

POPULATION ECOLOGY OF *ANAEA RYPHEA*
(NYMPHALIDAE): IMMATURES AT CAMPINAS, BRAZIL

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ABSTRACT. I studied a population of *Anaea ryphea* (Nymphalidae) for three years in Campinas, Brazil, to identify the extent and causes of egg and larval mortality. Life tables showed the highest level of mortality occurred during the first instar, but key-factor analysis demonstrated that both first and third instars were responsible for the greatest variation in density between months. There was a high and significant correlation between monthly rainfall indices and total k-values for larvae ($r^2=0.79$ and 0.88 for 1989 and 1991 respectively), suggesting a strong influence of rainfall on mortality. Disappearance accounted for a reduction of 53.7% in the total number of marked eggs, and parasitism was observed in 55.9% of the remaining eggs. A positive and significant correlation was found between the number of new eggs and the relative humidity of the previous night ($r^2=0.55$, $p<0.05$), suggesting that changes in humidity could serve as a cue for oviposition.

Additional key words: *Bracon*, mortality, parasitism, predation, *Trichogramma*.

There are few published papers treating population ecology and dynamics of phytophagous insects in natural tropical systems. Most of the information about phytophagous insect population dynamics comes from pests of agroecosystems (Stiling 1988, Hassell et al. 1989). These studies suggest that regulation through natural enemies—predators, parasitoids, pathogens—is common (Risch 1980, Crawley 1989). Results obtained from agroecosystem studies usually cannot be applied to natural systems because natural hosts usually are more dispersed and present more effective defenses against herbivores. These defenses may slow development (Rausher et al. 1993) and help natural enemies control outbreaks (Hassell & Waage 1984).

Parasitism in larvae is reported commonly for temperate and tropical insects (Young & Moffett 1979, Maltais et al. 1980, Strong et al. 1984, Stiling 1988, Hassell et al. 1989) and is seen as a typical mechanism of regulation in herbivorous species (Clark et al. 1967, Dempster 1984, Strong et al. 1984, Hassell 1986, Waage & Greathead 1986). Recent works (Crawley 1989, Karban 1993) also show that, for many herbivorous insects, population densities are maintained at a low level by quality and not quantity of food.

Variation in population numbers also can be due to meteorological and climatic factors. Seasonal variations in insect population size are usually a consequence of climatic conditions. These can act directly as stress or mortality sources, serve as cues and induce adaptive changes (such as diapause), or influence resources and natural enemies. Even in the tropics there can be highly unfavorable abiotic conditions, such

as a strong dry season or wind and rain storms. These can cause density dependent or density independent mortality (Clark et al. 1967), and there are several possibilities of indirect effect of climate on the interactions with enemies, competitors, and resources (Wolda 1978). These can influence the population size in the present (Courtney & Duggan 1983) or future generations (Pollard 1979).

I studied a population of the tropical butterfly *Anaea ryphea* (Nymphalidae: Charaxinae) for 3 years, in order to gather information on natural regulation of a tropical insect. The goal was to assess the main mortality factors acting on immature stages and to search for evidence of density dependent processes.

Adult Charaxinae butterflies feed on rotten fruits, carrion, and feces, rarely on nectar (DeVries 1987). They have a cryptic coloration ("dry leaf" pattern) on the underside of the wings that seems to confer protection from predators when the butterflies are perched. Larvae feed on various families of plants, but mainly Euphorbiaceae. These butterflies can be found from sea level to 2000 m, and some species have large geographic distributions. *Anaea ryphea* was used for this study because it is a phytophagous insect easily found in tropical forests from Mexico to southern Brazil (DeVries 1987). Its abundance and widespread occurrence will allow further studies about variation in mortality factors among populations under different climatic and geographic conditions. Although this butterfly is common in my study area, and its larval food plant was abundant along the forest margins, little is known about the population ecology of this and related species (DeVries 1987, Caldas 1994).

