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TEMPERATURE DETERMINES DIAPAUSE TERMINATION IN *PAPILIO TROILUS* (LEPIDOPTERA: PAPILIONIDAE)

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ABSTRACT.– Diapausing pupae of the spicebush swallowtail butterfly, *Papilio troilus* Linnaeus, were individually set up in screened cages under one of 4 temperature and 2 photoperiod treatments (14°, 18°, 22°, or 26°; in 18:6 or 12:12 photo/scotophase). The breaking of diapause and eclosion of adult butterflies was significantly faster at 26°C (13-24 days) than 22°C (18-36 days) and 18°C (36-69 days) regardless of the photoperiod conditions. As of 88 days, none of the pupae eclosed as adults in either 14°C chamber (18:6 or 12:12). While diapause induction for these Ohio populations was known to be determined in the larval stages by a critical photoperiod of less than 15 hours, it did not matter whether the photophase was 12h or 18h for termination of diapause and eclosion of adults. In every treatment (n = 6), the majority of the first 10 individuals eclosed were males, suggesting that protandry is likely in the field.

KEY WORDS: biology, diapause termination, hostplants, Lauraceae, Michigan, Nearctic, North America, Ohio, photoperiod, pupal diapause, scotophase, spicebush swallowtail, temperature, USA.

The spicebush swallowtail, *Papilio troilus* Linnaeus, is distributed across the eastern half of the United States from Florida to central Wisconsin, northern Michigan and into New England (Scriber, 1996a). While red bay, *Persea borbonia* (L.) Spreng is the only host plant available in southern Florida, spicebush, *Lindera benzoin* (L.) Blume, and sassafras, *Sassafras albidum* (Nutt.) Nees are the preferred hosts northward to the Great Lakes region (Nitao *et al.*, 1991; Lederhouse *et al.*, 1992) (all Lauraceae). Swamp bay, *Persea palustris*, and the introduced camphor tree are also used in central Florida (M. Minno, pers. comm.).

While temperature, moisture and food quality are known to alter voltinism patterns (Beck, 1980; Tauber *et al.*, 1986; Danks, 1987; Leather *et al.*, 1993; Scriber, 1994; Hunter and McNeil, 1997), strong photoperiodic control of diapause in *Papilio troilus* has been demonstrated (Tidwell, 1995; Valella and Scriber, 1998). In Florida, the critical photophase for inducing diapause is about 12h, in northern Georgia it is about 14-14.5h, in southern Ohio it is 14.5-15h and in Michigan it is 16h. However, the spring diapause termination conditions are basically unknown for this butterfly. In this study, we examined potential temperature and photoperiod factors that could control diapause termination in *Papilio troilus*.

METHODS

In 1996, we reared larvae from field-captured females of *P. troilus* on sassafras in our laboratory at Michigan State University under controlled environmental conditions for diapause induction (12:12 photo:scotophase, 25°C). Individual pupae resulting from this rearing were weighed on a Mettler Analytical Balance and stored for the winter in separate petri dishes at 2-4°C in total darkness. All pupae were brown (no green pupae) under our rearing conditions (West and Hazel, 1985; Hazel, 1995).

In 1997, we brought out pupae (128 males and 128 females) and distributed them individually into screen cages in one of our 8 temperature/photoperiod treatments (14°, 18°, 22°, 26°C at either 12:12 or 18:6 photoperiod). Six different maternal broods were used with equal and random distribution across each of the 6 treatments for any maternal lineage. All pupae were misted with water daily to enhance eclosion. The source of these 6 females (12422, 12427, 12428, 12429, 12438, and 12439) was Lawrence County, Ohio. Upon adult eclosion, we measured the forewing lengths, determined the sex, and recorded the date for each individual.

RESULTS

After overwintering at 2-4°C in darkness, the emergence of adult *P. troilus* from diapausing pupae was fastest in the 26°C chamber (13-24 days) compared to 22°C (18-36 days) and 18°C (where the first eclosion was at 36 days and the last at 69 days). The results are virtually identical for the 2 photoperiod conditions at each temperature (Fig. 1). Of the first 10 (of 32) possible adults to eclose, males were in the majority in every treatment. In fact, all 12 of the first 12 adults were males in the 26°C (12:12) treatment.

After 2-1/2 months (88 days), we terminated the study. All pupae at 14°C (n = 32 in 18:6, n = 32 in 12:12 photoperiods) apparently remained in diapause. At that time there were also a few pupae that died or still had not eclosed as adults at the other 3 temperatures. These uneclosed pupae (of n = 32 original) were as follows: 6 in the 18°C (18:6), 3 in the 22°C (18:6), 1 in the 26°C (18:6), 5 in the 18°C (12:12), 3 in the 22°C (12:12), and none in the 26°C (12:12) treatments. Again, as with the times for eclosion, the results are virtually identical for the different photoperiods at any temperature. Also notable is that 14 of these 18 dead or greatly delayed eclosions were females.

