoot weekly (Stewart and Payne 1975). Fifteen trees in each of three replications were marked and then monitored throughout the 1995 growing season. Because of increasing tree height (2.5 – 3.5 m) and canopy complexity after the seventh week of sampling, only 10 of the 15 trees per replicate were sampled during the remainder of the growing season. Seasonal abundance, as determined by the number of active attacks per selected tree, was not recorded in 1996. The number-of-active-hits data were analyzed using a three-way (sampling date, generation, replicate) ANOVA.

Beginning 29 July 1995, active attacks were classified further by recording the following designations: (1) presence or absence of frass at the entrance hole of an active attack, and (2) location of active attacks (terminal growth node, growth node, or internode). In 1996, active attacks were classified as in 1995; however, attacks by first instars in the midveins of leaves also were examined.

When active attack counts are recorded, a similar type of damage caused by the poplar branch borer, *Oberea schaumii* (LeConte) (Coleoptera: Cerambycidae) must be subtracted from the active attacks. Poplar branch borer holes are distinct from cottonwood twig borer active attacks because they can be found at the bases of petioles with scarring on the outside of the entrance hole, and with wood shavings instead of red frass.

RESULTS

Phenology of cottonwood twig borer. Similar to Morris' (1967) findings, five instars were detected in Iowa (Table 1). First instars had head capsules that varied in width from 0.16 to 0.22 mm compared with Morris (1967) who found a very nearly constant head capsule width of 0.19 mm. In addition, the range of head capsule widths for fifth instars was larger during both years of the study than in Mississippi. In Iowa, more variation (greater range) in head capsule width occurred during the fourth and fifth stadia than during the second and third stadia. This greater variation corresponded

Table 1.   Head capsule width of cottonwood twig borer larvae in Mississippi and Iowa and corresponding volume of feeding gallery.

Instar	Mississippi[*] (n)	Iowa 1995 Mean (SE)	Iowa 1995 Range (n)	Iowa 1996 Mean (SE)	Iowa 1996 Range (n)	Gallery volume (mm^3) 1996 (SE)
			Head Capsule Width (mm)			
1	0.19 (87)	0.19 (0.01)	0.16–0.22 (2)	0.20 (0.02)	0.16–0.22 (2)	—
2	0.26–0.32 (172)	0.31 (0.03)	0.26–0.35 (7)	0.32 (0.02)	0.28–0.35 (8)	4.13 (1.39)
3	0.38–0.45 (242)	0.43 (0.03)	0.37–0.47 (36)	0.43 (0.03)	0.37–0.47 (40)	10.56 (2.28)
4	0.51–0.83 (988)	0.68 (0.09)	0.49–0.81 (147)	0.67 (0.09)	0.48–0.80 (133)	34.86 (3.97)
5	0.90–1.15 (604)	0.98 (0.09)	0.82–1.18 (132)	0.97 (0.09)	0.81–1.18 (154)	68.37 (6.21)