STUDY SITE AND METHODS

The study area consisted of a semideciduous subtropical moist forest in successional mosaic, left undisturbed for the past 12 years. Along the central 1,160 m trail of the 2.5 km² Reserva Mata de Santa Genebra, Campinas, São Paulo state, Brazil (22°54'S and 47°05'W, elevation 650m), I marked 186 individuals of *Croton floribundus* (Euphorbiaceae), the main larval food plant of *A. ryphea*, with numbered plastic tags. Plants more than 2 m high were not included in the study because of difficulty in examining the higher leaves. Observations on larvae started in November 1988 and observations on eggs started in November 1990; both continued until May 1991. Census information on adults was difficult to obtain. Trapping within the reserve was not very successful (insufficient number of individuals for population studies), and capture with an aerial net was not practical because adults usually fly high in the canopy for most of the time, and hence are very difficult to see. No

adults were observed within the reserve from June to November. Since adjoining forested areas have the same climatic pattern, with dry and wet seasons occurring at the same time of the year as in Campinas, adults would have to fly long distances in order to find suitable food plants to oviposit and remain reproductively active. Migration does not seem to occur in Charaxinae butterflies (Baker 1969), and no individuals were captured in traps located around the reserve. A likely explanation for the absence of adults is reproductive diapause, as observed in *Anaea andria* populations (Riley 1988). This diapause was observed by the author when a reduction in the number of females occurred, toward the end of the reproductive season, when the whole population was declining. Further studies are needed to clarify whether the apparent absence of adults from June to November is due to a low number of reproductive diapausing females or to a complete absence of the species in the area.

The number of plants censused varied from year to year because of plant mortality during the dry season and recruitment during the rainy season. However, the total number of plants included in the study never deviated by more than 10% from the initial 186 marked in November 1988.

During each weekly census, all leaves of tagged plants were searched for larvae, and during 1990 and 1991 for eggs and larvae. Eventually censuses reached a 2-day interval, so that each individual's fate could be assessed more accurately. All larvae were individually identified by tying a numbered plastic band on the petiole of the host leaf, and, if more than one egg or larva were on the same leaf, the position and stage of each was noted. Eggs were identified by writing numbers next to them on the leaf with indelible ink.

This methodology allowed easy identification of parasitized larvae and eggs. Larvae attacked by *Bracon* sp. (Hymenoptera: Braconidae) could be recognized from the fourth instar by two characteristic features: 1) they became lethargic and stayed inside their rolled leaves, and 2) the leaf tube, which usually is clean inside, was covered with silk and accumulated debris. Eggs parasitized by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) changed color from translucent green to yellowish and finally to a metallic gray.

Because no adults or immatures of *A. ryphea* were found during August and September of 1988, 1989, and 1990, the biological year used for the analyses was from September of one year to August of the next.

Vertical (time-specific) life tables (see Table 1) were constructed for *A. ryphea* larvae for the seasons 1988/89 and 1990/91. The intermediate season (1989/90) was not analyzed by this method because of sampling

problems, namely a gap in data because larvae were not followed. Life tables provide an easy way of displaying mortality in different age intervals for a constantly reproductive population. It shows the age interval (X), the total number of individuals belonging to that age interval found in the field (N), the number of individuals that "die" in a certain age interval (meaning do not reach the next age interval), and the most probable causes for the deaths in each age interval. After calculating the log of each N, the difference between logs of successive N's is called the "killing power" (k-value) of the mortality factor(s) acting in that age interval. Vertical life tables are constructed from the age distribution in the field for each census, as opposed to horizontal (age specific) life tables, where a cohort is marked and followed. From direct observations in the field and from inferences based on the pattern of larval disappearance, the probable main causes of mortality throughout the season were listed for each age class.

The life tables built for each month were then used for a Key-factor analysis (Morris 1959, Varley and Gradwell 1960), a method meant to show which factor would be responsible for changes in numbers of individuals between generations in that population. This is not necessarily the factor that kills most (has higher "killing power"), but the one whose "killing power" correlates more with the overall mortality of the population (the total value for k).

Meteorological data on monthly temperature, rainfall, and relative humidity were obtained from FEAGRI/UNICAMP meteorological station, approximately 3 km from the Reserve. All data obtained from *A. ryphea* in the field and subsequent calculations (estimates of mortality, percent parasitism) were analyzed in relation to the meteorological data using correlation analysis and multiple regression (Sokal & Rohlf 1981). With these procedures I tried to identify possible climatic and weather factors associated with changes in population density.

