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thesis entitled

LATITUDINAL COMPARISONS OF DIAPAUSE INDUCTION BETWEEN POPULATIONS OF PAPILIO GLAUCUS AND PAPILIO TROILUS (LEPIDOPTERA: PAPILIONIDAE)

presented by

Patricia A. Tidwell

has been accepted towards fulfillment of the requirements for

MS degree in Entomology

J. Mark Scriber

Major professor

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By

Patricia Ann Tidwell

A THESIS

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in partial fulfillment of the requirements
for the degree of

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1995

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ABSTRACT

LATITUDINAL COMPARISONS OF DIAPAUSE INDUCTION BETWEEN POPULATIONS OF PAPILIO GLAUCUS AND PAPILIO TROILUS (LEPIDOPTERA: PAPILIONIDAE)

By

Patricia Ann Tidwell

The multivoltine butterflies *Papilio glaucus* and *P. troilus* rely on seasonal environmental cues to avert or induce diapause. The most reliable seasonal cue in their range is thought to be photoperiod. Offspring of butterflies from different latitudinal populations were reared in the laboratory under varying photoperiods. Data were collected as to whether pupae did or did not diapause. The purpose of this research was to determine critical photoperiods for diapause and variation between these populations and to better understand diapause induction in these two species. Statistical analysis indicated that *P. glaucus* likely used a cue(s) in addition to photoperiod to induce diapause, and that *P. troilus* used photoperiodic cues to induce diapause. Understanding diapause dynamics can shed light on the evolution of a species and on adaptations to the local environment. Diapause dynamics can limit or allow wider distribution of a species by eliminating nondiapausing individuals during adverse conditions.

To Penny Tidwell, my mother-in-law, who told me,
"Never let anyone tell you, 'You can't do it.'"

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about stupid things. Thanks to my husband, Craig, for bringing me Happy Meals from McDonalds when I had to stay late, and for helping me feed butterflies when he wasn't harrassing the larvae. And a special thanks to Reneé Brenner for staying with me for three seasons through thousands and thousands of eggs, larvae, pupae and adults. She was a blessing without which I would not have been able to do this research on such a big scale.

"The significance of photoperiodic adaptations in the formation of physiologically different local races has also been demonstrated, enabling us to approach from a new angle the problems of understanding the first stages of intraspecific differentiation and of evaluating the possibilities of acclimatization and extending the distribution range of insects."

Danielevskii 1965

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INTRODUCTION

Preface

Diapause in insects is important because it gives insects the ability to withstand adverse conditions; it also synchronizes insect life cycles to the changing seasons. I investigated photoperiodic induction of diapause in two species of swallowtail butterflies, *Papilio glaucus* and *P. troilus*. Butterflies were collected from different latitudinal populations in the United States and their offspring were reared in the laboratory under varying photoperiods until pupation, the stage at which they diapause. Data were taken as to whether pupae diapaused or not, and used to determine the critical photoperiod for diapause.

This paper is divided into three chapters with the general introduction at the beginning. The first chapter describes diapause in *P. glaucus*, and the second describes the experiments with *P. troilus*. The third chapter is a discussion comparing and contrasting the two species of butterflies with respect to diapause induction.

Background

Many insects diapause to avoid adverse conditions (Lees 1955). Tauber et al. (1986) define diapause as

> a neurohormonally mediated, dynamic state of low metabolic activity. Associated with this are reduced morphogenesis, increased resistance to environmental extremes, and altered or reduced behavioral activity. Diapause occurs during a genetically determined stage(s) of metamorphosis, and its full expression develops in a species-specific manner, usually in response to a number of environmental stimuli

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Diapause should not be confused with "quiescence," a rapidly reversible state of suppressed metabolism, allowing insects to withstand aseasonal periods of stress. The environmental cues regulating diapause are called "token stimuli." Token stimuli are not themselves favorable or unfavorable for growth but indicate that a change in environmental conditions is forthcoming. The most widely used token stimulus is photoperiod (Tauber et al. 1986, Danks 1987). The critical photoperiod for diapause, as defined by Tauber et al. (1986), is the photoperiod which induces 50% of a population to enter diapause, given other constant conditions. The critical photoperiod may be changed due to factors such as different temperatures, host plant quality or moisture levels.

Diapause in insects can occur at various stages of development, but the stage is usually species-specific (Danilevskii 1965, Danks 1987). Most swallowtail butterflies (Lepidoptera: Papilionidae) in temperate regions overwinter as diapausing pupae, but *Parnassius* swallowtails overwinter as eggs (Tyler et al. 1994). Diapause in *Papilio* can be "obligate" and genetically determined, or it can be "facultative" and influenced by environmental factors (Scriber 1994). Thus the number of generations per year, or voltinism patterns, are genetically determined as well as environmentally cued (Slansky 1974, Scriber and Slansky 1981, Scriber and Lederhouse 1992). The genotype can determine whether a population is univoltine or multivoltine, but voltinism can be altered by environmental factors such as temperature, moisture, food quality, or day length (Beck 1980, Danks 1987).

The onset of diapause in insects can occur during the sensitive developmental stage when a critical environmental stimulus(i) is experienced (Eizaguirre et al. 1994). However, Tauber et al. (1986) have shown that in

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that insects can detect changes in the environment long before the diapause stage and can store this information. Some species can integrate different types of stimuli to determine when to enter diapause. The Mediterranean corn stalk borer, *Sesamia nonagrioides* (Noctuidae), which spends most of its larval development inside plant tissue, integrates both photoperiod and thermoperiod cues before diapausing (Eizaguirre et al. 1994). The anise swallowtail, *Papilio zelicaon*, can also use more than one cue. It has both univoltine and multivoltine populations, which occur respectively in ephemeral habitats or in more stable environments (Sims 1983). The incidence of diapause in *P. zelicaon* can be modified by temperature and host plant species (Sims 1980).

Diapause and Voltinism

At any given location, the maximum number of generations per season depends on environmental conditions such as thermal unit accumulations (season length) and host plant quality (Scriber and Lederhouse 1992, Scriber 1994). If the host plant quality declines after one brood and the growing season is short, the butterflies in that area are probably univoltine and will diapause after one generation. If food plant quality remains high for many months and the thermal accumulations are sufficient, then the butterflies in that area will be able to produce at least two generations before going into diapause. This is the case for *P. zelicaon* in habitats with a more permanent host species.

Diapause in other *Papilio*

P. demoleus:

Insects use different cues as indicators of season, depending on the biome which they inhabit. Ishii (1987) showed that for tropical species such as *Papilio demoleus*, seasonal changes in temperature trigger diapause. When larvae reared at 14:10 (L:D cycle) were compared to larvae reared at 10:14 at the same temperature, there were no diapause differences. When he investigated different temperatures, all larvae reared at 25°C direct-developed, while 66% of the larvae reared at 20°C went into diapause. This shows that *P. demoleus* relies partially on temperature as opposed to a change in day length as a cue to seasonal change.

In tropical regions, seasonal change in day length is nominal and may be inadequate as an indicator of season (Figure 1) (Beck 1980). In temperate regions however, day length is a reliable seasonal cue, and photoperiod plays a major role in diapause induction in insects occurring there (Andrewartha 1952, Danilevskii 1961, Beck 1980, Saunders 1982). Temperature (thermoperiod) may also play a role in season determination in temperate environments, but it may not be an accurate indicator because of warm spells in winter or cold spells in summer.

P. canadensis:

Papilio canadensis R & J, the northern tiger swallowtail butterfly, occurs in northern latitudes from 42° to 45° from Minnesota to New England and northward. It is an "obligate" diapauser having only one generation per season. The growing season in these regions is very short and host plant quality can usually only support one generation per season. *P. canadensis* is univoltine for all photoperiods (Scriber 1982, Scriber and Lederhouse 1983, Rockey et al. 1987a, Scriber and Lederhouse 1992). Even when larvae were

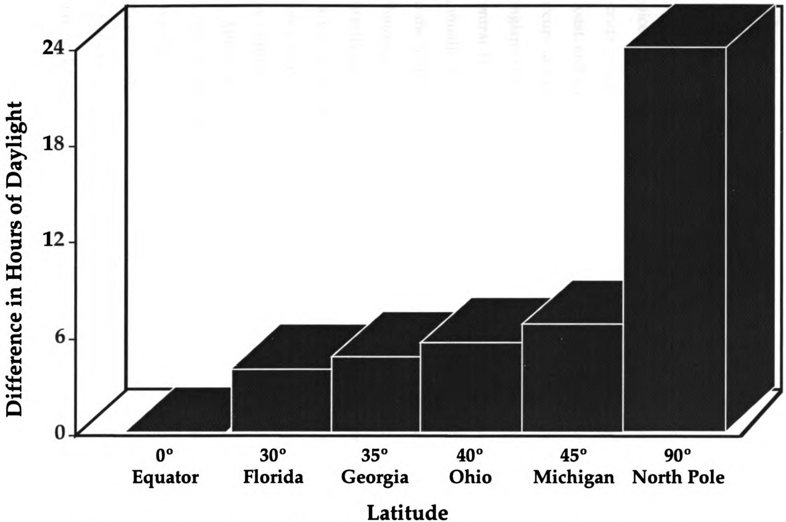

Figure 1. Difference in Hours of Daylight between the longest and shortest days, vs. N° Latitude. Twilight is not included.

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reared under 24h of light in the laboratory, almost 100% of the pupae diapaused (Rockey et al. 1987a, b). They also showed that in the *P. glaucus* group, diapause behavior was a sex-linked gene and inherited from the male (in butterflies, females are heterogametic).

P. glaucus* and *P. troilus

Papilio glaucus L., the eastern tiger swallowtail, and *Papilio troilus* L., the spicebush swallowtail, are two multivoltine species of butterflies. *P. glaucus* occurs in eastern North America from southern Ontario south to the Gulf Coast, and west to eastern Colorado and central Texas (Figure 2). *P. troilus* occurs in eastern North America from southern Ontario and southern New England south to Florida and the Gulf Coast, and west to Oklahoma and central Texas (Figure 2) (Opler 1992). Each has three or more generations annually in the southern-most portions of their ranges and two generations in the northern-most portion of their ranges (Scriber and Lederhouse 1992). Photoperiod experienced during larval development cues pupae to develop directly into adults or diapause until the next season (Tauber et al. 1986). Larvae reared under short day conditions generally go into diapause when they pupate, whereas larvae reared under long day conditions direct-develop into adults.

Much of the range and many of the flight periods of *P. glaucus* and *P. troilus* overlap, which prompted my interest in comparing these two species. I wanted to know how these two closely related species respond to the same photoperiodic conditions, given that they are located in the same areas and fly at the same time. Results from a preliminary experiment indicated that there may be differences between these two species with respect to diapause.

Data were collected to determine the critical photoperiod for diapause in

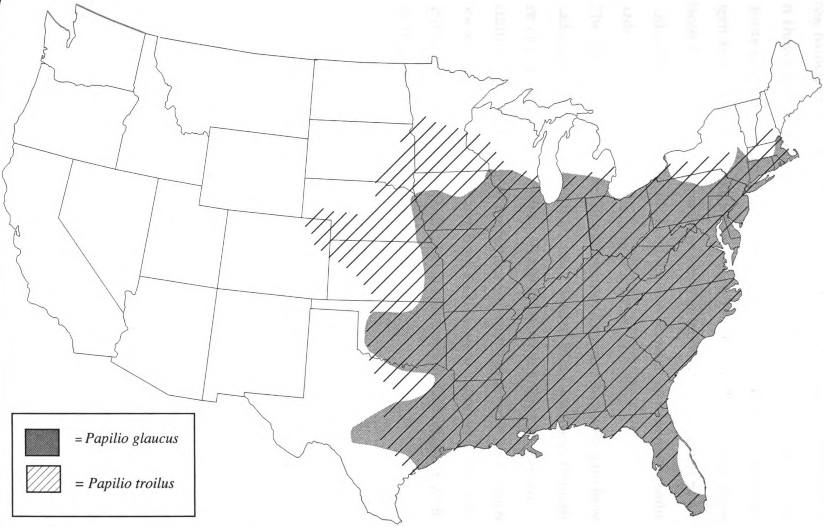

Figure 2. Range of *Papilio glaucus* and *Papilio troilus*.

populations of both species occurring at different latitudes in the United States, namely from Florida, Georgia, Ohio, and Michigan (Figure 3). It has been shown that at a 16:8 photo:scotophase, a decline in direct development (ie. more diapause) occurs in populations of *P. glaucus* at higher latitudes (Hagen and Lederhouse 1985, Rockey et al. 1987a), and this research allowed for both 1) intraspecific comparisons between populations at different latitudes and for 2) interspecific comparisons between species at the same latitude.

The above locations were chosen because I wanted to investigate how latitude affects diapause induction, and I needed to do a transect through the range of *P. glaucus* and *P. troilus* . These areas were known to contain populations of both species of butterflies and could also serve as a recurrent source of butterflies. Populations from Florida were included to serve as a comparison between populations from a sub-tropical environment with those from a temperate environment.

