Neotrop Entomol
DOI 10.1007/s13744-013-0170-0

ECOLOGY, BEHAVIOR AND BIONOMICS

Egg Phenology of a Host-Specialist Butterfly in the Western Slopes of the Northern Chilean Andes

HA Vargas[1], HA Benítez[2,3]

[1]Depto de Recursos Ambientales, Fac de Ciencias Agronómicas, Univ de Tarapacá, Arica, Chile
[2]Fac of Life Sciences, The Univ of Manchester, Manchester, UK
[3]Instituto de Alta Investigación, Univ de Tarapacá, Arica, Chile

Keywords
Folivorous, Lepidoptera, monophagous, Pieridae, temporal abundance

Correspondence
HA Vargas, Depto de Recursos Ambientales, Fac de Ciencias Agronómicas, Univ de Tarapacá, Casilla 6-D, Arica, Chile; havargas@uta.cl

Edited by Fernando L Cônsoli – ESALQ/USP

Received 2 January 2013 and accepted 29 August 2013

Abstract

Phenological studies are especially important in order to understand the ecological process operating at temporal level. The western slopes of the northern Chilean Andes at about 3,500 m asl are a mosaic of arid environments in which precipitations are highly seasonal, mostly concentrated in summer. *Teriocolias zelia andina* Forbes (Lepidoptera: Pieridae) is one of the most conspicuous and regularly observed butterflies flying in this region; it is a host specialist associated with the native shrub *Senna birostris* var. *arequipensis* (Fabaceae). The objectives of this study were (1) to characterize the temporal variations in the relative abundance of eggs of this host-specialist butterfly and (2) to examine the relationship of these variations with leaf phenology. Monthly samplings of eggs were carried out from February 2011 to January 2012. Circular statistical analyses of the relative abundance of eggs indicated clustered distribution along the year with the mean vector in June. Temporal variation in the relative abundance of eggs was correlated (Spearman rank correlation test) with the availability of plant substrate for egg laying and larval feeding.

Introduction

Phenology is the temporal distribution of a phenomenon (Wolda 1988). Phenological studies, in which characterizations of temporal patterns of abundance of organisms are provided and underlying causes are determined, are extremely important in order to adequately understand the ecological process operating at temporal level. This knowledge is useful when forecasting is required for any purpose, either in anthropic or natural environments (Primack *et al* 2009).

Temporal suitability of the environments is an important factor modelling temporal patterns of animals and plants because successful reproduction and growth may be often limited to a short period each year (Visser & Both 2005). Dealing with insect–plant relationships, the host plant phenology may have a key role as a determinant of many aspects of the ecology of the associated phytophagous insects (Hodkinson *et al* 2001). This situation could be especially relevant when highly specialized insects are involved. Furthermore, plant phenology is also a strong selective force (Wood *et al* 1990, Mopper 2005), affecting directly the survival of immature stages or indirectly the success of adult mating (Mopper 2005).

Insect phenology is best known and understood in temperate than in tropical zones (Wolda 1988, Hilt *et al* 2007). In general, seasonality of tropical insects has been reported for locations with clearly defined dry and wet seasons (Wolda 1988, Kitching *et al* 2000, Aguirre *et al* 2011, Muniz *et al* 2012). However, different patterns may occur in the same location (Pinheiro *et al* 2002, Hilt *et al* 2007, Aguirre *et al* 2011, Silva *et al* 2011).

Few phenological studies have been carried out on insects of the tropical environments in South America; however, some interesting patterns have been already detected, either at population or at community level. Some of these studies have included immature stages or adults of Lepidoptera, one of the most diverse orders of insects, whose importance as subject of ecological studies has been largely acknowledged (DeVries *et al* 1997). The most important factors associated

Published online: 04 October 2013

with seasonality of these organisms in these locations are average temperature, seasonality of rain, and food resource availability (Pinheiro *et al* 2002, Bendicho-Lopez *et al* 2003, Hilt *et al* 2007, Pessoa-Queiroz *et al* 2008, Ribeiro *et al* 2010, Silva *et al* 2011, Muniz *et al* 2012).

The western slopes of the northern Chilean Andes at about 3,500 m above sea level (masl) are a mosaic of arid landscapes largely dominated by shrubs (Luebert & Pliscoff 2006, Muñoz &Bonacic 2006). Despite the great biological interest of this area, associated with the aridity, the ecology of invertebrates inhabiting these environments is poorly known. Changes in temperature along the year are minimal in these landscapes, while the water input is determined by the highly seasonal rainfall, which is mostly concentrated between December and February, while a long dry season goes from March to November (Luebert & Pliscoff 2006). Under these conditions, temporal abundance patterns of host-specialist phytophagous insects inhabiting these arid environments are expected to be highly seasonal and mostly determined by food availability. The aim of this study was to characterize the temporal variation in the relative abundance of eggs of one host-specialist butterfly and to examine its relationship with leaf phenology.