RESULTS AND DISCUSSION

In each of the three years of study, oviposition started in November/December, reaching levels considered suitable for my study ($n > 5$ new eggs and larvae per census) in January. Although strongly variable, maximum recruitment happened between February and April. After that, oviposition declined more or less rapidly before winter started. The oviposition pattern each year did not provide evidence of pulses that could be identified as generations.

There was dramatic variation in the number of larvae censused in each year of study (Fig. 1). In the first year (September 1988 to August 1989), the population increased abruptly in January, and de-

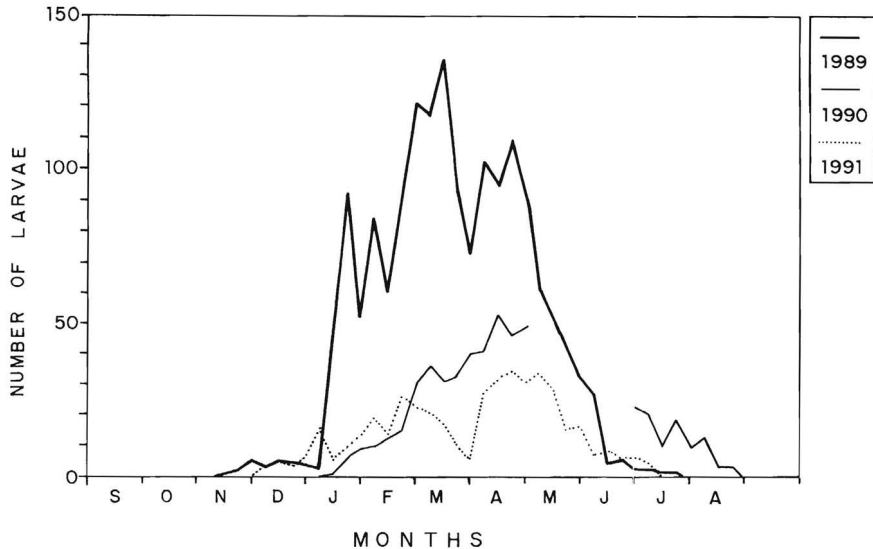


FIG. 1. Total number of *Anaea ryphea* larvae censused at Campinas during the three years of study.

creased abruptly in May. The abundance peak was in late February/early March. In the second year (September 1989 to August 1990) population growth was gradual from January to April and so was the decrease, from late June until September, with a peak in April. During the third year (September 1990 to June 1991), increase was still more gradual. The highest densities were found in April and May.

The beginning of the rainy season affects many tropical species' abundance (Wolda 1983). For *A. ryphea* this could be the best time for a populational increase, because the host plant population not only grows during this period, but also the individuals leaf out. But strong rains are not favorable to the survivorship of *A. ryphea* younger imatures, which can be mechanically dislodged from the leaves by the force of the raindrops. Strong rains might also affect oviposition behavior, although this was not measured. In this sense, an *A. ryphea* population might be adversely impacted during the beginning of the rainy season and prevented from reaching higher density levels. Perhaps the observed variations in population growth in this study could be

FIG. 2. Climographs for the three years of study at Campinas.