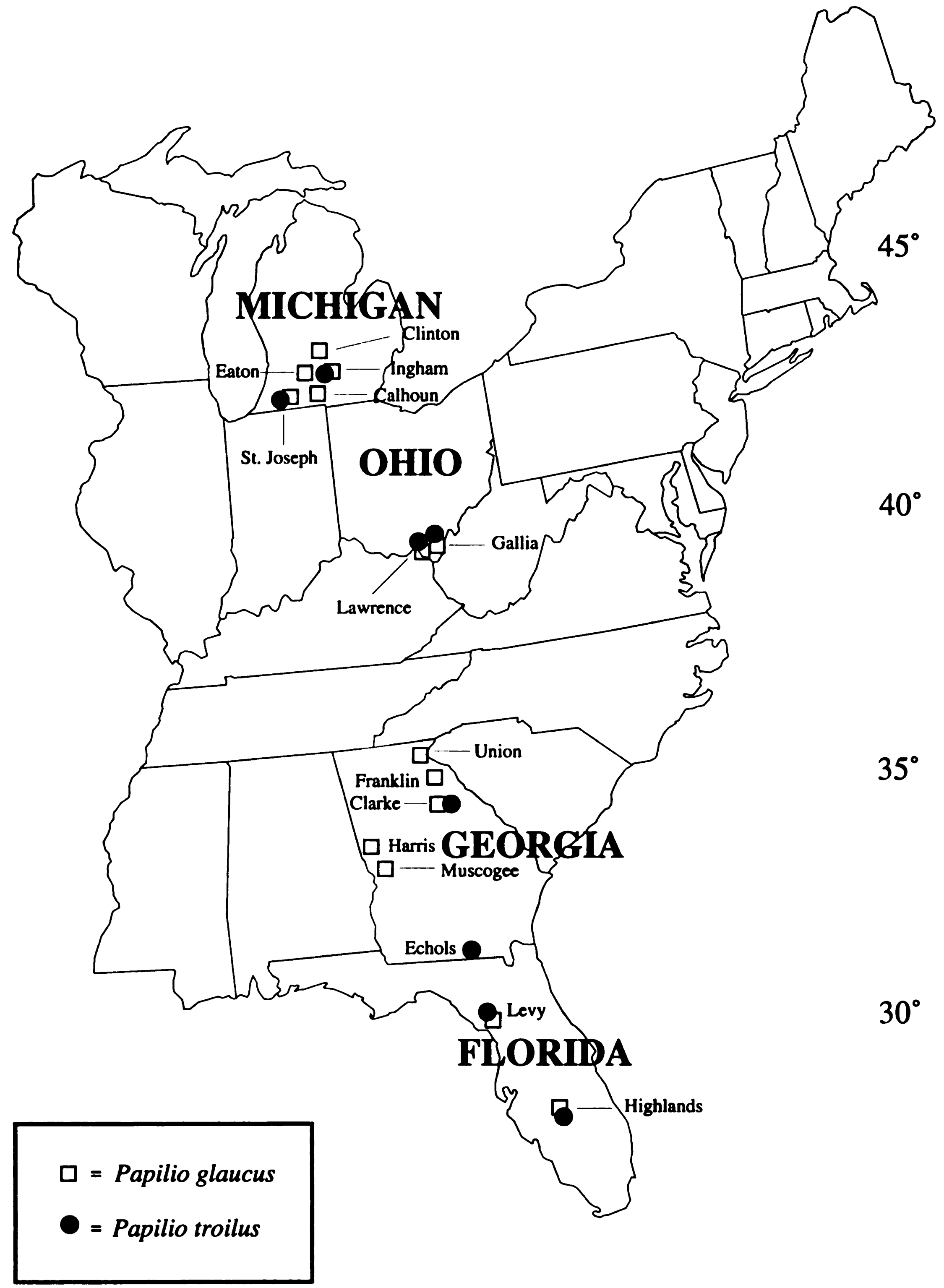

Figure 3. Counties where butterflies were collected from the wild.

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CHAPTER 1

Photoperiodic Induction of Diapause in Populations of *Papilio glaucus* Occurring at Different Latitudes

Abstract

Facultative diapause in *P. glaucus* is induced by seasonal environmental cues. The most reliable seasonal cue in temperate regions is day length. Females were collected from Florida, Georgia, Ohio and Michigan and their offspring were reared in controlled environments with constant temperatures, at different photoperiods. The purpose of this research was to determine the extent of differences among populations in the critical photoperiod for diapause and to better understand diapause induction in this species. The critical photoperiod for diapause for the Florida population appeared to be between 12.0h and 12.5h of light. For the Georgia population, the critical photoperiod appeared to be between 14.5h and 15h of light. The critical photoperiod for the Ohio population was different for the two years tested, but when averaged together the critical photoperiod was between 14.5h and 15.0h of light. For the Michigan population, the critical photoperiod was at least 18h of light, even though Michigan larvae never experience 18h days. The R^2 values obtained from the logistic regression suggest that *P. glaucus* used a cue(s) in addition to photoperiod to induce diapause. These data help explain the diapause dynamics of this species which can shed light on adaptations to local environments and on the evolution of this species.

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Introduction

P. glaucus is a facultative diapauser, that relies on environmental cues to determine the season (Scriber 1985, Lederhouse et al. 1995). *P. glaucus* is a multivoltine species, producing one to three generations annually depending on the environmental conditions and host plant quality. Photoperiod is the major seasonal indicator for larvae of temperate butterflies that overwinter as pupae (Tauber et al. 1986). The purpose of this research was to determine the critical photoperiod that induced diapause in populations of *P. glaucus* occurring in Florida, Georgia, Ohio and Michigan, and to determine if there are any differences between these populations.

Materials and Methods

Florida Population, 1993

In March, female butterflies were collected from Highlands and Levy Counties, Florida (Figure 3) and shipped via Federal Express or brought back to Michigan in a cooler. Females were allowed to oviposit in the laboratory and were fed a 1:4 honey:water solution. See Scriber, 1993 for oviposition methods. Eggs were collected daily, put in a petri dish and placed in an 18°C chamber, with a photoperiod of 18:6 (L:D). Eggs hatched in five to seven days. Larvae were reared in controlled environmental Percival® chambers with four 40 watt fluorescent bulbs at a constant 25°C. The neonates were set up in 150 mm wide x 25 mm deep petri dishes, one per dish, with foliage from the field. The nine photoperiod treatments were as follows: 12:12 (L:D), 13:11, 13.5:10.5, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8, and 18:6. Twelve family lines were set up, for a total of 490 larvae, which were set up between 3 - 7 April. Family lines were followed throughout the experiment.

P. glaucus larvae were reared on sweet bay (*Magnolia virginiana*) collected in Florida and shipped to Michigan via Federal Express or brought back to Michigan in a cooler. Leaves were placed in aquapics (rubber-capped, water-filled vials) to maintain turgor. Fresh leaves were provided 1-2 times per week for early instars and 2-3 times per week for later instars, until pupation. Pupae were weighed one day after pupation and then placed in individual petri dishes with a 175 mm high x 125 mm wide screen cylinder, returned to the same treatment chamber as they were reared in and checked daily for emergence. Pupae that develop directly take about 14 days to eclose. After six weeks live pupae were assumed to be in diapause and dead pupae were discarded. Pupae were considered to be alive if the abdomen was still flexible. All butterflies, eggs, larvae and pupae in subsequent experiments were treated similarly unless otherwise stated.

Florida Population, 1994

Females were collected from Highlands and Levy counties, Florida in March. The first instars were set up across six treatments with five siblings per dish instead of one per dish to increase efficiency of space utilization. Each female's offspring were randomly assigned as done in the previous experiment to one of six treatments, and reared at 25°C. One to two dishes were set up per female. After 10d, larvae were placed into individual dishes to complete their development. The six photoperiod treatments tested were as follows: 8:16 (L:D), 9:15, 10:14, 11:13, 12:12, and 13:11. These photoperiods were selected because in Florida, the longest day is about 14.5h (Beck, 1980), so all of the treatments used in 1993 were "long" days to the larvae except the 12:12 and 13:11 treatments. The modified photoperiodic treatments more closely match and bracket what a Florida larva would experience (Table 1).

Table 1. Larval development periods of *P. glaucus* and *P. troilus* in the wild, based on flight periods of adults. These dates are approximate due to annual variation in the accumulation of degree days.

Dates	Range in Photoperiod[1]	Change in daylength[2]
FLORIDA (27-29 N° Latitude)		
March 25 - April 30	12.2 -13.2 h	increase 1 h
May 10 - June 15	13.60 - 14.05 h	increase 27 min
June 25 - July 30	14.06 - 14.05 h	decrease < 1 min
Aug. 10 - Sept. 15	13.3 - 12.3 h	decrease 1 h
GEORGIA[3] (33 N° Latitude)		
Aug. 5 - Sept. 10	13.7 - 12.6 h	decrease 1 h 6 min
Aug. 20 - Sept. 25	13.30 - 12.03 h	decrease 1 h 16 min
Sept. 10 - Oct. 15	12.6 - 11.3 h	decrease 1 h 14 min
OHIO[3] (38 N° Latitude)		
June 5 - July 10	14.87 - 14.80 h	decrease 4 min
July 30 - Sept. 5	14.23 - 12.86 h	decrease 1 h 22 min
Aug. 10 - Sept. 15	13.90 - 12.48 h	decrease 1 h 25 min
MICHIGAN (41-42 N° Latitude)		
May 25 - June 30	15.18 - 15.57 h	increase 23 min
July 25 - Aug. 30	14.48 - 13.25 h	decrease 1 h 37 min
Aug. 20 - Sept. 25	13.84 - 12.00 h	decrease 1 h 50 min

1 Range given in hours of light.
2 Daylength does not include twilight.
3 There may be more broods in Georgia and Ohio, but they were not included.

Ten different family lines were set up between 5 - 11 April for a total of 479 larvae.

Florida Population, 1995

Butterflies were collected from the same counties as previous years and allowed to oviposit in the laboratory. Larvae were set up in petri dishes with three to five siblings per dish and one to four dishes per female per treatment. Larvae were place into individual dishes at 10d to complete development. A total of 682 larvae in fifteen family lines were set up between 4 - 12 April. Results from the previous two experiments (in 1993 and 1994) suggested that the critical photoperiod for diapause was likely to be between 12h and 13h of light; therefore a 12.5:11.5 treatment was added and the treatments were modified to the following five: 10:14(L:D), 11:13, 12:12, 12.5:11.5, and 13:11. Each female's offspring were randomly assigned as done in previous experiments. Those pupae that did not eclose within six weeks were considered to be in diapause.

Georgia, Ohio and Michigan Populations, 1993

Butterflies were collected from Clarke County, Georgia; Gallia and Lawrence Counties, Ohio; and St. Joseph and Clinton Counties, Michigan in July 1993 (Figure 3). The offspring from twenty-three different females were set up for a total of 973 larvae. First instar siblings were set up two to five per dish, one to three dishes per female per treatment, and at 10d larvae were subsequently placed into individual dishes. Each dish was randomly assigned to one of the nine treatments so that each female line was represented in each treatment. The treatments were: 12:12 (L:D), 13:11, 13.5:11.5, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8 and 18:6. Larvae were reared to pupation on black cherry

(*Prunus serotina*), collected in Michigan. Black cherry was used because sweet bay is not available to northern populations of *P. glaucus*. Georgia offspring were set up between 19 - 22 August, Ohio offspring set up between 11 - 13 August, and Michigan larvae were set up between 10 - 19 August.

Georgia, Ohio and Michigan Populations, 1994

Butterflies were collected from Clarke County, Georgia; from Gallia and Lawrence Counties, Ohio; and from St. Joseph, Eaton, Calhoun, and Ingham Counties, Michigan in July. There was also one female butterfly used from each of Columbus, Franklin, Harris, and Union Counties, Georgia. Offspring were randomly assigned to treatments as described above. The treatments were as follows: 12:12 (L:D), 13:11, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8, and 18:6. Larvae were reared to pupation on black cherry. Twenty-eight different family lines were set up across the eight photoperiod treatments for a total of 1,948 larvae. Georgia offspring were set up between 9 - 12 August and 16 - 22 August. Ohio neonates were set up between 7 - 10 August and 17 - 20 August. Michigan larvae were set up between 31 July and 8 August. Larvae and pupae were treated as in other experiments.

Statistical Analysis

Data were analyzed using logistic regression analysis. Analysis was done by testing percent diapause versus photoperiod for each state (with all years lumped together). R^2 values were used to determine how fit the model was for predicting diapause induction, where 1 is a perfect fit and 0 means there is no gain by using the model (JMP 1995).

Results

The following results should be interpreted with caution. High mortality rates and possible interactions with pathogens may have biased the results.

Florida Population, 1993, 1994 and 1995

In 1993, 50% of the pupae diapaused when reared at 12h of light; only 11% of the pupae reared at 13h diapaused. At 13.5h of light and longer all pupae direct developed into adults except for 20% (n= 1/5) at 15h of light (Figure 4, Table 2).

In 1994, when larvae were reared at shorter day lengths, 25% of the pupae in the 13h treatment diapaused and 60% diapaused in the 12h treatment (Figure 5, Table 3). Percent diapause increased from 60% to 80% with a decrease in day length from 11h to 8h of light, respectively. In no treatment was there 100% diapause for Florida butterflies for either year. Therefore, the critical photoperiod for diapause for these experiments appeared to be less than 13h of light.

In 1995, the critical photoperiod was more closely approximated and appeared to be between 12.0h and 12.5h of light. Percent diapause decreased from 70% at 12.0h to 0% at 12.5h of light (Figure 6, Table 4).

Georgia, Ohio, and Michigan Populations, 1993 and 1994

In the 1993 Georgia population experiment, at 16h of light, 50% (n= 1/2) of the pupae diapaused. No pupae diapaused at 15.5h and only 14% diapaused at 15h of light (Figure 7, Table 5). At 14.5h of light, percent diapause went up to 67%; the critical photoperiod appears to be between 15h and 14.5h of light. In 1994, the Georgia population responded with a low incidence of diapause at 18h, 16h and 15h, although 75% diapaused (n= 3/4) at 15.5h. Diapause

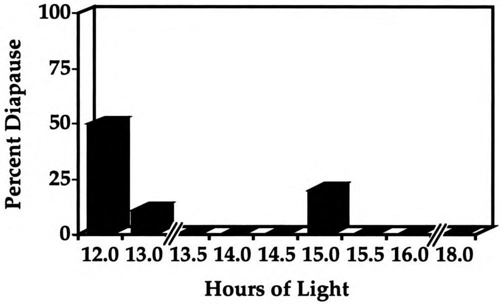

Figure 4. *Papilio glaucus*, Florida Population, Spring 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 7, range in number of pupae= 4-12.

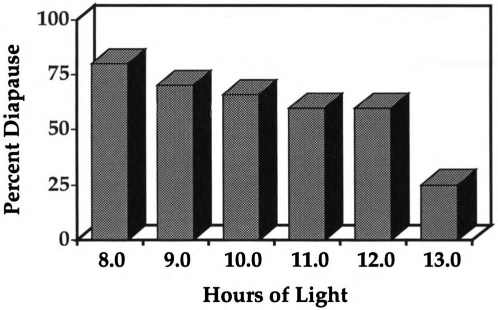

Figure 5. *Papilio glaucus*, Florida Population, Spring 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 7, range in number of pupae= 4-10.