Material and Methods

Study site

The work was carried out in the western slopes of the northern Chilean Andes, at about 3,300 m asl, in the surroundings of Socoroma village (18°16′S, 69°35′W), Parinacota Province. This area is characterized by a tropical xeric bioclimate (Luebert & Pliscoff 2006). In general, vegetation cover is seasonal, reaching higher levels after rains, during April–May (Muñoz & Bonacic 2006).

Study species

Teriocolias zelia andina Forbes (Lepidoptera: Pieridae) is one of the most conspicuous and regularly observed native butterflies flying in this area (Peña & Ugarte 1996). It has been reported as a monophagous species in the study site, with egg laying and subsequent larval feeding and development restricted to the shrub *Senna birostris* var. *arequipensis* (Fabaceae) (Vargas 2012), which is a very important component of the native flora in this location. Eggs are placed singly on new or mature leaflets; the time elapsed from egg laying to adult emergence is about 45 days.

Sampling

Eggs were collected at monthly intervals from February 2011 to January 2012. Fifty plants were randomly selected each time, and a careful examination was carried out to determine the total number of eggs on each. Phenological studies on the host plant are unknown for the study area; thus, the number of plants with new or mature leaves was monthly recorded in order to estimate the availability of substrate for egg laying and larval feeding. New leaves were those not completely expanded or that have just completely expanded, typically dark green and lightly flexible. Mature leaves were completely expanded, whitish green, and coriaceous. Additionally, the number of plants with old (light yellow or light brown) leaves was also determined.

Statistical analyses

A chi-square test was performed to determine if eggs were randomly distributed among new and mature leaves. Descriptive circular analyses were performed to characterize the monthly relative abundance of eggs. The Rayleigh uniformity test was conducted to verify if the eggs were uniformly distributed along the year. Circular statistics is a tool of great importance for data analysis in studies of temporal variation of biological phenomena, although these procedures have not been frequently employed in published contributions dealing with phenology (Hudson 2010). However, these tools have been successfully used in studies of temporal variation in the Neotropics, either with plants (Morellato *et al* 2000) or insects (Pinheiro *et al* 2002, Silva *et al* 2011), including butterflies (Ribeiro *et al* 2010).

In order to determine if the relative abundance of eggs is related with leaf phenology and/or some abiotic factor, Spearman rank correlation analyses were conducted with the following variables: maximum and minimum temperature (in degrees Celsius), precipitation (in millimeters), number of plants with new leaves, number of plants with mature leaves, and number of plants with old leaves. Following Pinheiro *et al* (2002), analyses were performed with the variables measured in the respective sampling month and the variables measured in the previous month (delayed variable), because the time elapsed from egg laying to adult emergence of this butterfly is about 45 days. Climatic information was obtained from the Instituto de Investigaciones Agropecuarias, INIA-URURI (Fig 1). Statistical analyses were performed with the software BioEstat 5.0 (Ayres *et al* 2007).

Results

Relative age of leaves used for egg laying

Overall, 274 eggs were counted during the sampling period, 233 of which were found on new leaves, while the remaining 41 were on mature leaves. No eggs were found on old leaves.

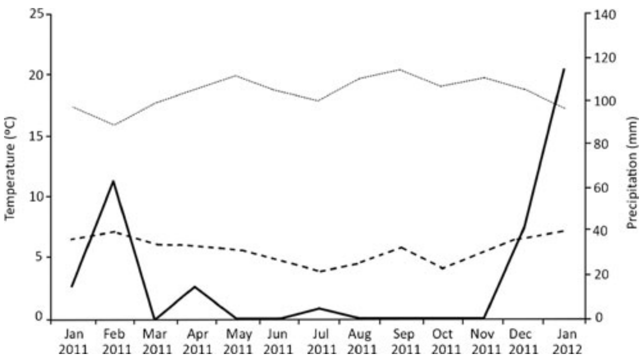

Fig 1 Climate data from the meteorological station of Socoroma (18°16′ S, 69°35′W), Parinacota Province, occidental slopes of northern Chilean Andes, from January 2011 to January 2012; *dotted line*-maximum temperature; *dashed line*-minimum temperature; *solid line*-precipitation.