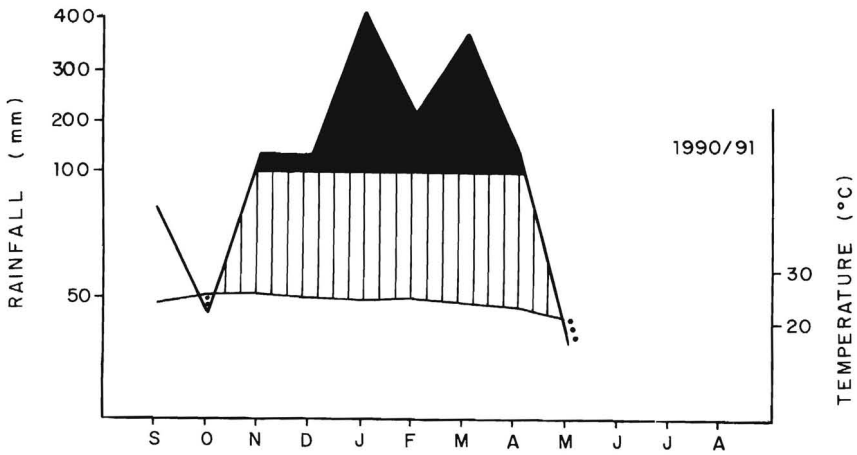
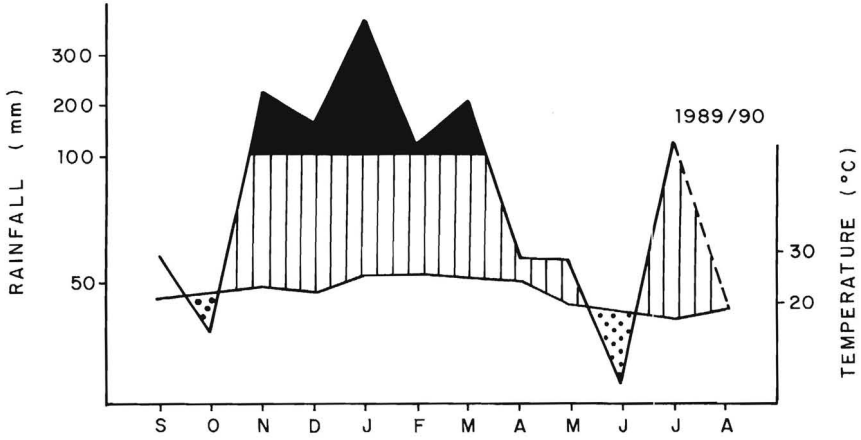
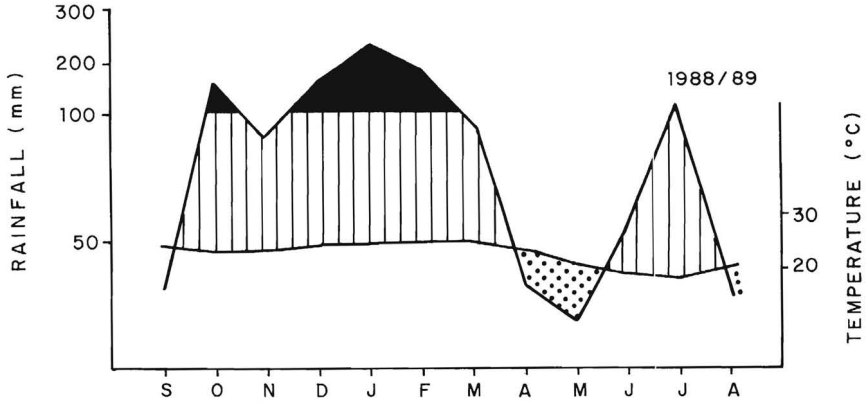


TABLE 1. Vertical life tables for *Anaea ryphea* for the three years of study at Campinas (* = total value for k ; 1S, first instar; 2S, second instar; etc.).

X	Total (N)	"Dead"	Mortality facator	$\log N_x$	k_x
Year 1988/89					
1S	1161	563	Rain(+)/Predation	3.065	0.288
2S	598	290	Rain(-)/Predation	2.777	0.288
3S	308	7	Virus (?)	2.489	0.010
4S	301	28	Parasitism	2.479	0.043
5S	273			2.436	0.629*
Year 1989/90					
1S	184	110	Rain(+)/Predation	2.265	0.396
2S	74	8	Rain(-)/Predation	1.869	0.050
3S	66	3	Virus (?)	1.819	0.020
4S	63	5	Parasitism	1.799	0.036
5S	58			1.763	0.502*
Year 1990/91					
1S	154	61	Rain(+)/Predation	2.187	0.219
2S	93	6	Rain(-)/Predation	1.968	0.029
3S	87	9	Virus (?)	1.939	0.047
4S	78	42	Parasitism	1.892	0.336
5S	36			1.556	0.631*