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Table 2. *Papilio glaucus*, Florida Population, Spring, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	40	40	38	38	41	39	39	39	39
9701	---	---	---	0/1	0/2	0/1	---	---	---
9703	---	0/1	---	---	---	---	---	---	---
9718	2/4	0/1	0/3	0/4	0/3	1/3	0/3	0/2	0/3
9719	0/2	0/2	---	0/2	0/1	0/1	0/3	0/3	0/1
9720	2/2	1/2	---	0/2	0/2	---	0/1	0/2	0/1
9744	---	0/2	---	0/2	0/1	---	---	---	---
9751	---	0/1	0/1	0/1	---	---	---	---	---
Total	**4/8**	**1/9**	**0/4**	**0/12**	**0/9**	**1/5**	**0/7**	**0/7**	**0/5**
%Diapause	50	89	0	0	0	20	0	0	0

Table 3. *Papilio glaucus*, Florida Population, Spring, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase					
Mother #	**8.0**	**9.0**	**10.0**	**11.0**	**12.0**	**13.0**
# neonates	35	37	39	38	39	43
10318	1/1	2/2	3/3	1/1	---	1/1
10323	---	---	---	---	---	0/1
10327	4/4	3/3	2/3	2/3	2/4	---
10329	---	---	---	---	---	0/1
10335	3/5	2/5	1/3	0/1	1/1	0/1
Total	**8/10**	**7/10**	**6/9**	**3/5**	**3/5**	**1/4**
%Diapause	80	70	67	60	60	75

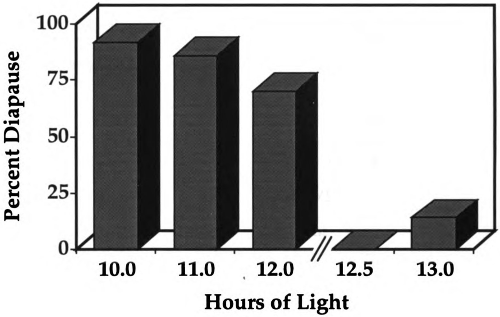

Figure 6. *Papilio glaucus*, Florida Population, Spring 1995.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 12, range in number of pupae= 8-16.

Table 4.
in diapa

Table 4. *Papilio glaucus*, Florida Population, Spring, 1995; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase				
Mother #	**10.0**	**11.0**	**12.0**	**12.5**	**13.0**
# neonates	129	122	111	118	126
11004	---	1/3	---	0/3	1/3
11046	---	4/4	---	---	---
11048	1/1	---	---	---	0/4
11053	5/5	1/1	---	0/1	0/3
11064	2/2	---	3/6	0/3	0/1
11065	1/1	---	1/1	---	---
11066	---	---	---	0/1	1/3
11070	---	1/1	1/1	---	---
11097	---	3/3	1/1	---	---
11099	---	1/1	---	---	---
11104	1/2	---	1/1	---	---
11106	2/2	1/1	---	---	---
Total	**12/13**	**12/14**	**7/10**	**0/8**	**2/14**
%Diapause	92	86	70	0	14

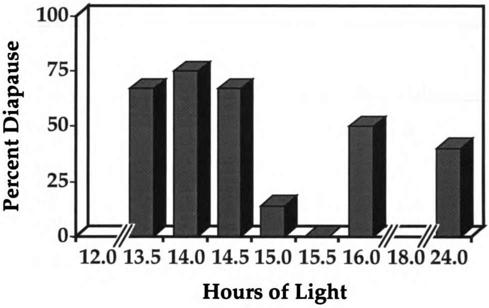

Figure 7. *Papilio glaucus*, Georgia Population, Late Summer 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 4, range in number of pupae= 1-7.

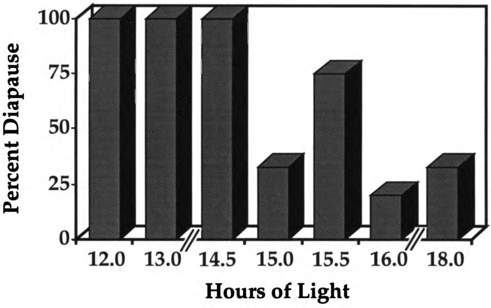

Figure 8. *Papilio glaucus*, Georgia Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 5, range in number of pupae= 2-9.

Table 5. *Papilio glaucus*, Gerogia Population, Late Summer, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**24.0**
# neonates	22	15	19	25	25	30	26
10021	1/1	---	---	---	---	---	1/1
10025	1/1	3/3	1/1	0/3	---	0/1	---
10026	0/1	---	---	0/1	---	---	---
10027	---	0/1	0/1	0/2	0/1	1/1	1/3
10029	---	---	1/1	1/1	---	---	0/1
Total	**2/3**	**3/4**	**2/3**	**1/7**	**0/41**	**1/0**	**2/5**
%Diapause	67	75	67	14	0	50	40

Table 6. *Papilio glaucus*, Georgia Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**12.0**	**13.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	100	101	100	99	99	99	101
10727	---	---	---	---	---	---	0/1
10728	1/1	3/3	---	0/2	---	0/2	0/1
10730	---	---	---	---	---	---	1/2
10731	1/1	---	1/1	0/1	---	---	0/3
10737	---	---	---	---	---	---	1/1
10757	1/1	---	1/1	1/1	1/2	0/2	---
10760	---	---	---	0/1	1/1	---	---
10779	---	---	---	---	1/1	---	---
10787	---	1/1	---	---	---	---	---
10790	---	---	---	1/1	---	---	---
10799	1/1	---	---	---	---	1/1	1/1
Total	**4/4**	**4/4**	**2/2**	**2/6**	**3/4**	**1/5**	**3/9**
%Diapause	100	100	100	33	75	20	33

increased from 33% at 15h to 100% at 14.5h of light (Figure 8, Table 6). There appears to be no difference in the critical photoperiod between 1993 and 1994 for the Georgia population.

In the 1993 Ohio population, the the critical photoperiod was between 15h and 14.5h of light, same as for the Georgia population; diapause at the longer photoperiods ranged from 14% to 36% and increased up to 100% at 13.5h of light (Figure 9, Table 7). In the 1994 Ohio population the long days 16h and 18h induced 60% and 50% diapause respectively; although only 17% diapaused under the 15.5h photoperiod (Figure 10, Table 8). Many of these larvae died of disease, with only about 5% survival.

For the Michigan population, diapause was induced in at least 55% of the larvae reared at photoperiods as long as 18h in both years (Figures 11 and 12, Tables 9 and 10). Only in the 24h photoperiod was there no diapause. (The 13:11 treatment was actually a 24:00 treatment due to a malfunction with the lighting dial, which was not discovered until most of the pupae direct developed where diapause was expected.) In 1993, 55% of the pupae diapaused under 18h, while 80% diapaused in 1994. In addition, percent diapause increased from 60% at 15.5h to 94% at 15h of light in 1993. 100% diapause occurred under photophases shorter than 14.5h of light in 1993. Percent diapause was at 100% from the 16h photophase down to the 12h photophase, in 1994.

There appears to be an absolute photoperiod, for the populations north of Florida, where all pupae will diapause, which was at 13h days or shorter (Figure 13).

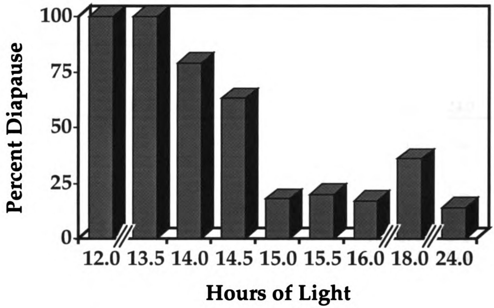

Figure 9. *Papilio glaucus*, Ohio Population, Late Summer 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 17, range in number of pupae= 11-22.

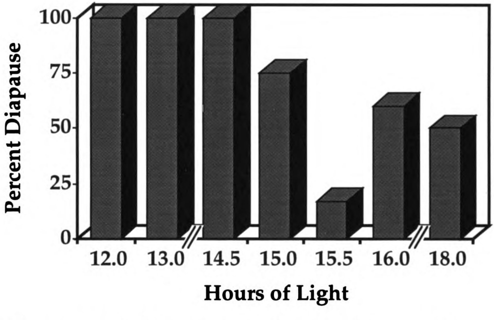

Figure 10. *Papilio glaucus*, Ohio Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 4, range in number of pupae= 1-10.

Table 7. *Papilio glaucus*, Ohio Population, Late Summer, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**	**24.0**
# neonates	45	42	48	48	44	48	44	43	44
9953	2/2	2/2	1/1	0/1	0/1	1/2	0/3	0/1	---
9960	---	---	1/1	2/2	---	1/3	---	---	---
9965	2/2	1/1	2/2	2/2	1/1	2/3	3/3	2/3	3/3
9967	2/2	---	1/3	1/1	1/3	0/1	0/2	---	0/2
9968	1/1	3/3	2/3	1/1	0/3	0/3	0/1	0/1	0/5
9972	3/3	6/6	3/3	0/2	---	0/3	0/4	0/2	0/4
9974	4/4	1/1	---	2/2	1/4	---	---	0/1	0/3
9975	---	---	1/2	0/2	0/1	---	---	---	0/1
9976	2/2	4/4	4/4	2/3	0/4	0/6	0/5	2/3	0/4
Total	**16/16**	**17/17**	**15/19**	**10/16**	**3/17**	**4/21**	**3/18**	**4/11**	**3/22**
%Diapause	100	100	79	63	18	20	17	36	14

Table 8. *Papilio glaucus*, Ohio Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**12.0**	**13.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	87	95	86	90	87	90	85
10705	---	---	---	---	0/1	---	---
10708	---	---	---	1/1	---	---	---
10710	---	---	---	---	1/1	2/3	---
10711	2/2	---	---	1/2	0/1	---	1/1
10713	4/4	1/1	---	---	0/1	1/2	0/1
10717	1/1	1/1	1/1	1/1	---	---	---
10747	1/1	---	---	---	0/1	---	---
10748	1/1	---	---	---	0/1	---	---
10750	---	1/1	---	---	---	---	---
10758	1/1	---	---	---	---	---	---
Total	**10/10**	**3/3**	**1/1**	**3/4**	**1/6**	**3/5**	**1/2**
%Diapause	100	100	100	75	17	60	50

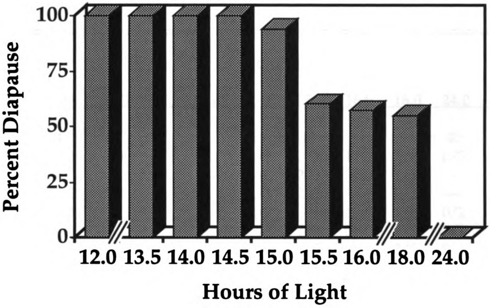

Figure 11. *Papilio glaucus*, Michigan Population, Late Summer 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 12, range in number of pupae= 7-20.

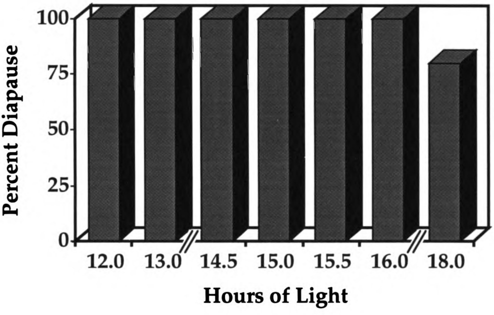

Figure 12. *Papilio glaucus*, Michigan Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 5, range in number of pupae= 1-9.

Table 9. *Papilio glaucus*, Michigan Population, Late Summer, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**	**24.0**
# neonates	43	47	39	36	39	44	39	39	40
9992	3/3	4/4	5/5	3/3	3/4	0/4	1/2	2/4	0/3
9994	---	---	---	---	---	2/3	0/1	1/1	---
9995	2/2	1/1	---	2/2	1/1	2/2	---	0/2	---
9996	1/1	---	---	2/2	2/2	---	---	1/3	0/2
10002	---	---	1/1	---	3/3	1/2	0/1	2/3	0/1
10005	2/2	1/1	---	---	3/3	2/2	---	3/5	0/1
10008	---	---	---	---	--	0/1	---	---	---
10015	3/3	1/1	1/1	1/1	2/2	2/3	1/1	2/2	0/1
10017	2/2	---	1/1	---	1/1	3/3	2/2	---	---
Total	**13/13**	**7/7**	**8/8**	**8/8**	**15/16**	**12/20**	**4/7**	**11/20**	**0/8**
%Diapause	100	100	100	100	94	60	57	55	0

Table 10. *Papilio glaucus*, Michigan Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**12.0**	**13.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	55	56	55	53	55	55	53
10657	---	1/1	---	---	---	---	---
10658	5/5	2/2	1/1	1/1	---	2/2	0/1
10660	---	---	---	1/1	---	---	2/2
10662	1/1	2/2	---	---	---	---	2/2
10663	---	1/1	---	---	---	---	---
10674	---	1/1	---	---	---	---	---
10675	---	2/2	2/2	3/3	1/1	1/1	---
Total	**6/6**	**9/9**	**3/3**	**5/5**	**1/1**	**3/3**	**4/5**
%Diapause	100	100	100	100	100	100	80