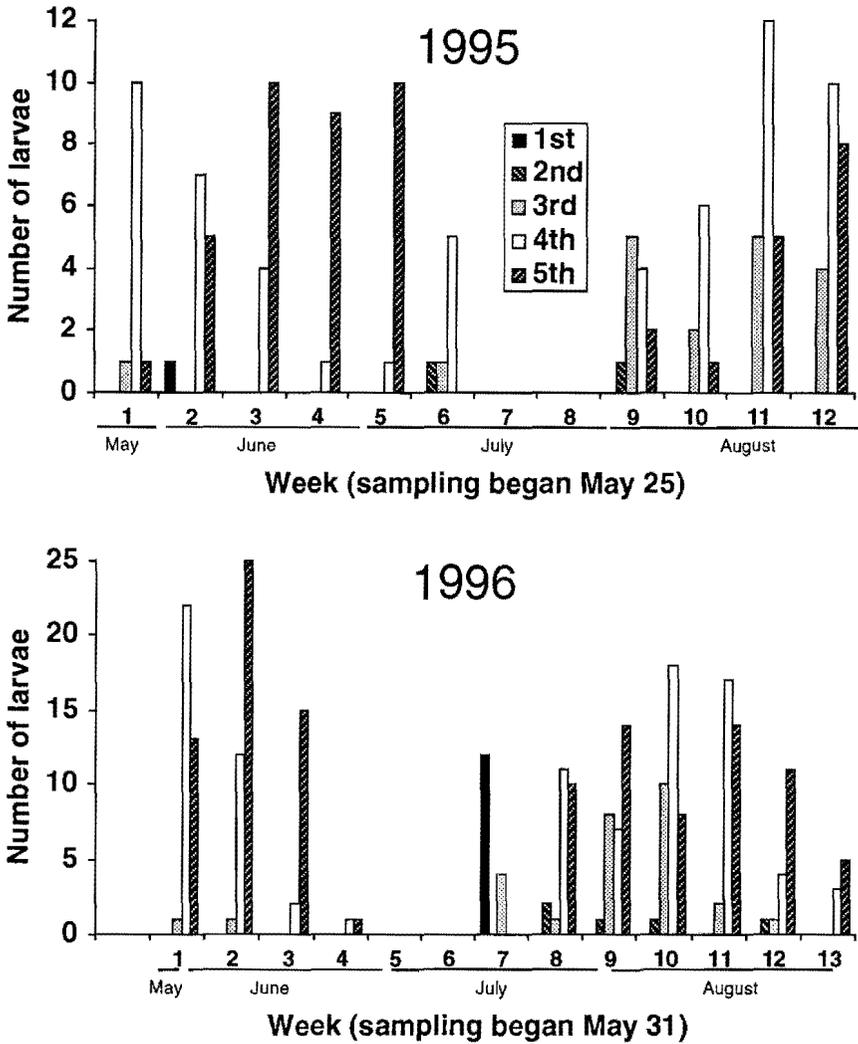*Data reported by Morris (1967).

Figure 1. Instar frequency of cottonwood twig borer by sampling date during the 1995 and 1996 growing seasons.

with the period of greatest increase in head capsule size of the developing larvae.

The mean width of head capsules varied by sampling date in both years (P < 0.001). Based on the measurements and frequencies of head capsule widths, there were two generations per year in Iowa (Fig. 1). The first larval

generation was completed by the end of June and the second larval generation occurred from July through August. Because a majority of larvae during week 1 were already in the fourth stadium (Fig. 1), second or third instars probably overwinter as in the southern United States (Stewart and Payne 1976). However, an inspection of shoots in February 1998 failed to find any hibernacula or larvae in dormant buds. Only one second instar was found and it occurred inside a hollowed area of the shoot, 20 cm below the terminal bud.

The volume of feeding gallery varied by instar (F ratio = 14.5, $P < 0.001$, df = 3). Regression analysis revealed a significant curvilinear relationship between the volume of feeding gallery (y) and head capsule width (x) (y = $4.08x^{2.78}$, d.f. = 236, F ratio = 200.3, $P < 0.001$, r^2 = 0.58, Table 1). Mean gallery volume also varied by sampling period in 1996 (F ratio = 6.8, $P < 0.001$, df = 10) with a greater volume of plant tissue being consumed during the early season generation (Fig. 2).