related to the rainfall pattern of each year, which follow. In the first year, the rainy season started in mid-September (Fig. 2), reaching a maximum of 229.2 mm in January 1988; the dry season started between March and April 1989, and lasted for 2 months. The following year was different: it was dry until mid-October 1989, then a very humid period (maximum 373.1 mm in January 1990) persisted until May. The third year was similar to the second: a dry period somewhat milder in September and October 1990, then a strong rainy period (maximum 418.3 in January 1991) until April. The fact that the rainy season started earlier and was of lower intensity in 1988 could have allowed a higher recruitment of *A. ryphea* in 1988/1989, leading to a population peak higher than during the other two years, when it rained heavily during spring and recruitment levels were low. Indeed, correlation coefficients between monthly k -values and rainfall proved significant both in 1989 and 1991 ($r^2=0.79$ and 0.88 respectively, $p<0.05$), but this hypothesis needs further examination during shorter intervals of time, in order to assess the full impact of rain on a daily basis.

Highest mortality was recorded during the first stadium, as shown by vertical life tables over the 3 years of study (Table 1). Recruitment pattern in first instar larvae of *A. ryphea*, which is the percent of

TABLE 2. *K*-values for *Anaea ryphea* life tables constructed at Campinas. *n* = number of individuals marked; k_x = *k*-value for instar *x*; T, total *k*-value for the month.

	Jan 89 n = 144	Feb 89 n = 371	Mar 89 n = 445	Apr 89 n = 167	May 89 n = 34
k_1	0.266	0.382	0.266	0.214	0.171
k_2	0.194	0.396	0.308	0.197	0.282
k_3	0.066	0.102	0.011	0	0
k_4	0.520	0.430	0	0	0
T	1.046	1.310	0.585	0.411	0.453
	Jan 91 n = 45	Feb 91 n = 23	Mar 91 n = 53	Apr 91 n = 35	May 91 n < 5
k_1	0.423	—	0.278	0.266	
k_2	0.23	0.158	0.031	0	
k_3	0	0.090	0	0	
k_4	0	0.336	0.375	0.278	
T	0.654	0.584	0.684	0.544	

individuals in this instar that survive to the next one, is in part dependent on the abundance of females and their oviposition behavior in the study area, which were not fully assessed. Nevertheless, their level of mortality was high. Survivorship of second and later instars was generally high (90% or higher per instar), but in 1988/89 second instar individuals had a 48.5% mortality, and in 1990/91 53.7% of fourth instar larvae died. There was no record of high mortality for this instar in prior years of the study. In 1991, first instar mortality was highest in January but fourth instar parasitism was the dominant source of mortality in February and March. Fifth instar could not be evaluated because individuals abandoned the foodplant to pupate.

Monthly life tables show higher mortality in the rainy period of January and February 1989, followed by relatively high survivorship between March and May (Table 2). The decrease in mortality was due to a strong reduction in parasitism of fourth instar larvae together with increased survivorship of first and second instars, the latter apparently related to the reduction of rainfall during the same period. Pollard (1979) found that, in four years of study of the white admiral in England, weather conditions were the main cause of population fluctuations between years. When weather conditions became unfavorable, larvae took a longer time to complete development and were exposed to predators for a longer period of time. Thus, the combination of adverse weather and increased exposure to predation led to striking differences in population sizes between years.

Key-factor analysis for monthly life tables showed that first and third instars were the stages most responsible for variation in abundance between months for the years of 1989 and 1991 respectively (Table 3).

TABLE 3. Key-factor analysis through coefficient of variation using vertical monthly life tables in different years.

Age	Average number of individuals per census	Coefficient of variation per census
a. 1989		
1S	232.20	73%
2S	119.62	69%
3S	61.66	62%
4S	57.00	66%
5S	44.80	90%
b. 1991		
1S	39.00	33%
2S	21.75	22%
3S	17.75	37%
4S	18.75	29%
5S	8.50	28%

It is important to note that in vertical life tables (counting individuals in the same stadium at certain time intervals), the same larva may be counted more than once. That is, because parasitism and disease tend to lengthen the duration of the affected instar, attacked individuals are likely to remain in the same instar (before they died) longer than "normal" larvae, and may be responsible for an underestimation of the mortality for those instars in sequential counts, which were then gathered within each month. Because I knew which larvae had been parasitized, they could be excluded from counts made after the average time of duration for that stadium. This means that there is no underestimation of mortality for any instar.