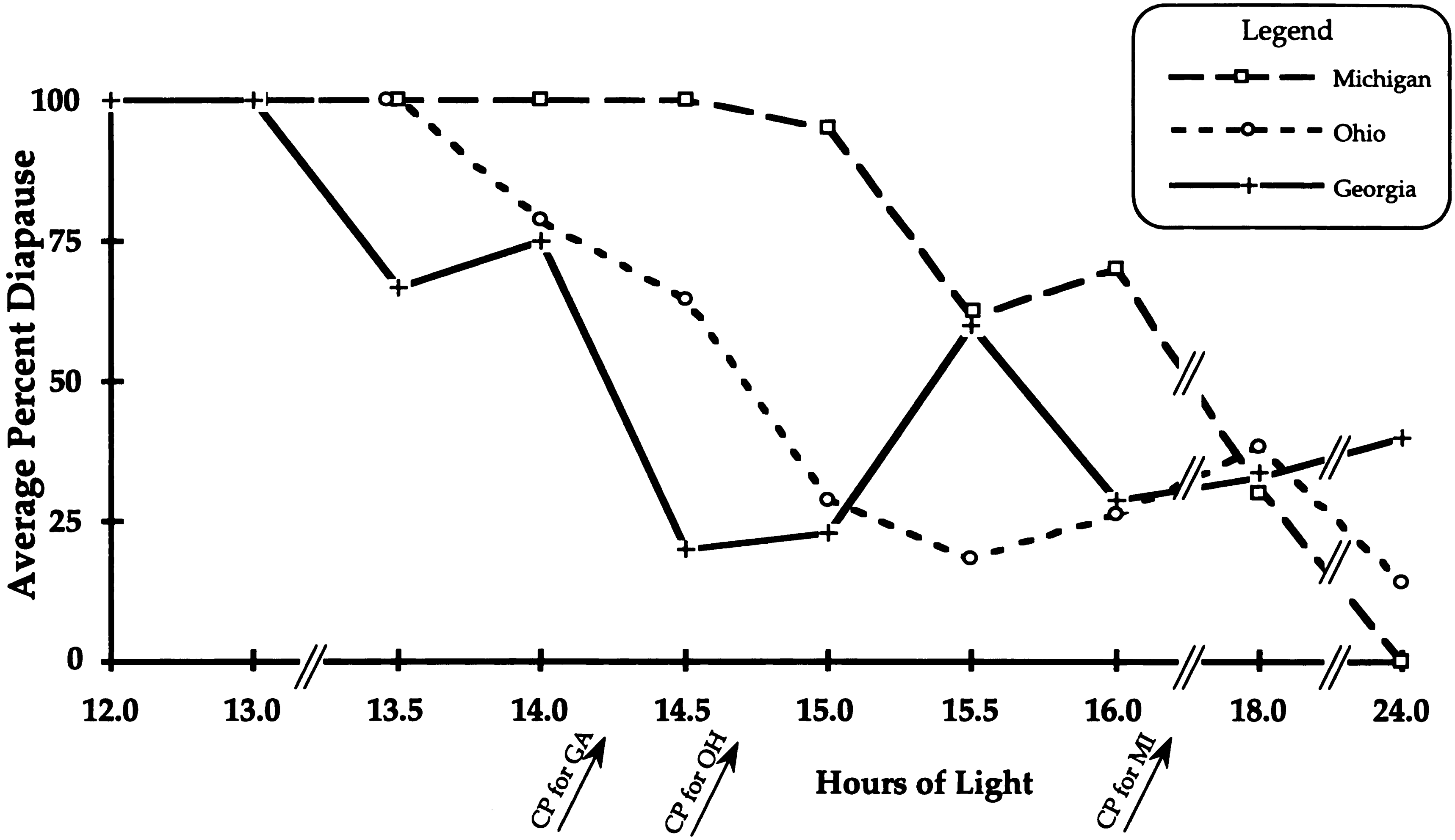

Figure 13. *P. glaucus* Average Percent Diapause vs. Hours of Light larvae were reared at. A comparison between populations from Michigan, Ohio and Georgia. CP = Critical Photopeirod.

Statistical Analysis

The results of the analysis are contained in Table 11. All R^2 values were less than 0.5.

Table 11. Results determined by logistic regression analysis, by state.

State	R^2	-Log Likelihood	Chi-square	p-value
Florida	.3672	24.98	49.96	0.00
Georgia	.0544	2.22	4.44	0.03
Ohio	.0252	26.72	53.44	0.00
Michigan	0.3427	24.84	49.69	0.00

Discussion

Disease

Disease may play a role in diapause induction. Hanski (1988) noted that insects can go into diapause to avoid adverse conditions other than weather, such as high disease conditions. The diseases that caused a high mortality rate amongst the larvae may have altered the experimental conditions. One pathogen was determined to be a bacteria, *Seratia marsescens*. I believe other pathogens were also present such as viruses and other bacteria due to the differences in the ways the larvae and prepupae died.

Michigan and Ohio Population

The critical photoperiod for the Ohio population was lower in 1994 than in 1993, and more pupae diapaused from the Michigan population in 1994 than in 1993. The difference between 1993 and 1994 may be attributed to small sample size, but a high rate of disease in 1994 could have altered the critical photoperiod, inducing diapause on longer days in the presence of disease. Tauber et al. (1986) stated that the critical photoperiod determined by experiments are for specific conditions. Here the presence of disease altered the specific conditions set up for the experiment.

Georgia Population

The critical photoperiod for both 1993 and 1994 was between 14.5h and 15.0h of light. The photoperiods that larvae in the wild would likely be experiencing at the time of year the tests were done are between 13.7h and 11.3h of light (Table 1). The critical photoperiod determined by these experiments seems high for this latitude. Perhaps there is some token stimulus in addition to photoperiod responsible for this result. See further discussion.

Florida Population

The Florida population seems to be different from its northern counterparts. The critical photoperiod determined by these experiments for Florida populations was between 12.0h and 12.5h of light, and all northern populations tested diapaused under these conditions. This may be due to being reared on a different host, sweet bay, than the other populations. A more likely explanation is that Florida is a subtropical environment and experiences less change from season to season, including smaller changes in day length. Cockrell et al. (1994) found that monarch butterfly (*Danaus plexxipus*) populations in Gainesville, FL avoid the very hot weather, while other populations north and south of the area continue to reproduce without interruption by diapause or quiescence. It was suggested that maybe *P. glaucus* also avoids the hottest days of summer between June and September.

However the data do not support this hypothesis. Butterflies are flying in Florida in March when days are about 12.1h long. The subsequent offspring would experience days longer than 12.5h–above the critical photoperiod–and direct develop into adults. Butterflies would be flying again by mid-May when temperatures are frequently in the 90°s (Climates of the States 1985). These butterflies may be using some cue other than photoperiod, otherwise the butterflies and their offspring would be out in the midst of the Florida heat.

In addition, not all Florida pupae direct develop on long days or all diapause on short days. These butterflies may be "bet-hedging" by spreading a few offspring over the season. Normally pupae emerge in about two weeks, but I have observed Florida pupae reared in the laboratory emerge 45 to 50 days after pupation. Florida butterflies may have a slightly different genetic make up that allows for an extended pupal stage that other populations do not have, thus increasing the butterfly's chances for survival over the longer, sub-tropical season.

Statistical Analysis

According to the R^2 values obtained from the logistic regression, photoperiod alone cannot explain diapause induction, at least for these experiments. All values were less than 0.5, indicating that *P. glaucus* may be using a token stimulus in addition to photoperiod to induce diapause. It seems that photoperiod would override any other cue, especially in Michigan, since the climate is not as predictable or stable as it is in the other locations tested (Table 12 and 13).

Probit and Logit were not used to analyze data because I did not have enough 'non-zero' data points. Also, contingency table analysis or Chi-square

Table 12. Climatic information about collection sites.

State	Florida		Georgia	Ohio		Michigan	
County	Highlands	Levy	Clarke	Gallia	Lawrence	Ingham	St. Joseph
Latitude	27°	29°	33°	38°	38°	42°	41°
Elevation (ft)	113	sea level	802	673	555	841	984
Daily max[1] (°C)	31.3	3039	27.2	29.4	29.7	24.8	25.1
Daily min[1] (°C)	19.2	17.7	15.8	15.2	16.4	12.1	12.2
Rainfall[2] (in)	53.1	67.9	50.1	41.2	42.3	30.4	33.4

1 Average daily minimum and miximum temperatures during flight and larval development periods. See Table 1.
2 Average annual precipitation.

From Climates of the States, 1985.

Table 13. General climatic information about collection states.

State	Florida	Georgia	Ohio	Michigan
Average January Temperature (°C)	10 to 20	10 to 20	-10 to 0	-10 to 0
Average July Temperature (°C)	20 to 30	20 to 30	20 to 30	20 to 30
Climatic Region	humid/warm	humid/warm	humid/warm	humid/cold

From Comparative World Atlas, 1992.

analysis were mathematically impossible because I had expected values of zero.

Twilight

Twilight was not included in any of the given photoperiods. The importance of twilight varies among species depending on their individual light intensity thresholds (Beck 1980), and the chambers in which the larvae were reared did not take into account twilight. The lights in the chambers came on and went off at the prescribed times. The effect of twilight was not investigated in this experiment.

A LATITUDINAL COMPARISON

The longest days that Michigan larvae would experience is 15.6h and by 1 October the days are about 11.7 hours long (Nautical Almanac). The critical photoperiod determined by these experiments was 18h, even though Michigan larvae never experience a day this long. Photoperiod may not be the only cue Michigan larvae or the Ohio or Georgia populations use to determine season, and thus to trigger diapause.

It is not known what the sensitive stage is for the tiger swallowtail. Although we know it is probably the larval stage, we do not know if it is limited to one or more instars. We also do not know how larvae interpret the photoperiodic cue, e.g., whether they can detect decreasing day lengths or take an average of the total light hours experienced. Some insects can only detect change in day length instead of absolute day length, so the proper cue of decreasing light hours would not have been available to the larvae reared at a constant photoperiod (Tauber et al. 1986). Larvae take about one month to reach the pupal stage and the day length can change by almost one and a half hours from 1 August to 1 September in Michigan, which is when wild larvae would be growing outside.

Alternative hypotheses follow in the discussion as to why the critical photoperiod in Michigan is greater than 18.0h of light and why there were differences and similarities between the populations tested.

Hybridization and Introgression

One explanation why the critical photoperiod for the Michigan population seems high is that there may be hybridization and introgression between *P. glaucus* and *P. canadensis.* Their ranges overlap in mid-Michigan at a "hybrid zone" and there may be gene flow between these two species, which would account for the higher incidence of diapause at longer photoperiods. *P. canadensis* is an obligate diapauser, but even a few individuals direct-developed when reared at 24h of light (Rockey 1987b). This gene flow may only go in one direction. If a female *P. glaucus* hybridizes with a male *P. canadensis* most females and some males diapause because they will all inherit the obligate diapause gene from their father (Rockey et al. 1987a,b). If a female *P. canadensis* hybridizes with a male *P. glaucus* some of the males will diapause, since they are heterozygous for the X chromosome, and all of the females will be facultative diapausers, inheriting the diapause gene on the X chromosome of their *P. glaucus* father. It is not known to what extent hybridization and introgression takes place between *P. glaucus* and *P. canadensis,* although Hagen et al. (1991) believe it is not very common. But some *P. canadensis* genes may be increasing the genetic variability and thus influencing this high critical photoperiod. Scriber (1990) has noted a "Spring form" of *P. glaucus* which resembles *P. canadensis* in color and wing pattern. He proposes that this similarity is due to *P. glaucus* containing *P. canadensis* genes obtained through introgression. So it is possible that there is influence from *P. canadensis*, at least in the Michigan population.

Gene Flow

This experiment could not detect a difference between the Georgia and Ohio populations. Both populations appear to have a critical photoperiod of 14.5h of light. The fact that the critical photoperiod is the same may be due to small sample size, or that there really is no difference between these populations. This homogeneity may be maintained by gene flow between Georgia and Ohio populations. Larger sample sizes, with less disease in laboratory rearing could help differentiate the relative causes and the critical photoperiod for diapause.

Host Plant Quality

As stated earlier, *P. glaucus* is probably not primarily using day length as a token stimulus. Host plant quality may be influencing diapause induction as well. If larvae are reared on a "good" host, they will grow fast and pupate while the days are still long. If a larvae that hatched at the same time was reared on a lower quality host species, they will grow slower and pupate while when the days are getting shorter. The larvae on good hosts will subsequently become adults earlier in the season and produce another generation. The larvae on the lower quality host will pupate and probably go into diapause because it is too late in the season to reproduce another generation. Host plant quality and host plant species may be more important in diapause induction than photoperiod, especially in areas where three generations could be possible on high quality hosts, such as Ohio and Georgia.

There could be another explanation. *P. glaucus* larvae may incorporate more than one cue to determine change of season. Host plant quality may be influencing diapause induction (Hagen and Lederhouse 1985). They reported a greater than 50% diapause induction in all late season populations of *P. glaucus* north of about 38° latitude when reared at 16:8. This would coincide with the results from the Michigan population.

The pink bollworm, *Pectinophora gossypiella,* was affected by diet. When fed a diet of 1% lipid content versus 5% lipid content, the higher lipid content diet favored induction of diapause (Ankersmit and Adkisson 1967). In addition to the bollworm, *P. zelicaon* can take into account the quality of the host plant and enter diapause on an ephemeral host or direct develop on a more enduring host species. The *P. glaucus* larvae may have detected a change in the host plant (black cherry) quality which would override any photoperiodic stimulus (Sims 1983).

Maternal Effect

Another hypothesis as to why there was an overall high percentage of diapause in the Michigan population is that there may have been a maternal effect. I refer to maternal effect in the sense that "offspring of diapausing females direct develop into adults, and offspring of direct developing females go into diapause." The Michigan butterflies collected from the field for these experiments were second generation adults, offspring of diapausing mothers. This second generation came from direct developing females. If there is a maternal effect on diapause induction, all of the offspring should have diapaused and most of them did from the Michigan population. Only under 24h of light was there 100% emergence of pupae. A maternal effect would also ensure that there would be pupae diapausing late in summer for the next generation in spring. A maternal effect is probably not apparent in the Ohio or Georgia population where the climate is milder than in Michigan (Table 13). Further experiments are needed to confirm this hypothesis.

Conclusion

P. glaucus is likely using a cue(s) other than photoperiod or in addition to photoperiod to determine the season and to induce or avert diapause. Other cues that *P. glaucus* may be sensitive to are changes in thermoperiod or host plant quality. Further investigation is needed to determine what the extent of gene flow is, how host plant quality or host plant species can affect diapause induction and the role the mother plays in diapause induction.

CHAPTER 2

Photoperiodic Induction of Diapause in Populations of *Papilio troilus* Occurring at Different Latitudes

Abstract

For *P. troilus*, facultative diapause is induced by seasonal changes in the environment. The most reliable seasonal cue in its range is day length. Females were collected from Florida, Georgia, Ohio and Michigan and their offspring were reared at different photoperiods. The purpose of this research was to determine the extent of population differences with respect to the critical photoperiod for diapause and to better understand diapause induction in this species. The critical photoperiod for diapause for the Florida population appeared to be between 12.0h and 12.5h of light. The critical photoperiod for the Georgia population could not be determined but it was longer than 13.0h and shorter than 14.5h of light. The critical photoperiod for the Ohio population appeared to be between 13.0h and 14.5h of light. For the Michigan population it was between 14.5h and 15.0h of light. There were differences between populations. The more northern populations entered diapause on longer days than more southern, and the R^2 values obtained from the logistic regression suggest that *P. troilus* is using photoperiod as a cue to avert/induce diapause. These data support the idea that diapause induction by photoperiod is synchronizing the life cycle of *P. troilus* with the seasonal changes in the environment.