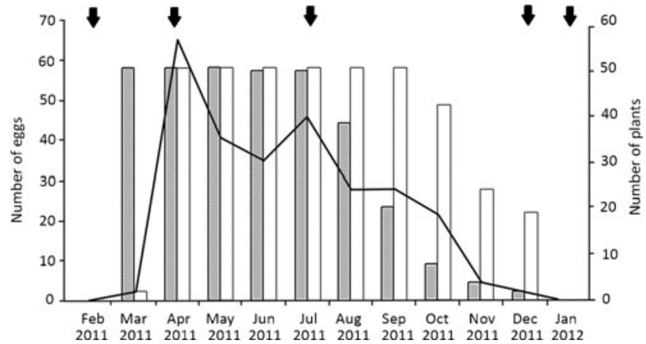

Fig 2 Number of eggs (*black line*) of *Teriocolias zelia andina* (Lepidoptera: Pieridae) and number of plants with new (*gray bars*) or mature (*white bars*) leaves in 50 individuals of *Senna birostris* var. *arequipensis* (Fabaceae) in the occidental slopes of the Andes, Parinacota Province, northernmost Chile, from February 2011 to January 2012. *Black arrows* indicate rains.

Then, relative ages were differentially used for egg laying (χ^2=134.54; *df* =1; p <0.0001).

Phenological pattern

The 274 eggs counted during the sampling period were found between March and December 2011 (Fig 2). These were not uniformly distributed throughout the year (Rayleigh test, p <0.001; Table 1); the mean vector (127.3555°, Table 1) was located in June. Interestingly, 88.7% of the eggs were concentrated in autumn (n =141) and winter (n =102) samplings, whereas no eggs were found in summer months February 2011 and January 2012.

Relationships with biotic and abiotic factors

The relative abundance of eggs was positively and significantly correlated with the two variables associated to substrate availability in the collecting month: number of plants with new leaves (p =0.0048; Table 2) and number of plants with mature leaves (p <0.0001; Table 2). A similar situation was observed with resource availability measured in the previous month, but it was restricted to the number of plants with new leaves (p <0.0001; Table 2). Availability of plants with old leaves was not significantly correlated with the relative abundance of eggs, either in the sampling month or in the previous month (Table 2). The only climatic variable significantly associated with the relative abundance of eggs was minimum temperature measured in the sampling month, but this relationship was negative (p =0.0250; Table 2).

Discussion

The differential use of leaf relative age suggests that females search and select new leaves for oviposition. This specialized behavior could be underlying the temporal pattern here detected. Then, as expected for a highly host-specialist butterfly living in a location with highly seasonal rain distribution, eggs were not uniformly distributed throughout the year (Table 1 and Fig 1), and it was strongly correlated with the level of availability of host plant substrate for egg laying and larval development (Table 2). The high relative abundance of eggs during the first half of the dry season could be understood as a cumulative effect of leaf production prompted by the wet season. Surprisingly, the scant precipitations during the wet season, in January and February 2011 (77.3 mm; Fig 1), were enough to stimulate leaf growth (Fig 2). Interestingly, the 7 months previous to January 2011 were extremely dry, with just 0.2 mm precipitation in November 2010. During 2011, "unusual" rains were recorded during the (normally) dry season (Fig 1), in April (14.4 mm) and July (4.4 mm), which could have contributed, at least partially, in maintaining the active leaf growth, because a great quantity of plants with new leaves was found until August, decreasing quickly from September (Fig 2). Thus, the subsequent decrease in the relative abundance of eggs during the second half of the dry season could be understood as the inevitable effect of reduction in the availability of adequate leaves. The absence of significant correlation between the relative abundance of eggs and old leaves, either in the sampling month or in the previous month (Table 2), was expected because these leaves are not used for egg laying or larval feeding. The negative relationship between the relative abundance of eggs and minimum temperature in the collecting month (Table 2) is not well understood.

The higher relative abundance of eggs during the dry season recorded for *T. zelia andina* resembles patterns reported for lepidopteran larval abundance in the Brazilian Cerrado, with peaks mostly reached in the first half of the dry season (Morais *et al* 1999, Diniz *et al* 2007). In the same biome, a similar pattern was documented for larvae of the bivoltine micro-moth *Chlamydastis platyspora* (Meyrick)

Table 1 Descriptive circular statistics and Rayleigh one-sample test for egg abundance of *Teriocolias zelia andina* (Lepidoptera: Pieridae) on *Senna birostris* var. *arequipensis* (Fabaceae) in the neighboring area of Socoroma village (18°16′S, 69°35′W), at about 3,300 m asl, in the occidental slopes of the Andes, Parinacota Province, northernmost Chile, from February 2011 to January 2012.