In each of the 3 years of study there was a different oviposition pattern, probably due to variation in the abundance of females during the season and to meteorological conditions. The correlation coefficient between number of new eggs and relative humidity of the night before censuses in the third year of study was 0.74 ($r^2=0.55$, significant for $p<0.05$), suggesting that relative humidity positively influences oviposition. It has been shown that, for some lepidopteran species, certain environmental conditions trigger or enhance oviposition. The number of eggs of the pierid *Leptidea sinapis* found in the field in a 8-year study was highly correlated with the maximum temperature of that day ($r = 0.73$) (Warren et al. 1986), and for *Anthocaris cardamines* the number of eggs laid is dependent on the insolation during the oviposition period (Courtney & Duggan 1983). Whether relative humidity acts as a cue or just influences oviposition rates cannot be assessed with the present data.

Censuses enabled me to calculate the percentages of parasitized,

TABLE 4. *Anaea ryphea* egg survivorship at Campinas in 1990/91 (actual values; * = total k -value for eggs).

Total (N)	"Dead"	Mortality factor	log N	k
653	351	disappearing	2.814	0.334
302	169	parasitized	2.480	0.357
133	31	nonfertile	2.123	0.115
102			2.008	0.806*

disappeared, and non-eclosing eggs (Table 4). The percentage of disappeared eggs (53.7%) was almost the same as the incidence of parasitism in the remaining ones (55.9%), an unusually high level for Lepidoptera. In most cases, parasitism was under 20%, predation being the main mortality factor. Hassel et al. (1989) found only 3 cases in 28 Lepidoptera studies where egg parasitism was considered an important mortality source, acting in a density-dependent way. But even in those cases its impact was low, as opposed to my study where it is almost as high as predation, the main mortality factor.

Competition is seldom considered a mortality factor in life table studies. Interspecific competition is rarely seen among phytophagous insects in the field, at least directly. *Hypna clytemnestra* (Lepidoptera: Nymphalidae) could be considered a potential competitor when it co-occurs with *A. ryphea* (Caldas 1991, 1994). Observations on asymmetric competition between these two species indicate a low-intensity interaction, because the *H. clytemnestra* population was never higher than 10% of the *A. ryphea* population in the area (Caldas 1991). Yamamoto (1981) found that the co-occurrence of two pierid species on the same foodplant affects the number of eggs laid on these plants and the larval survivorship.

The population of *A. ryphea* at Campinas seems to be well below the limits imposed by availability of its larval foodplant, as not a single defoliated plant was found. Larval feeding damage by *A. ryphea*, although not measured or tested, is not thought to adversely affect the food plants in a significant way. Larvae do not feed on apical parts of the plants (Caldas 1994), and therefore have little direct impact on plant growth or reproduction. Also, the fact that females tend to oviposit on leaves free of other eggs or larvae (Caldas 1994), together with high larval mortality, results in reduced herbivore impact on the plants.