Introduction

P. troilus is multivoltine and a facultative diapauser which relies on environmental cues. Photoperiod is thought to be the most reliable indicator of season throughout its range. The purpose of this research was to determine the critical photoperiod for diapause for populations of *P. troilus* occurring in Florida, Georgia, Ohio and Michigan, and to determine if there were any differences between these populations.

Materials and Methods

Florida Population, 1993

Butterflies were collected from Highlands and Levy counties, Florida in March (Figure 3) and shipped via Federal Express or brought back to Michigan in a cooler. Females were allowed to oviposit in the laboratory and were fed a 1:4 honey:water solution (see Scriber 1993). Eggs were collected daily, put in a petri dish and placed in an 18°C chamber, with a photoperiod of 18:6 (L:D). Hatching occurred in five to seven days. The neonates were set up one per dish in petri dishes with foliage from the field. Each female's offspring were equally distributed amongst the treatments, but assigned to a treatment in random order. This was done to avoid any bias from early versus older offspring. The larvae were reared at 25°C, and the nine photoperiod treatments were as follow: 12:12 (L:D), 13:11, 13.5:10.5, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8, and 18:6. After nine larvae from a female were set up across the treatments, the next nine larvae were randomly assigned in a similar manner, until all larvae were evenly distributed. This was done to eliminate bias between younger vs. older larvae. Offspring from four different females were set up for a total of 456 larvae, which were set up between 3 - 10 April. Family lines were followed throughout the experiment.

P. troilus larvae were reared to pupation on redbay, *Persea borbonia,* collected in Florida and shipped to Michigan via Federal Express or brought back to Michigan in a cooler. Leaf turgor was maintained using aquapics (rubber-capped, water-filled vials). Fresh leaves were provided 1-2 times per week for early instars and 2-3 times per week for later instars, until pupation. Pupae were weighed one day after pupation, placed in individual petri dishes with a 175 mm high x 125 mm wide screen cylinder, returned to the same treatment chamber and checked daily for emergence. After six weeks live pupae were assumed to be in diapause and dead pupae were discarded. Pupae were considered to be alive if the abdomen was still flexible. All butterflies, eggs, larvae and pupae in subsequent experiments were treated similarly otherwise stated. All larvae were reared at the same time, in the same chambers with the *P. glaucus* larvae.

Florida Population, 1994

Females were collected from Highlands and Levy counties, Florida in March. Butterfly adults were treated in a similar manner as in the previous year. The first instars were set up five per dish instead of one per dish to increase efficiency of space utilization. At 10d, larvae were put into individual dishes to complete their larval development. Neonates were randomly assigned to one of six photoperiod treatments, and reared at 25°C. The six photoperiod treatments were as follows: 8:16, 9:15, 10:14, 11:13, 12:12, and 13:11. These photoperiods were used because in Florida, the longest day is about 14.5h (Beck, 1980), so all of the treatments used in 1993 were "long" days to the larvae except the 12:12 and 13:11 treatments. The modified photoperiod treatments more closely match what a Florida larva would experience. After six dishes of larvae from each female were set up across the treatments, another six were randomly assigned to a treatment, and so on until three dishes per treatment per female were set up. Larvae were reared to pupation and treated in the same manner as in 1993. Three family lines were set up across the six treatments for a total of 61 larvae, which were set up between 7 - 10 April.

Florida Population, 1995

Butterflies were collected from the same counties as in previous years. Larvae were originally set up five per dish and then placed into individual dishes at 10d. Due to the results in the previous two experiments, it was hypothesized that the critical photoperiod for diapause may be between 12 and 13h of light, therefore the 12.5:11.5 treatment was added and modified to the following five treatments: 10:14, 11:13, 12:12, 12.5:11.5, and 13:11. Offspring from five different females were set up; one to three dishes per female per treatment were randomly assigned as done in previous experiments. A total of 232 larvae was set up between 4 - 16 April. Larvae were reared to pupation on redbay and pupae were treated as before.

Ohio and Michigan Populations, 1993

Butterflies were collected from Gallia and Lawrence counties, Ohio and from St. Joseph county, Michigan during July. No butterflies were collected from Georgia in 1993. A total of 547 larvae was set up from twelve different female lines. Larvae were set up two to three per dish, two to three dishes per female per treatment. After 10d larvae were placed into individual dishes to complete their development. Larvae were reared to pupation on sassafras (*Sassafras albidum*), which was collected from the field, because redbay is not available to northern populations of *P. troilus*. Leaf turgor was maintained using aquapics. Each dish was randomly assigned as done before to one of the following photoperiods (L:D): 12:12, 13:11, 13.5:10.5, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8, and 18:6. Larvae were reared to pupation and pupae were treated in a manner similar to the Florida population. Ohio larvae were set up between 10 - 16 August, and Michigan neonates were set up between 13 - 14 August and 21 - 22 August.

Georgia, Ohio and Michigan Populations, 1994

Butterflies were collected from Clarke and Echols counties, Georgia; Gallia and Lawrence counties, Ohio; and from Ingham county, Michigan during July. Fourteen family lines were set up, for a total of 768 larvae. Larvae were reared on sassafras collected from the field. Five neonates per dish and one to three dishes per female per treatment were set up. Larvae were placed into individual dishes at 10d to complete development. Each female's offspring were randomly assigned to one of the following L:D treatments: 12:12, 13:11, 14:10, 14.5:9.5, 15:9, 15.5:8.5, 16:8, 18:6. Georgia larvae were set up between 18 - 25 August, Ohio neonates were set up between 7 - 12 August and Michigan offspring were set up between 31 July and 8 August. Pupae were treated as before.

Statistical Analysis

Data were analyzed using logistic regression analysis. Analysis was done by testing percent diapause versus photoperiod for each state (with all years lumped together). R^2 values were used to determine how fit the model was for predicting diapause induction, where 1 is a perfect fit and 0 means there is no gain by using the model (JMP 1995).

Results

The following results should be interpreted with caution. Small sample size or possible interactions with pathogens may have biased the results.

Florida Population, 1993, 1994 and 1995

All larvae that survived to pupation in the 1993 experiment directly developed into adults (Table 14). All larvae that survived to pupation in the 1994 experiment diapaused (Table 15). The critical photoperiod for diapause appeared to be between 12h and 13h of light; The 1995 experiment more closely approximated the critical photoperiod to be between 12h and 12.5h of light (Figure 14, Table 16). Percent diapause dropped from 100% at 12h to 0% at 12.5h of light.

Georgia, Ohio, and Michigan, 1993 and 1994

For the Georgia population, only two family lines were represented and only in 1994 (Figure 15, Table 17). All larvae reared in the 14h treatment died, which was likely close to the critical photoperiod for diapause. At 14.5h of light all pupae emerged and at 13h of light all pupae diapaused. The critical photoperiod for diapause was either between 14.5h and 14h or between 14h and 13h of light.

In the 1993 Ohio experiment, percent diapause increased from 3% at 15h to 64% at 14.5h of light (Figure 16, Table 18). The critical photoperiod for diapause appears to be between 15h and 14.5h of light. In 1994, percent diapause increased from 3% at 15h, to 28% at 14.5h and to 100% at 13h of light (Figure 17, Table 19). All the larvae in the 14h treatment died. There were insufficient data to determine whether the critical photoperiod for diapause

Table 14. *Papilio troilus*, Florida Population, Spring, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	51	52	51	51	50	51	50	49	51
9711	---	0/1	---	---	0/2	---	---	---	---
9713	---	---	---	0/2	0/2	---	0/2	---	---
9714	---	0/1	---	---	0/2	---	0/1	0/2	0/1
Total	**---**	**0/2**	**---**	**0/2**	**0/6**	**---**	**0/3**	**0/2**	**0/1**
%Diapause	---	0	---	0	0	---	0	0	0

Table 15. *Papilio troilus*, Florida Population, Spring, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase				
Mother #	**8.0**	**9.0**	**10.0**	**11.0**	**12.0**
# neonates	29	32	34	31	32
10315	1/1	2/2	1/1	5/5	4/4
10316	1/1	2/2	1/1	---	1/1
10332	2/2	1/1	1/1	1/1	1/1
Total	**4/4**	**5/5**	**3/3**	**6/6**	**6/6**
%Diapause	100	100	100	100	100

Table 16. *Papilio troilus*, Florida Population, Spring, 1995; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase				
Mother #	**10.0**	**11.0**	**12.0**	**12.5**	**13.0**
# neonates	37	40	43	42	40
11008	1/1	3/3	2/2	0/3	1/6
11071	1/1	2/2	---	0/1	---
11090	---	---	1/1	0/1	1/3
11114	---	4/4	---	---	---
11131	1/1	---	---	---	0/1
11135	---	1/1	---	---	---
Total	**3/3**	**10/10**	**3/3**	**0/5**	**2/10**
%Diapause	100	100	100	0	20

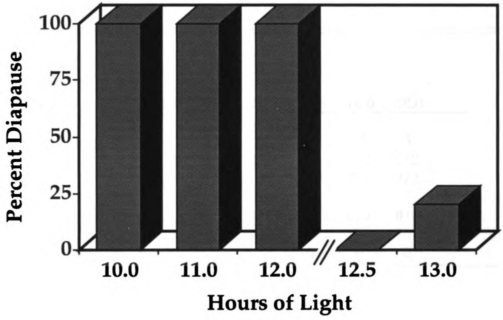

Figure 14. *Papilio troilus*, Florida Population, Spring 1995.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 6, range in number of pupae= 3-10.

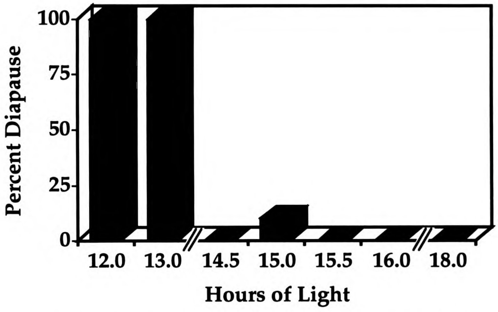

Figure 15. *Papilio troilus*, Georgia Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 7, range in number of pupae= 3-11.

Table 17. *Papilio troilus*, Georgia Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**12.0**	**13.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	7	10	11	11	11	5	5
10800	2/2	2/2	0/3	1/3	0/3	0/2	0/2
10859	1/1	4/4	0/8	0/7	0/8	0/1	0/2
Total	**3/3**	**6/6**	**0/11**	**1/10**	**0/11**	**0/3**	**0/4**
%Diapause	100	100	0	10	0	0	0

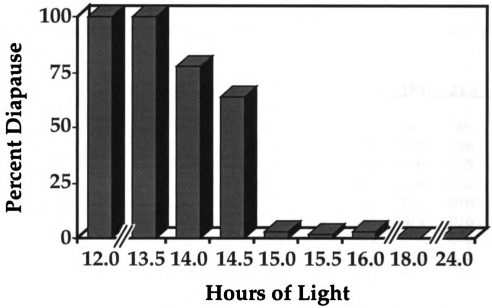

Figure 16. *Papilio troilus*, Ohio Population, Late Summer 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 37, range in number of pupae= 39-45.

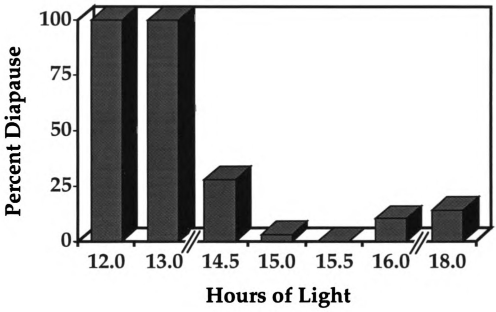

Figure 17. *Papilio troilus*, Ohio Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatment= 47, range in number of pupae= 36-59.

Table 18. *Papilio troilus*, Ohio Population, Late Summer, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**	**24.0**
# neonates	45	50	46	46	49	43	45	44	48
9978	5/5	2/2	1/4	6/6	0/6	1/6	0/6	0/5	0/6
9980	5/5	6/6	5/5	3/4	0/6	0/6	1/6	0/6	0/5
9981	6/6	5/5	4/5	2/5	0/4	0/6	0/5	0/6	0/5
9983	3/3	4/4	5/6	5/6	0/5	0/6	0/5	0/6	0/6
9984	6/6	3/3	6/6	2/3	0/1	0/4	0/4	0/4	0/6
9985	3/3	1/1	1/2	1/3	1/4	0/6	0/2	---	0/4
9989	4/4	2/2	4/4	4/5	0/5	0/5	0/4	0/5	0/4
9990	4/4	6/6	3/5	0/4	0/4	0/6	0/6	0/5	0/4
Total	**36/36**	**29/29**	**29/37**	**23/36**	**1/35**	**1/45**	**1/38**	**0/37**	**0/40**
%Diapause	100	100	78	64	3	2	3	0	0

Table 19. *Papilio troilus*, Ohio Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase						
Mother #	**12.0**	**13.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	57	65	58	55	67	66	66
10684	4/4	8/8	1/3	1/10	0/8	1/9	3/8
10687	5/5	6/6	1/7	0/4	0/5	0/8	0/5
10690	7/7	8/8	1/8	0/8	0/8	0/10	0/7
10691	6/6	5/5	2/4	0/2	0/6	0/4	1/4
10692	4/4	5/5	0/1	0/3	0/4	3/9	2/7
10696	4/4	7/7	0/2	0/4	0/4	0/2	1/3
10698	---	3/3	0/2	---	0/4	0/4	0/5
10699	---	---	---	---	---	---	0/2
10702	9/9	8/8	3/6	0/7	0/8	2/10	0/7
10742	---	4/4	2/3	0/3	0/2	---	---
10744	---	---	---	---	0/1	0/3	---
Total	**39/39**	**54/54**	**10/36**	**1/41**	**0/50**	**6/59**	**7/49**
%Diapause	100	100	28	3	0	10	14

was between 14.5h and 14h of light, or between 14h and 13h of light.