Infestation patterns of cottonwood twig borer. Corresponding to the two generations per year, monitoring of the previously marked trees during the 1995 growing season showed two peaks of active attacks (Fig. 3). Significant effects of sampling date, generation, and replication on the number of active hits were detected (Table 2). The mean number of active attacks was larger in generation 1 (4.76 ± 0.27 SE) than in generation 2 (2.90 ± 0.23). The peak for generation 1 occurred during week 3 and the peak for generation 2 occurred during week 11. A significant linear relationship was found between the number of active attacks (y) and the number of actively growing shoots
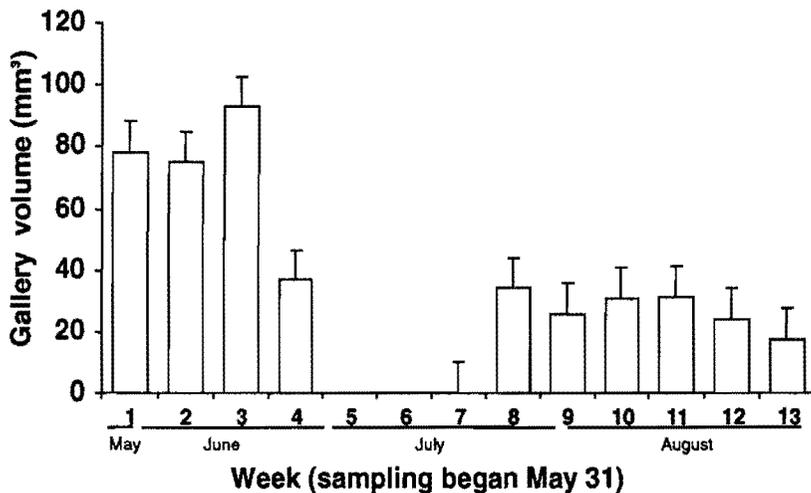

Figure 2. Feeding gallery volume (mm^3) of cottonwood twig borer larvae by sampling date in 1996. Bars represent mean + SE of the mean.

Table 2. Three-way ANOVA (sampling date × generation × replication) of the number of cottonwood twig borer active attacks per tree during the 1995 growing season.

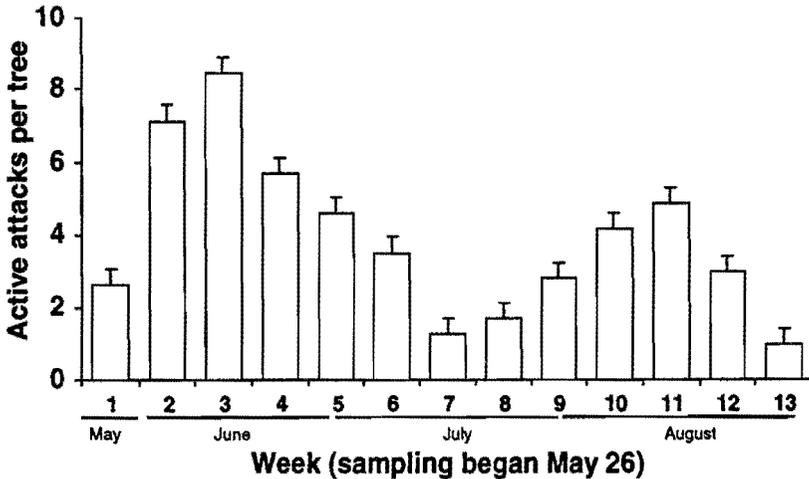| Source of variation	d.f.	F ratio	P value
Sampling date	11	13.5	< 0.001
Generation	1	28.4	< 0.001
Replicate	2	8.0	< 0.001

Figure 3. Number of cottonwood twig borer attacks per tree by sampling date during the 1995 growing season. Bars represent mean + SE of the mean.

(x) during week 7 in 1995 (y = -0.40 + 0.17x, d.f. = 43, F ratio = 23.50, P < 0.001, r^2 = 0.31). The significant difference in the number of active attacks among replicates was caused, in part, by a greater number of actively growing shoots in one of the three replicates.

In general, only one active attack was observed per infested shoot (Table 3). More than 80% of infested shoots contained only one active attack and less than 2% of infested shoots contained three or more active attacks. For shoots that had more than one attack, the percentage of active attacks that contained actively feeding larvae increased toward the apex of a shoot. Of the three active attacks closest to the terminal apex, feeding larvae were found in 97.1% of the first active attack, 2.6% in the second active attack, and 0.3% in the third. Greater than 90% of active attacks had frass at the entrance hole of the twig-boring larvae. Furthermore, more than 85% of active attacks housed only one larva per feeding gallery (Table 3).

Table 3. Frequency of active attacks per infested shoot for generation 2, 1995 and the frequency of cottonwood twig borer larvae per feeding gallery in 1995 and 1996.