After the 6-treatment experiment was terminated (88 days), we removed all of the pupae from both photoperiod chambers at 14°C and set them up at room temperature (24-27°C) and natural photoperiod (May 2) for East Lansing, Michigan. Adults began eclosing within one to three weeks in one family, but only after 6-7 weeks for all other families under these conditions. From pupae originally tested under the 18:6, 14°C conditions adults eclosed over a range of 88-151 total days. However, under 12:12, 14°C (original 88 days) they were constrained in eclosion over a total day range of 154-174 days. Many of these died in the process of eclosion or had crumpled wings and would be unable to fly.

DISCUSSION

Diapausing pupae eclose as adult *Papilio troilus* at times of 2-3 weeks at 26°C, 2.5-5 weeks at 22°C, and at 7-10 weeks at 18°C, re-

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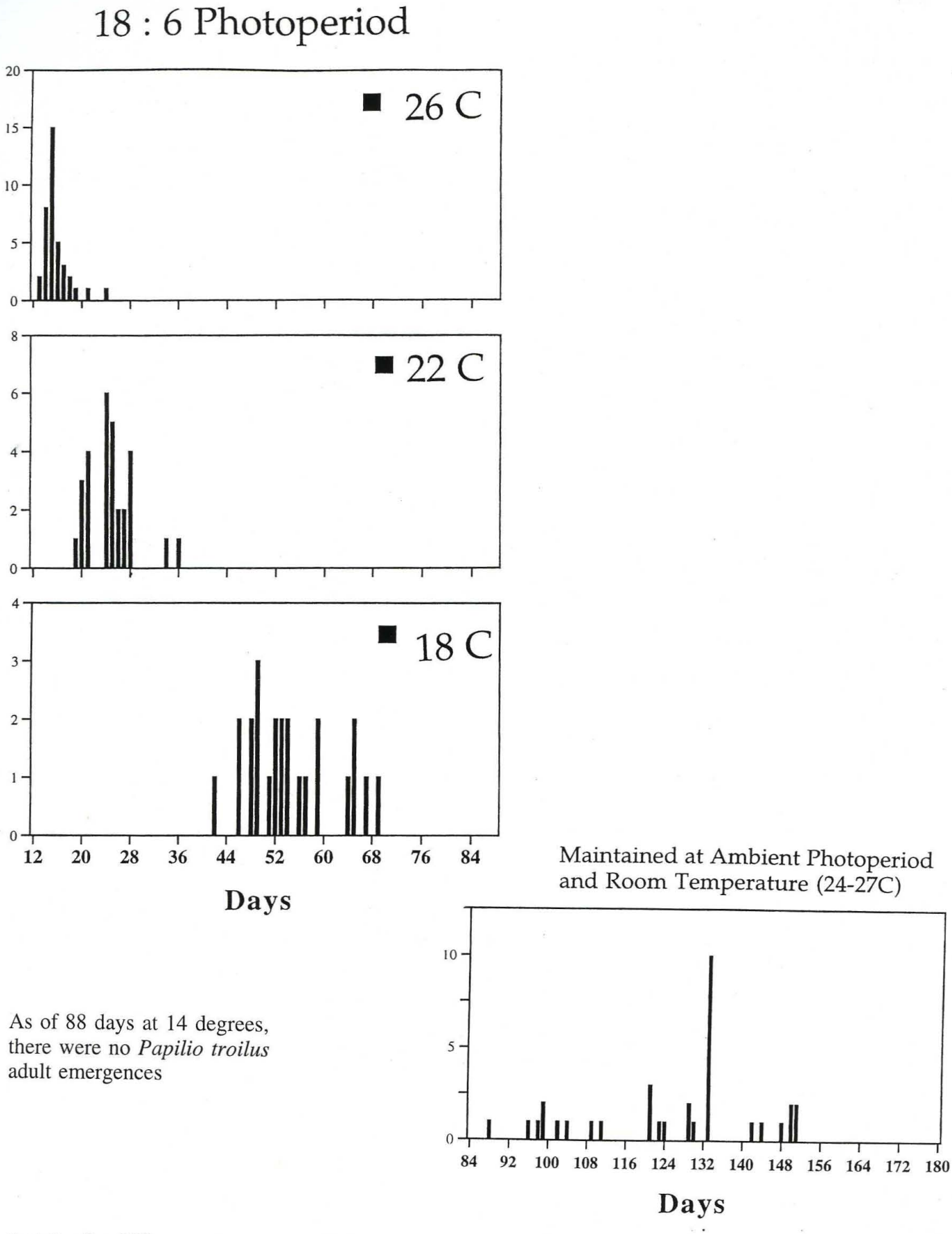
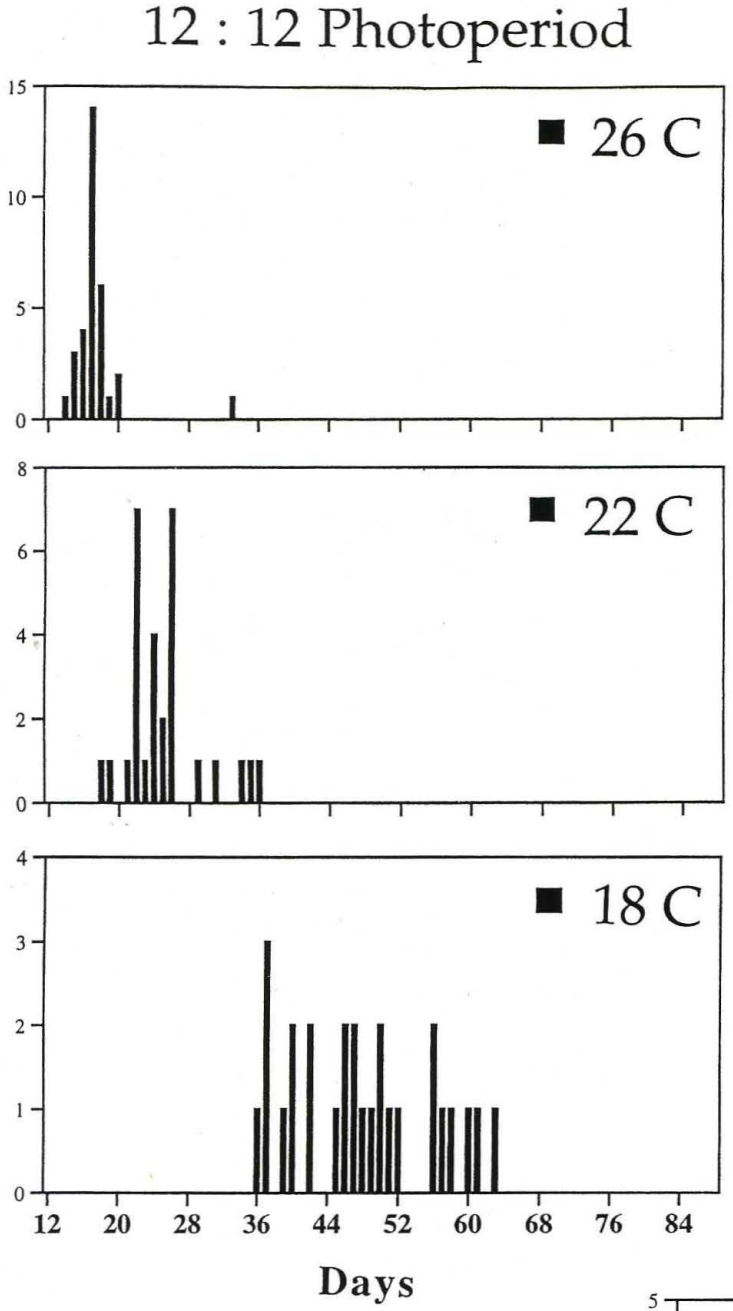