In the 1993 Michigan experiment, percent diapause increased from 33% to 100% between the 15h and the 14.5h treatment (Figure 18, Table 20); in 1994, percent diapause increased from 0% at 15.5h, to 50% at 15h and to 60% at 14.5h (Figure 19, Table 21). There is a discrepancy between these two years of data as was also the case for the Ohio population. The 1993 data implies that the critical photoperiod for diapause is between 15h and 14.5h of light and the 1994 data implies that the critical photoperiod for diapause is between 15h and 15.5h of light.

In Figure 20, average percent diapause vs. hours of light is graphed for *P. troilus* populations from Georgia, Ohio and Michigan. The critical photoperiod seems to change by about one half hour as you move from one population to the next: 15.0h for Michigan, 14.5h for Ohio and between 14.5h and 13.0h for Georgia.

Statistical Analysis

The results of the analysis are contained in Table 22. All R^2 values were greater than 0.5.

Table 22. Results determined by logistic regression analysis, by state.

State	R^2	-Log Likelihood	Chi-square	p-value
Florida	.9845	26.92	53.84	0.00
Georgia	.7715	18.95	37.90	0.00
Ohio	.5607	n/a	n/a	0.00
Michigan	.6361	100.94	201.88	0.00

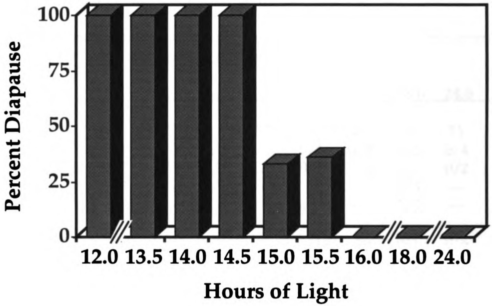

Figure 18. *Papilio troilus*, Michigan Population, Late Summer 1993.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 10, range in number of pupae= 6-17.

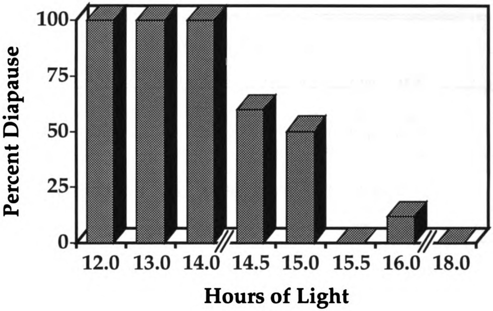

Figure 19. *Papilio troilus*, Michigan Population, Late Summer 1994.
Percent Diapause vs. Hours of Light larvae were reared under.
Average number of pupae per treatement= 17, range in number of pupae= 6-24.

Table 20. *Papilio troilus*, Michigan Population, Late Summer, 1993; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase								
Mother #	**12.0**	**13.5**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**	**24.0**
# neonates	16	16	11	12	17	16	11	21	11
9997	5/5	3/3	5/5	5/5	1/3	0/6	0/2	0/6	0/4
10040	5/5	3/3	5/5	4/4	1/3	0/1	0/4	0/1	0/2
10041	---	3/3	---	1/1	---	1/2	---	0/5	---
10042	3/3	---	---	---	---	4/5	---	0/5	---
Total	**13/13**	**9/9**	**10/10**	**10/10**	**2/6**	**5/14**	**0/6**	**0/17**	**0/6**
%Diapause	100	100	100	100	33	36	0	0	0

Table 21. *Papilio troilus*, Michigan Population, Late Summer, 1994; number of pupae in diapause per number of pupae formed, for each family line.

	Hours in the Photophase							
Mother #	**12.0**	**13.0**	**14.0**	**14.5**	**15.0**	**15.5**	**16.0**	**18.0**
# neonates	25	25	25	25	25	24	22	26
10659	12/12	11/11	6/6	9/12	7/12	0/10	1/15	0/12
10680	5/5	7/7	---	5/8	3/8	0/5	2/9	0/6
Total	**17/17**	**18/18**	**6/6**	**14/20**	**10/20**	**0/15**	**3/24**	**0/18**
%Diapause	100	100	100	60	50	0	12	0

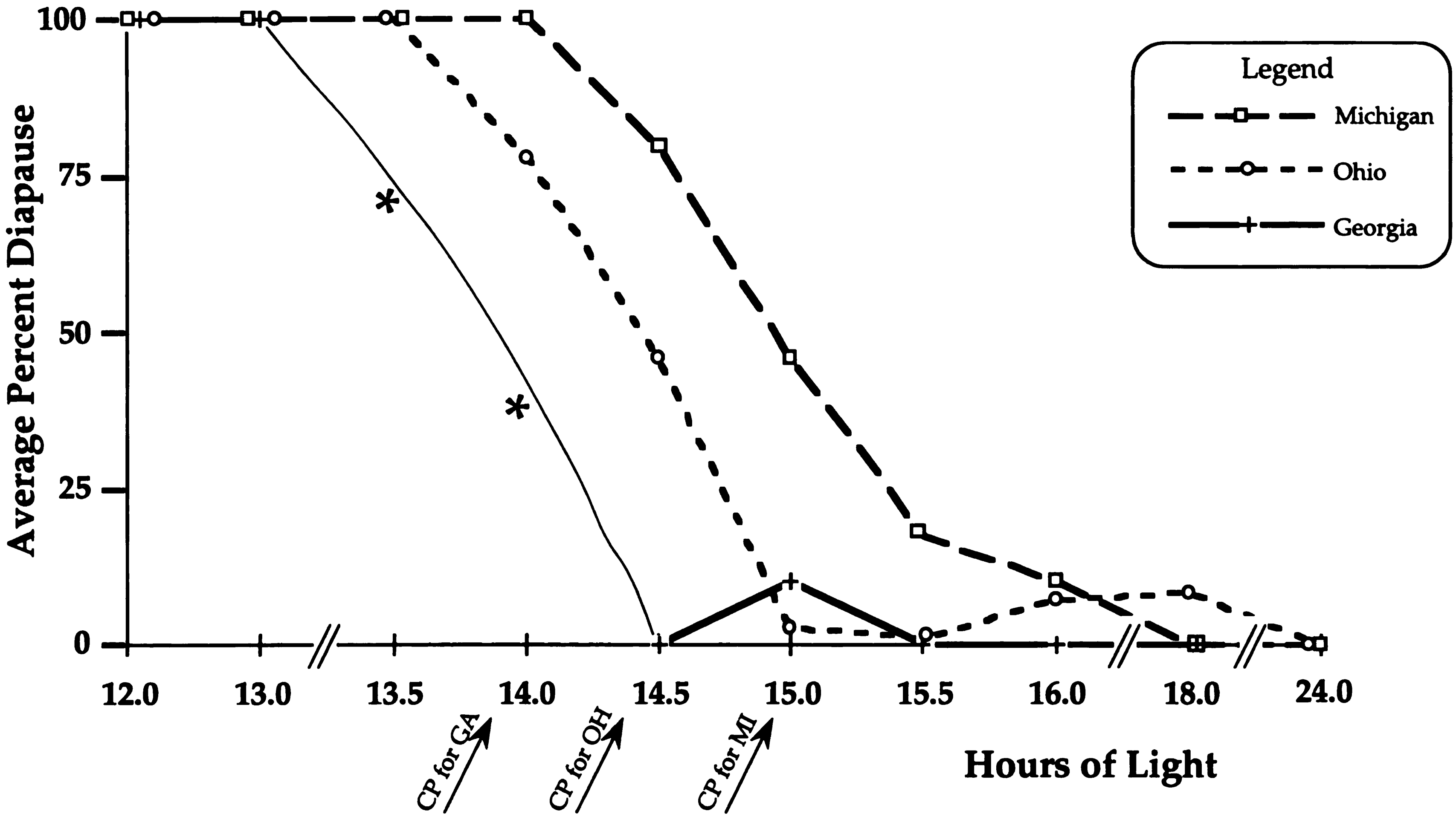

Figure 20. *P. troilus*. Average Percent Diapause vs. Hours of Light larvae were reared at. A comparison between populations from Michigan, Ohio and Georgia. CP = Critical Photopeirod.
* These two points were estimated; all Georgia larvae reared at 13.5h and 14h died.

Discussion

Michigan and Ohio Population

The difference in the Michigan population between the two years may be due to sampling error. Only two family lines were represented in 1994 and four family lines in 1993, although twice as many pupae survived in 1994 as in 1993. Perhaps the second experiment better represents individual family lines better than the population as a whole; although four family lines does not represent a population very well; but those were the only females that produced eggs that hatched.

For the Ohio population, the difference in the critical photoperiod between 1993 and 1994 is not likely based on sample size; eight family lines were represented in 1993 and eleven family lines in 1994. In addition to the number of families represented, the number of pupae per treatment ranged from 29 to 45 in 1993 and from 36 to 50 in 1994. One reason why there was a difference between the two trials was that the *P. troilus* larvae were reared in the same chambers as the *P. glaucus* larvae. *P. troilus* seemed to be unaffected in terms of survival by being in the same chamber in which *P. glaucus* were dying from disease. Perhaps the presence of a high level disease may have somehow altered the *P. troilus* diapause response.

Georgia Population

Only one year of data were available for the Georgia population. Another experiment in which the larvae survive to pupation in all treatments would enable me to determine the critical photoperiod for diapause. These results fall in nicely with what has been determined for the Michigan and Ohio populations, indicating that the critical photoperiod is probably less than for the more northern populations.

Florida Population

The Florida population seems to be different from its northern counterparts. The critical photoperiod determined by these experiments for Florida populations was between 12.0h and 12.5h of light, and all northern populations tested diapaused under these conditions. This may be due to being reared on a different host, redbay, than the other populations. A more likely explanation is that Florida is a subtropical environment and experiences less change from season to season, including smaller changes in day length. The critical photoperiod corresponds with what larvae in the wild would be experiencing in the wild (Table 1). Only in the Florida population did percent diapause drop directly from 100% to 0% and in no other populations tested. This indicates that Florida butterflies probably integrate the token stimulus differently than their northern counterparts.

Statistical Analysis

According to the R^2 values obtained from the logistic regression analysis (Table 22) *P. troilus* reared in this experiment primarily used photoperiod as a cue to induce or avert diapause, since all R^2 values were greater than 0.5. Results also indicated that the proportion of variation in diapause percent is mainly explained by the model.

Probit and Logit were not used to analyxe data because I did not have enough 'non-zero' data points. A contingency table analysis or Chi-square test were mathematically impossible because I had expected values of zero.

A LATITUDINAL COMPARISON

Pupae generally diapaused when larvae were reared under short days and direct developed when reared under long days. I could not pinpoint the critical photoperiod for the Georgia population because no larvae survived to pupation in the 13.5h and 14.0h treatments, but the critical photoperiod is between 13.0h and 14.5 hours of light because percent diapause decreases from 100% at 13.0h to 0% at 14.5h of light. The critical photoperiod would also be less than the critical photoperiod for both Michigan and Ohio populations.

It appears from these data that the Michigan population begins to enter diapause at longer days than the Ohio population, which enters diapause at longer days than the Georgia population. This timing of diapause ensures that the populations are kept in phase with the changing seasons without limiting the potential for multiple generations by inducing diapause in the first generation when the growing season can still support another brood.

Conclusion

There appeared to be differences in the populations of *P. troilus* occurring at different latitudes with the northern populations going in to diapause at longer days. This serves to synchronize those populations with the earlier change of season while a shorter threshold for the southern populations ensures that they maximize their output within the longer growing season.

This also occurs in other species with populations occurring at different latitudes. Tauber and Tauber (1972) found that in the green lacewing, *Chrysopa carnea*, the critical photoperiod for diapause for a New York population at 42 N° was between 13.5 and 14.0 hours of light, and for an Arizona population at 33 N° the critical photoperiod was between 12.5 and 13.0 hours of light. This is an hour difference in critical photoperiod between these two populations reared under the same conditions.

Mousseau and Roff (1989) discovered a similar pattern in the striped ground cricket, *Allonemobious fasciatus*. They reared crickets, originating from different latitudes, at 14:10 (L:D), 30°C and found the percentage of crickets in diapause increased as the latitude of origin increased. And Bell et al. (1979) investigated 23 different populations of Indian meal moth, *Plodia interpunctella* ranging from 52 N° latitude to 35 S° latitude. They also found that there was a tendency for the critical photoperiod to increase in length as the latitude of origin increased. The longer critical photoperiods allow the northern populations of the green lacewing, the ground cricket, the Indian meal moth and the Spicebush swallowtail to enter diapause earlier in the season which adapts these species to the early occurring winter conditions at higher latitudes (Tauber and Tauber 1972). This ensures that these population are in synch with the unique seasonal changes of their environment.

CHAPTER 3

Interspecific Comparison of Diapause Induction in the Tiger Swallowtail and the Spicebush Swallowtail

As seen in the previous chapters *P. glaucus* and *P. troilus* did not respond similarly to varied photoperiods. This research has shown that there are differences between populations of the same species. The purpose of this chapter is to compare and contrast these two species at each location. Now I will look at and discuss the differences between species living in the same area, which are active at the same time of the year.

In Figure 21 the Florida populations of *P. glaucus* and *P. troilus* are graphed as Percent Diapause vs. Hours of Light, with all years lumped together. Both species responded with the same critical photoperiod, between 12.0h and 12.5h. The diapause threshold for *P. troilus* dropped off sharply between 12h and 12.5h of light, while for *P. glaucus* there was a more gradual slope down toward no diapause. In addition to the slope of the lines, *P. glaucus* never responded with 100% diapause, so some pupae always direct-developed. Florida populations of both species appear to have the same critical photoperiod, but *P. troilus* showed more of an "all or none" response while *P. glaucus* was more variable.

At the other three locations, there was a similar pattern (Figures 22, 23 and 24). Some *P. glaucus* diapaused in every treatment. Even when reared at 24h of light some pupae from Ohio and Georgia still went into diapause.