No. of active attacks per infested shoot	Frequency (% SE)	No. of larvae per feeding gallery	Frequency (% SE)	
			1995	1996
1	83.1 (2.0)	1	93.6 (4.0)	88.2 (1.4)
2	12.9 (1.7)	2	5.4 (3.0)	11.1 (1.4)
3	2.1 (0.7)	3	1.0 (1.0)	0.7 (0.4)
4	0.9 (0.1)			
5	0.2 (0.2)			
6	0.5 (0.5)			

DISCUSSION

Phenology of cottonwood twig borer. The results of this study were consistent with Morris' (1967) measurements of head capsule widths and number of instars, suggesting that there is not much geographical variation in body size of cottonwood twig borer larvae. One plausible explanation for the greater range in the size of fifth instar head capsules in Iowa compared with Mississippi is the possibility that an additional instar may occur for female cottonwood twig borer. Although Morris (1967) did not report any evidence of an additional instar for females, this is a common phenomenon for many insects (Coulson and Witter 1984). More work is needed to determine the occurrence of sexual dimorphism during the larval stage or the cause of the greater range in body size of larvae in Iowa.

Two generations of cottonwood twig borer occur annually in central Iowa based on the weekly sampling during 1995 and 1996. The shorter growing season in Iowa results in fewer generations per year compared with the southern United States. Week 1 in 1995 and week 3 in 1996 were the peaks or the start of decline of the fourth instar from the overwintering generation; therefore, future observations should commence in early May. During the intergenerational period (weeks 7 and 8 of 1995 and weeks 5 and 6 of 1996), it was difficult to locate cottonwood twig borer larvae because first instars are in the midvein of the leaves. The peak of fourth and fifth instars by the third week of August suggests that the generation would be completed before the average first frost (September 22). After this generation deposits eggs and larval eclosion occurs, the larvae probably begin to feed and molt in leaf midveins before moving to an overwintering location as second instars. The absence of any hibernacula in Iowa suggests that, because of the colder winters in Iowa compared with Mississippi, cottonwood twig borer may have evolved to overwinter inside terminal shoots or possibly in the leaf litter around the base of trees on the ground.

The mean gallery volume increased in a curvilinear fashion from the second instar to the fifth instar demonstrating that the late instars consume proportionately more than the early instars. The volume consumed increased from weeks 1 to 3 as the mean stage of instar increased. During the second generation, volume of feeding galleries was considerably less than during the first generation. This difference may have been caused by the first generation larvae having a longer feeding period and/or because of the impact of grasshopper damage during the second generation. In both years, larval feeding during the second generation was affected by damage to actively growing shoots from grasshopper feeding (*Melanoplus femurrubrum* DeGeer

and *Melanoplus differentialis* Thomas) (Orthoptera: Acrididae). Large populations of grasshoppers appeared in mid-July and were observed feeding on actively growing shoots. This grasshopper-caused damage most probably had a direct impact on twig borer populations by killing larvae that were feeding inside the shoots or an indirect impact by reducing their food resources (e.g. fewer actively growing terminals). In addition, possible differences in the nutritional value of shoots throughout the growing season may have influenced the feeding of larvae.

Infestation patterns of cottonwood twig borer. The results of this study suggest that lower densities of cottonwood twig borer are found in central Iowa than in the southern United States. In 1995, only one active attack per infested shoot was found, and, in general, only one larva per feeding gallery was found in 1995 and 1996. These findings are in contrast to the southern United States where a 15-inch terminal contained up to 25 larvae and three different instars during late summer (Morris 1967). These differences between geographic regions were probably caused by the greater number of generations per year and a longer history of cottonwood plantations in the south. In addition, Lorsban® was applied in July 1995 for controlling grasshopper populations and may have caused a direct impact on the larvae or on the natural enemy complex (Woessner and Payne 1971). The combination of this insecticide application with the grasshopper-caused damage to actively growing shoots probably resulted in the observed lower number of attacks per shoot in the second generation. The positive correlation between active attacks and the number of actively growing shoots indicates that factors leading to increases or decreases in the number of actively growing terminals affects cottonwood twig borer populations.

ACKNOWLEDGMENTS

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