As of 88 days at 14 degrees, there were no *Papilio troilus* adult emergences

Fig. 1. The effect of different temperatures upon the adult eclosion of *Papilio troilus* individual diapausing pupae under photoperiods of (A) 18:6 and (B) 12:12.



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Maintained at Ambient Photoperiod and Room Temperature (24-27C)

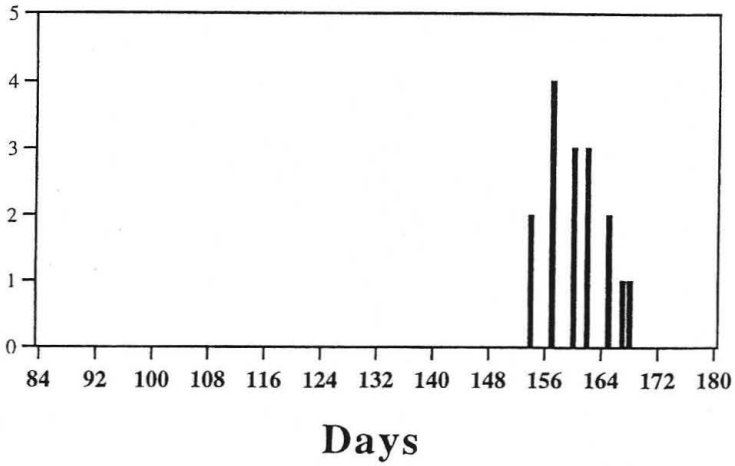

Table 1. Average number of days (± s.e.) to eclosion for *P. troilus* (male and female[1]) pupae from n = 5 or 6 different families (isofemale broods) reared under different photoperiods and temperature (East Lansing, MI 1997).