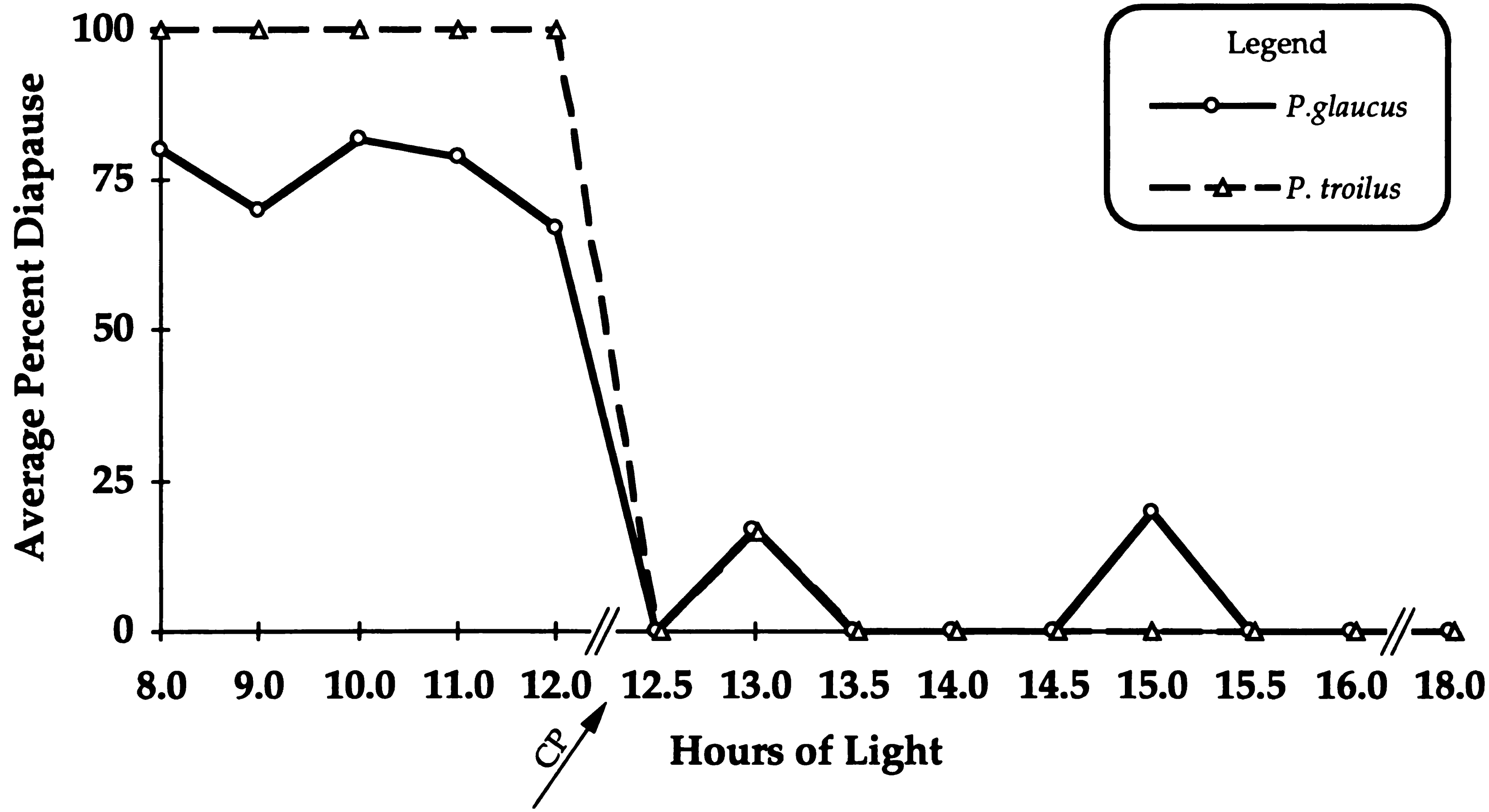

Figure 21. An Interspecific Comparison. Average Percent Diapause vs. Hours of Light larvae were reared at, for Florida Populations of *P. glaucus* and *P. troilus*. CP = Critical Photoperiod for diapause.

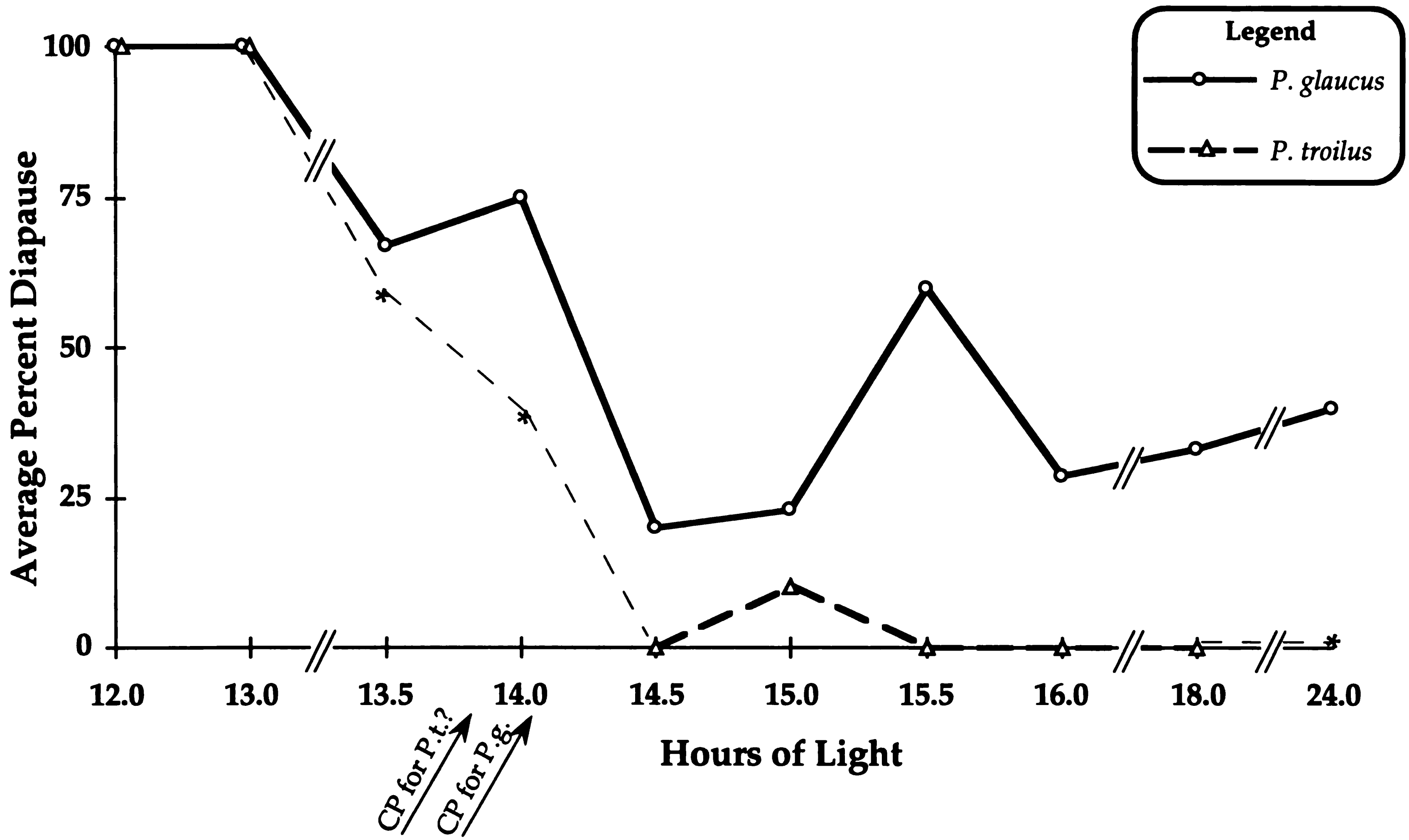

Figure 22. An Interspecific Comparison. Average Percent Diapause vs. Hours of Light larvae were reared at for Gerogia Populations of *P. glaucus* and *P. troilus*. CP = Critical Photoperiod for diapause.
*These points were estimated, all *P. troilus* larvae died in these treatments.

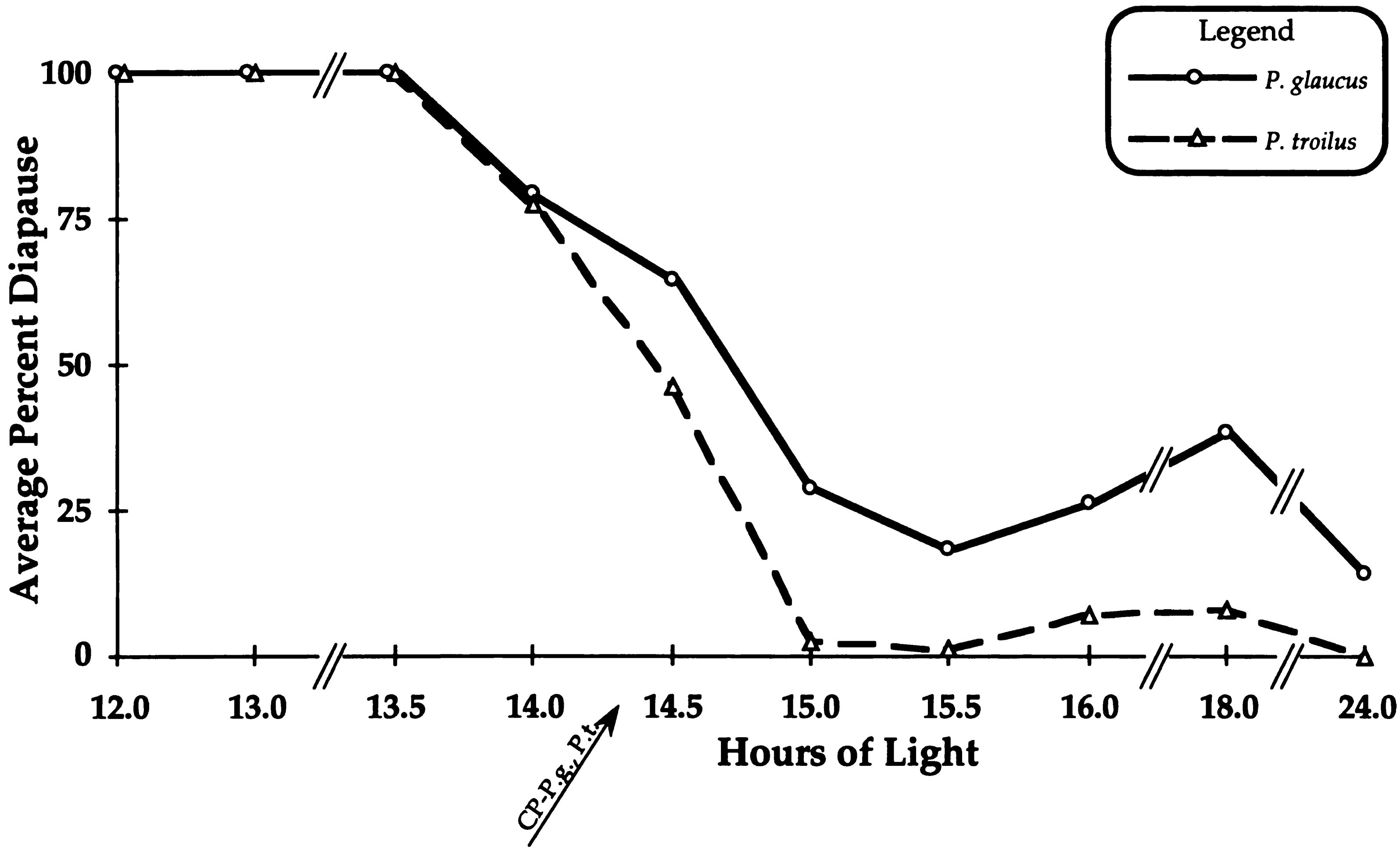

Figure 23. An Interspecific Comparison. Average Percent Diapause vs. Hours of Light larvae were reared at for Ohio Populations of *P. glaucus* and *P. troilus*. CP = Critical Photoperiod for diapause.

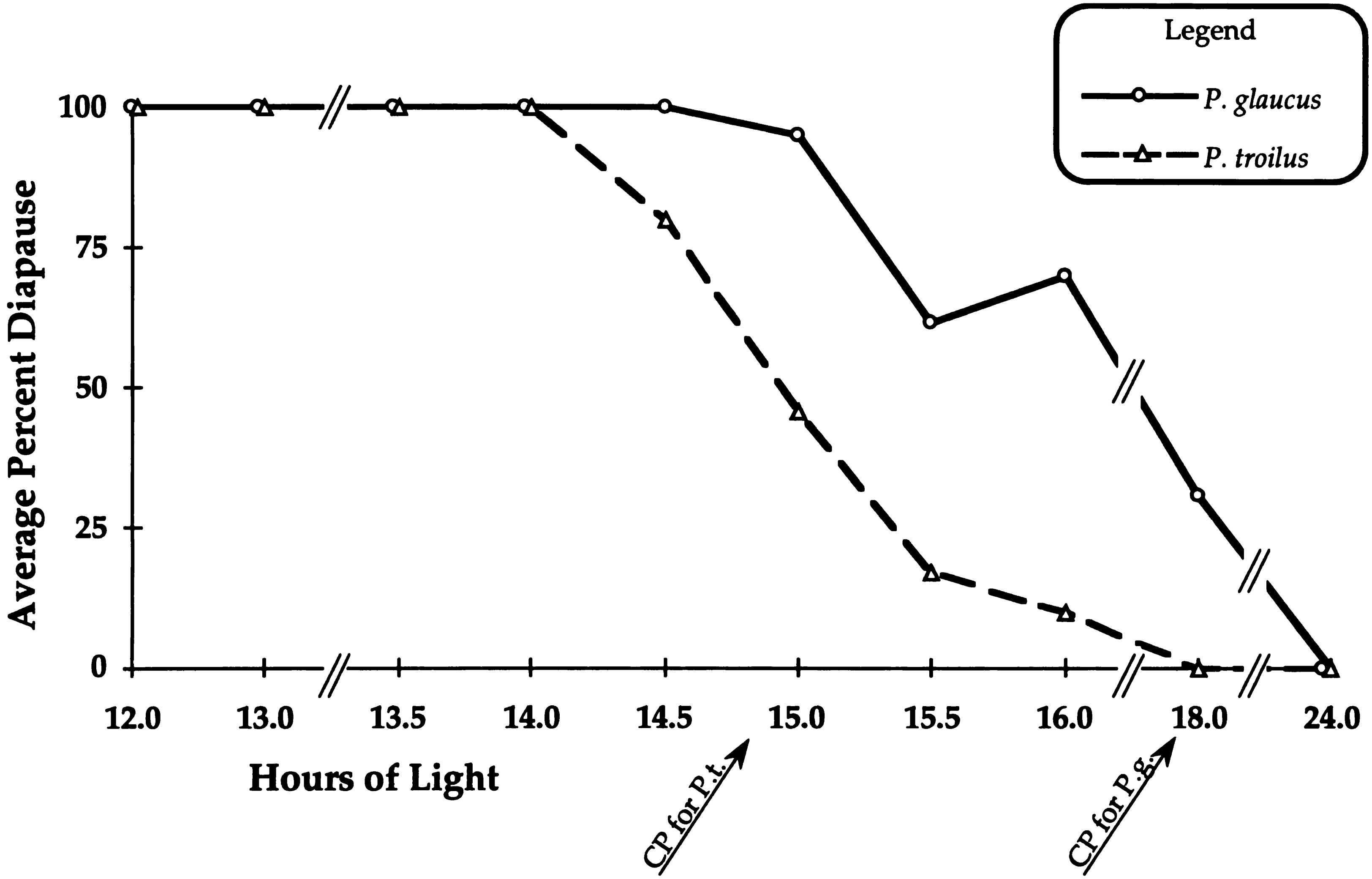

Figure 24. An Interspecific Comparison. Average Percent Diapause vs. Hours of Light larvae were reared at for Michigan Populations of *P. glaucus* and *P. troilus*. CP = Critical Photoperiod for diapause.