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LITERATURE CITED

- BAKER, R.R. 1969. The evolution of migratory habit in butterflies. *J. Anim. Ecol.* 38: 703-746.
- CALDAS, A. 1991. A population of *Anaea ryphea* (Nymphalidae) and its larval food plant at Campinas, Brazil. *J. Lepid. Soc.* 45:68.
- . 1994. Biology of *Anaea ryphea* (Nymphalidae) in Campinas, Brazil. *J. Lepid. Soc.* 48:248-257.
- CLARK, L. R., P. W. GEIER, R. D. HUGHES, & R. F. MORRIS. 1967. The ecology of insect populations in theory and practice. Chapman and Hall, London.
- COURTNEY, S. P. & A. E. DUGGAN. 1983. The population biology of the Orange Tip butterfly *Anthocaris cardamines* in Britain. *Ecol. Entomol.* 8:271-281.
- CRAWLEY, M. J. 1989. Insect herbivores and plant population dynamics. *Ann. Rev. Entomol.* 34:531-564.
- DEMPSTER, J. P. 1984. The natural enemies of butterflies, pp. 97-104. *In* Vane-Wright, R. I. & P. R. Ackery (eds.), *The biology of butterflies*. Academic, London.
- DEVRIES, P. J. 1987. *The butterflies of Costa Rica and their natural history*. Princeton University Press, New Jersey. 325 pp.
- HASSELL, M. P. 1986. Parasitoids and population regulation, pp. 201-224. *In* Waage, J. & D. Greathead (eds.), *Insect parasitoids*. Academic Press, London.
- , J. LATTO, & R. M. MAY. 1989. Seeing the wood for the trees: Detecting density dependence from existing life-table studies. *J. Anim. Ecol.* 58:883-892.
- & J. K. WAAGE. 1984. Host-parasitoid population interactions. *Ann. Rev. Entomol.* 29: 89-114.
- KARBAN, R. 1993. Induced resistance and plant density of a native shrub, *Gossypium thurberi*, affect its herbivores. *Ecology* 74: 1-8.
- MALTAIS, P. M., J. J. JUILLET, & D. D. OLIVEIRA. 1980. Écologie et dynamique des populations de la tenthrede du mélèze *Pristiphora erichsonii* (Htg.) au Parc National de Kouchibouguac au Nouveau-Brunswick. *Ann. Soc. Ent. Québec* 25:141-162.
- MORRIS, R. F. 1959. Single factor analysis in population dynamics. *Ecology* 40:580-588.
- POLLARD, E. 1979. Population ecology and change in range of the white admiral butterfly *Ladoga camilla* in England. *Ecol. Entomol.* 4:61-74.
- RAUSHER, M. D., K. IWAO, E. L. SIMMS, N. OHSAKI, & D. HALL. 1993. Induced resistance in *Ipomoea purpurea*. *Ecology* 74:20-29.
- RILEY, T. J. 1988. Effect of larval photoperiod on mating and reproductive diapause in seasonal forms of *Anaea andria* (Nymphalidae). *J. Lepid. Soc.* 42:263-268.
- RISCH, S. 1980. The population dynamics of several herbivorous beetles in a tropical agroecosystem: the effect of intercropping corn, beans and squash in Costa Rica. *J. Appl. Ecol.* 17:593-612.
- SOKAL, R. R. & F. J. ROHLF. 1981. *Biometry*. Freeman, San Francisco.
- STILING, P. 1988. Density-dependent processes and key factors in insect populations. *J. Anim. Ecol.* 57:581-593.
- STRONG, D. R., J. H. LAWTON, & T. R. E. SOUTHWOOD. 1984. *Insects on plants: Community patterns and mechanisms*. Blackwell, Oxford.
- VARLEY, G. C. & G. R. GRADWELL. 1960. Key factors in population studies. *J. Anim. Ecol.* 29:399-401.
- WAAGE, J. & D. GREATHEAD (eds). 1986. *Insect parasitoids*. Academic Press, London.

- WARREN, M. S., E. POLLARD, & T. J. BIBBY. 1986. Annual and long-term changes in a population of the wood white butterfly *Leptidea sinapis*. *J. Anim. Ecol.* 55:707-719.
- WOLDA, H. 1978. Fluctuations in abundance of tropical insects. *Am. Nat.* 112:1017-1045.
- . 1983. Spatial and temporal variation in abundance in tropical animals, pp. 93-105. *In* Sutton, S. L., T. C. Whitmore, & A. C. Chadwick (eds.), *Tropical rain forest: ecology and management*. Blackwell Scientific Publications, Oxford.
- YAMAMOTO, M. 1981. Comparison of population dynamics of two pierid butterflies, *Pieris rapae crucivora* and *P. napi nesis* living in the same area and feeding on the same plant in Sapporo, Northern Japan. *J. Fac. Sci. Hokkaido Univ. Ser. VI, Zool.* 22:202-249.
- YOUNG, A. M. & M. W. MOFFET. 1979. Studies on the population biology of the tropical butterfly *Mechanitis isthmia* in Costa Rica. *Am. Midl. Nat.* 101:309-319.

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