Temperature	Photoperiod			
	18:6		12:12	
	(n) Male	(n) Female	(n) Male	(n) Female
26°C	(6) 15.8 ± 0.5	(6) 15.9 ± 0.6	(6) 16.7 ± 0.4	(6) 19.7 ± 2.7
22°C	(5) 24.7 ± 0.6	(5) 25.6 ± 1.2	(5) 24.2 ± 0.9	(6) 25.6 ± 1.4
18°C	(6) 53.4 ± 2.0	(4) 54.2 ± 1.6	(5) 48.2 ± 2.5	(6) 51.9 ± 1.1
14°C*	(none emerged by day 88)		(none emerged by day 88)	
	(6) 132.7 ± 4.8	(5) 112.6 ± 4.9	(5) 158.4 ± 1.5	(4) 160.0 ± 2.2

(n) = The average time to adult eclosion for different *P. troilus* broods (the mean ± SE of family means).
* = After 88 days, the pupae from the 14°C treatments were removed from the chambers to room temperatures (24-27°C) for emergence.

1. None of the differences between the means for males and females were significant (t-test; P = 0.05) except for 14°C, 18:6 photoperiod.

gardless of whether this is under long (18:6) or short (12:12) photophases. Since photoperiod (and not temperature) seems to predominate for the induction of diapause in late summer (Tidwell, 1995; Valella and Scriber, 2005), it was surprising that temperate seems to regulate its termination. Since we did not investigate photoperiods of less than 12 hours in this study, we can not be sure of the minimum (or threshold) photoperiod required to initiate the breaking of the overwintering diapause state. However, other studies have suggested that temperature alone can induce the termination of Lepidoptera diapause (McLeod and Beck, 1963; Holtzer *et al.*, 1976).

The fact that at 88 days post-pupation, none of our pupae at 14°C had eclosed as adults suggests that they were below the threshold for diapause termination temperature. A separate study in Ohio, conducted by Jennifer Grim (1997; pers. comm.), found that *P. troilus* pupae held at 15°C all remained in diapause regardless of the photoperiod (16:8 or 8:16), while most of the pupae held at 28°C emerged from both photoperiods. It therefore seems that temperatures above 16°C but below 18°C may be the cue for breaking diapause in *P. troilus* from these Ohio latitudes (38-39°N). After 75 days when the 14°C pupae were moved to a warmer treatment (24-27°C), most of the pupae did eclose (within 21-76 additional days) which confirms that these pupae were in diapause and not dead. It is interesting that those pupae originally held at 12:12 photoperiod emerged synchronously, and after all pupae emerged from the 18:6 photoperiod treatment (Fig. 1-2). The combination of 14°C and 18:6 photoperiod apparently destabilized the synchrony of eclosion and the earlier (protandrous) emergence by males compared to females (Table 1).

It would be interesting to know what critical diapause termination factors are in effect further to the south. In south central Florida, for example, we have observed *P. troilus* in flight during all months except December and January (which is also possible). Such continuous flight potential suggests that winter diapause may not be occurring in these Highland County, Florida populations (Scriber *et al.*, 1998). However, Tidwell (1995) suggests that diapause is induced in Florida populations at photophases of 12 hours or less (see also Valella and Scriber, 2005). If 12:12 photoperiods didn't maintain diapause of Ohio *P. troilus* pupae as observed in this study, it is very unlikely they would do so for Florida populations. Hence, we would predict that southern Florida populations either do not enter a diapause, or perhaps may be induced by decline in hostplant quality during the winter. The only endemic hosts in this part of Florida are red bay and swamp bay, the leaves of both getting extremely "tough" in December and January. Tender new annual growth does not occur on these host plants until early March, which coincides with the large spring flight observed in this area (Scriber *et al.*, 1998). It is also possible that the Florida populations are more cued into rainfall (like some tropical butterflies) than are the Ohio populations we studied here.

The mating biology of *Papilio troilus* has not been studied directly; however, it is clear that the males are patrollers (Lederhouse, 1995). As such, the protandrous eclosion of adult males slightly before females would presumably serve to assure that females are quickly mated (Wiklund and Fagerström, 1977; Lederhouse *et al.*, 1982). In our results (Table 1), it is interesting that the average male in each of the 6 treatments (18°C, 22°C, and 26°C; 18:6 and 12:12 photoperiods) eclosed one to three days earlier than the corresponding female average. However, differences between male and female adult eclosions are statistically not significant, suggesting that protandry does not exit for these *P. troilus*. This was even observed after the removal from the 14°C treatment at 12:12 (158.4 versus 160.0 total days for males and females, respectively). It is important that males neither emerge very early or too late because they may have too few mating opportunities early or encounter small low-quality females later (Carvalho *et al.*, 1998).

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