Opler and Krizek (1984) stated that some offspring from every generation overwinter, so maybe *P. glaucus* is just following what it does in the wild despite the controlled conditions of a laboratory.

It seems clear from these results that *P. troilus* used photoperiod as a major indicator of season, and *P. glaucus* did not. *P. glaucus* may be using cues other than photoperiod to indicate what time of year it is, such as the quality of the host plant. This high level of variation may be attributed to the absence of natural selection for a specific diapause-inducing photoperiodic response (Harvey 1957). There may simply be some token stimulus not provided in laboratory rearing that would reduce the variation between *P. glaucus* and *P. troilus*.

Another explanation for the variation between the two species is that there may be more gene flow among populations of *P. glaucus* than among populations of *P. troilus*. Diapause is a genetic component and if there are genes coming in from other populations, this can influence what photoperiod is the critical photoperiod for each individual.

An alternative hypothesis is that diapause in *P. glaucus* is influenced by host species. *P. glaucus* larvae feed on host plants from at least nine different families while *P. troilus* only larvae feed on hosts only from one, the Lauraceae family (Nitao et al. 1991, Scriber 1994). *P. glaucus* were reared on only one host in these experiments, either sweet bay or black cherry, when in nature they are probably spread out amongst the many hosts available to it. Eggs would probably be distributed amongst high and low quality hosts. Since swallowtail larvae generally remain on the host and tree in which they were put, the offspring would be spread amongst various hosts. Each individual larvae will grow at a different rate thus exposing the larva to different amounts of light. By rearing larvae on only one host there may be some sort of artificial selection for an increase in the propensity to diapause not usually seen in nature when larvae are spread amongst many host plants.

As mentioned in the previous chapters, many *P. glaucus* larvae died of disease. Both the *P. glaucus* and *P. troilus* larvae were reared in the same chambers at all times, but almost all of the *P. troilus* larvae escaped death by disease. Even the adults that emerged produced fertile offspring. While over 90% of the *P. glaucus* larvae succumbed to disease, most of the *P. troilus* larvae survived. Most of the mortality of *P. troilus* was due to larvae not feeding or larvae crawling into the aquapic and drowning. *P. troilus* may simply escape disease by reaching the pupal stage faster than *P. glaucus*. In addition, *P. troilus* generally take about 20 days to pupate from date of hatch while *P. glaucus* usually take around 30 days. If *P. glaucus* do not pupate within a few days of their 30 day larval life, they usually don't survive to pupation, the pupae die, or if they happen to make it to adulthood, they're usually deformed. Differential disease interactions with diapause physiology may explain some of the differences between these two *Papilio* species.

[Currently the disease topic is under investigation, with initial steps taken to completely sterilize and repaint the rearing room and to sterilize foliage in a bleach solution. Other measures considered are creating an artificial diet, looking at the gut physiology of *P. troilus* to determine if it has any "special" disease-fighting enzymes, and rearing *P. glaucus* on sassafras to determine if sassafras is responsible for the low mortality rate in *P. troilus*.]

Understanding diapause dynamics and mechanisms determining voltinism patterns sheds light on adaptations of species to local environments and on the evolution of species. This also helps in the design of pest management systems, especially where multivoltinism is likely. Knowing the voltinism patterns of a pest species can help in the prescribing of pest management strategies. If the pest has more than one generation per season of a variable number, then special steps may have to be taken to predict and control the pest when it returns. The European Corn Borer, *Ostrinia nubilalis*, causes damaged to many crop species such as corn and potatoes. There are populations of both univoltine and bivoltine in some areas. Being able to predict potential insect damage to crops is important to insect management programs and the ability to predict this damage starts with diapause and voltinism patterns (Scriber and Lederhouse 1992).

Pest management tactics can also affect non-target species such as *P. glaucus* and *P. troilus*. Preliminary results of bioassays testing *Bacillus thuringiensis* on swallowtail larvae versus gypsy moth larvae (*Lymantria dispar*) revealed that the high doses of *Bt* needed to kill gypsy moth larvae are persistent for weeks and highly lethal to swallowtail larvae (Johnson et al. 1995). Even *Bt* in very small doses was lethal. If, in the areas where gypsy moths occur, *Bt* is being applied and non-target species are being affected, *Bt* could wipe out a species in that area. However, understanding the diapause dynamics and seasonal phenology of both the pest species and the non-target species could help in planning pest management strategies. By knowing when each species will be active in the areas targeted for management, strategies can be used that will maximize kill on the pest and minimize damage to the non-target species.

Diapause dynamics of an insect can limit or allow wider distribution of a species. The capacity of the Indian meal moth and the pink bollworm to diapause increases their chances that they will survive when moved to a new environment and has also facilitated in the spread of these pests (Ankersmit and Adkisson 1967, Bell et al. 1979). Diapause dynamics can also limit further distribution as it does for *P. glaucus*. *P. glaucus* has not extended its range farther north than the hybrid zone where it encounters *P. canadensis*. This limit is likely due to the inability of *P. glaucus* to adapt to a univoltine life cycle, which is necessary in regions north of the hybrid zone (Hagen et al. 1991).

Diapause is not just a way to avoid adverse conditions, but is also of prime adaptive significance in adjusting the insect's entire life cycle to seasonal change; it serves as the primary means whereby insect life cycles are kept in phase with the changing seasons (Tauber et al. 1986).

Understanding photoperiodic adjustments in animals and the interactions of biotic and abiotic environmental factors influencing animal behavior is also of importance in many other ways such as for determining hibernation or migration cycles.

APPENDICES

APPENDIX 1

Record of Deposition of Voucher Specimens*

The specimens listed on the following sheet(s) have been deposited in the named museum(s) as samples of those species or other taxa which were used in this research. Voucher recognition labels bearing the Voucher No. have been attached or included in fluid-preserved specimens.

Voucher No.: 1995-5

Title of thesis or dissertation (or other research projects):

LATITUDINAL COMPARISONS OF DIAPAUSE INDUCTION BETWEEN POPULATIONS OF PAPILIO GLAUCUS AND PAPILIO TROILUS (LEPIDOPTERA: PAPILIONIDAE)

Museum(s) where deposited and abbreviations for table on following sheets:

Entomology Museum, Michigan State University (MSU)

Other Museums:

Investigator's Name (s) (typed)
Patricia A. Tidwell

Date August 3, 1995

*Reference: Yoshimoto, C. M. 1978. Voucher Specimens for Entomology in North America. Bull. Entomol. Soc. Amer. 24:141-42.

Deposit as follows:

Original: Include as Appendix 1 in ribbon copy of thesis or dissertation.
Copies: Included as Appendix 1 in copies of thesis or dissertation. Museum(s) files. Research project files.

This form is available from and the Voucher No. is assigned by the Curator, Michigan State University Entomology Museum.

APPENDIX 1.1

Voucher Specimen Data

Page 1 of 1 Pages

Species or other taxon	Label data for specimens collected or used and deposited	Number of: Eggs	Larvae	Nymphs	Pupae	Adults ♀	Adults ♂	Other	Museum where deposited
Papilio glaucus	Lab Culture MI: St. Joseph Co. June 1995					2			Entomology Museum Michigan State University (MSU)
Papilio glaucus	Lab Culture MI: Ingham Co. August 1995					1			
Papilio glaucus	MI: St. Joseph Co. July 1995						1		
Papilio glaucus	GA: Clarke Co. July 1995						2		
Papilio troilus	Lab Culture MI: Ingham Co. August 1995					2	1		
Papilio troilus	MI: Ingham Co. July 1995					1			
Papilio troilus	MI: St. Joseph co. July 1995						1		
Papilio troilus	Lab Culture OH: Lawrence Co. August 1995						1		

(Use additional sheets if necessary)

Investigator's Name(s) (typed)

Patricia A. Tidwell

Date August 3, 1995

Voucher No. 1995-5

Received the above listed specimens for deposit in the Michigan State University Entomology Museum.

Curator Date

APPENDIX 2

Table 23. Information by mother number about female *P. glaucus*.

Mother #	County of Origin	Wear Class[1]	Date Collected	Color[2]
9701	n/a	2	3/22	yel
9703	n/a	3	3/23	yel
9718	Highlands	1	3/27	yel
9719	Highlands	1	3/27	yel
9720	Levy	1	3/31	dk
9744	Highlands	1	4/6	yel
9751	Levy	2	4/1	dk
10318	Levy	3	3/26	dk
10323	Levy	2	3/26	yel
10327	Highlands	2/3	3/27	yel
10329	Highlands	3/4	3/27	dk
10335	Highlands	2/3	3/27	yel
11004	Highlands	1	3/24	yel
11046	Highlands	3	3/26	yel
11048	Highlands	4	3/26	yel
11053	Levy	2/3	3/26	dk
11064	Levy	3	3/26	dk
11065	Levy	4	3/26	dk
11066	Levy	1	3/26	dk
11070	Levy	4	3/26	dk
11097	Levy	1	3/28	yel
11099	Levy	1	3/28	dk
11104	Levy	2	3/28	dk
11106	Levy	1	3/28	dk
10021	Clarke	2	8/11	dk
10025	Clarke	2	8/11	dk
10026	Clarke	2	8/11	dk
10027	Clarke	2	8/11	dk
10021	Clarke	1	8/11	dk

10727	Clarke	2	8/3	dk
10728	Clarke	2	8/3	dk
10730	Clarke	2	8/3	yel
10731	Clarke	1	8/3	dk
10737	Columbus	1	8/6	dk
10757	Union	2	8/7	dk
10760	Clarke	1	8/10	yel
10779	Clarke	1	8/10	dk
10787	Clarke	2	8/10	dk
10790	Clarke	1	8/10	dk
10799	Franklin	1	8/10	dk
9953	Gallia	2	8/4	dk
9960	Lawrence	1	8/4	dk
9965	Lawrence	2	8/4	dk
9967	Lawrence	1	8/4	dk
9968	Lawrence	2	8/4	dk
9972	Lawrence	1	8/4	dk
9974	Lawrence	1	8/4	yel
9975	Lawrence	1	8/4	yel
9976	Lawrence	2	8/4	yel
10705	Lawrence	2	7/30	dk
10708	Gallia	2	7/30	dk
10710	Gallia	3	7/30	dk
10711	Gallia	2	7/30	dk
10713	Gallia	2	7/30	dk
10717	Gallia	2	7/30	dk
10747	Gallia	2	8/9	dk
10748	Gallia	1	8/9	dk
10750	Gallia	2	8/9	dk
10758	Gallia	1	8/10	dk
9992	St. Joseph	2	7/30	dk
9994	St. Joseph	2	8/6	yel
9995	St. Joseph	2	8/6	dk
9996	St. Joseph	2	8/6	dk
10002	Clinton	2	8/7	yel
10005	Clinton	3	8/7	yel
10008	St. Joseph	2	8/8	yel
10015	Clinton	2	8/11	yel
10017	Clinton	2	8/11	yel

10657	Ingham	2	7/25	yel
10658	Ingham	1	7/25	yel
10660	Ingham	1	7/25	yel
10662	Calhoun	1	7/25	yel
10663	Calhoun	2	7/25	yel
10674	Eaton	2	7/28	yel
10675	Eaton	1	7/29	dk

Table 24. Information by mother number about female *P. troilus*.

Mother #	County of Origin	Wear Class	Date Collected
9711	n/a	3	3/22
9713	n/a	1	3/22
9714	n/a	1	3/22
10315	Levy	2	3/26
10316	Levy	1	3/26
10332	Highlands	2	3/26
11008	Highlands	1	3/24
11071	Highlands	1	3/26
11090	Highlands	2	3/27
11114	Levy	2	3/30
11131	Highlands	3	3/30
11135	Highlands	1	3/30
10800	Clarke	2	8/10
10859	Clarke	2	8/15
9978	Gallia	1	8/4
9980	Gallia	1	8/4
9981	Gallia	1	8/4
9983	Gallia	1	8/4
9984	Gallia	1	8/4
9985	Gallia	1	8/4
9989	Lawrence	2	8/4
9990	Lawrence	1	8/4
10684	Gallia	1	7/30
10687	Gallia	2	7/30
10690	Lawrence	1	7/30
10691	Lawrence	1	7/30

10692	Lawrence	1	7/30
10696	Lawrence	2	7/30
10698	Lawrence	1	7/30
10699	Lawrence	2	7/30
10702	Lawrence	2	7/30
10742	Gallia	2	8/9
10744	Gallia	2	8/9
9997	St. Joseph	1	8/6
10040	St. Joseph	1	8/14
10041	St. Joseph	2	8/14
10042	St. Joseph	3/4	8/14
10659	Ingham	3	7/25
10680	Ingham	1	7/29

1 The approximate age of a butterfly can be determined by the wear of wings and abdomen. 1 is a very fresh female and a 4 is a very old female, see Lederhouse and Scriber 1987.

2 *P. glaucus* females are dimorphic, either yellow or dark (black).

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