Descriptive circular statistics	
Observations (n)	274
Mean vector (μ)	127.3555°
Confidence interval (95%)	118.600–136.111
Length of mean vector (r)	0.5134
Circular variance	0.4866
Circular standard deviation	66.16097
One-sample test	
Rayleigh test (Z)	72.2209
Rayleigh test (p)	<0.01

(Lepidoptera: Elachistidae), whose higher larval abundance was found in the dry season (Bendicho-Lopez *et al* 2003). However, in another phenological study performed also in the Brazilian Cerrado, the peak of larval abundance of the micro-moth *Gonioterma exquisita* Duckworth (Lepidoptera: Elachistidae) was reached in the wet season (Pessoa-Queiroz *et al* 2008).

Table 2 Spearman rank correlation analysis between egg abundance of *Teriocolias zelia andina* (Lepidoptera: Pieridae) on *Senna birostris* var. *arequipensis* (Fabaceae) and some biotic and abiotic variables measured in the collecting month and previous (delayed) month in the neighboring area of Socoroma village (18°16′S, 69°35′W), at about 3,300 m asl, in the occidental slopes of the Andes, Parinacota Province, northernmost Chile, from February 2011 to January 2012.

Factor	n	r_s	df	p
NPNL	12	0.7518	10	0.0048[a]
NPML	12	0.9361	10	<0.0001[a]
NPOL	12	0.4116	10	0.1836 ns
MAXT	12	0.4148	10	0.1800 ns
MINT	12	−0.6397	10	0.0250[a]
PP	12	−0.3724	10	0.2332 ns
NPNL-D	12	0.9290	10	<0.0001[a]
NPML-D	12	0.5602	10	0.0581 ns
NPOL-D	12	0.0517	10	0.8732 ns
MAXT-D	12	0.0316	10	0.9223 ns
MINT-D	12	−0.3374	10	0.2834 ns
PP-D	12	−0.4969	10	0.1002 ns

NS nonsignificant correlation, *NPNL* number of plants with new leaves, *NPML* number of plants with mature leaves, *NPOL* number of plants with old leaves, *MAXT* maximum temperature, *MINT* minimum temperature, *PP* precipitation, *-D* "delayed" variable.

[a] Significant correlation.

Although the patterns of temporal abundance reported for *C. platyspora* and *G. exquisita* are too different, with higher larval abundance in the dry season for the first species and in the wet season for the second, it is important to indicate that larvae of both micro-moths are highly specialized in food use, preferring a specific leaf age. Host plants with mature and old leaves are more often used by larvae of *C. platyspora* than plants only with new leaves or only with mature leaves (Bendicho-Lopez *et al* 2003), whereas larvae of *G. exquisita* are specialized on mature leaves of their host plant, and their abundance is positively correlated with the availability of leaves at this phonological stage (Pessoa-Queiroz *et al* 2008).

Similarly, there is higher abundance of eggs and larvae of the specialist butterfly *Eunica bechina* (Hewitson) (Lepidoptera: Nymphalidae), whose larvae feed on new leaves of the host plant *Caryocar brasiliense* (Caryocaraceae), in the beginning of the rainy season in the Brazilian Cerrado, concurrently with the period of abundance of new leaves (Muniz *et al* 2012).

Therefore, regardless of dry or wet seasons, the temporal abundance pattern of larvae of these highly specialized folivorous species may be explained by the leaf phenology of the respective host plant, which is consistent with the results reported here for *T. zelia andina*.

No eggs of *T. zelia andina* were found in February 2011 and January 2012, which is coincident with the current absence of new or mature leaves in the study site. This fact suggests that diapause could be involved at some stage of the life cycle. However, more than 50 individuals have been reared until adult stage in laboratory from eggs or larvae collected in the field in different months of the year without observation of diapause at the egg, larval, or pupal stages (HAV, unpublished data). At adult stage, reproductive diapause has not been studied for *T. zelia andina*, although this is a strategy used by some tropical seasonal butterflies (Braby 1995, Canzano *et al* 2003, Pieloor & Seymour 2001). However, although adult abundance was not determined in this study, adults were searched each month in the same day of the egg samplings in order to verify their active presence in the study site. As a result, these were found in all sampling dates. Therefore, the absence of eggs in the samplings is not due to the absence of adults.

Furthermore, apparently reproductive diapause was not affecting these butterflies, because during the last days of November, when the relative abundance of eggs and frequency of plants with new or mature leaves were drastically reduced in the sampling site, one plant with abundant new and mature leaves was found in Socoroma village, at a distance about 3 km from the sampling site. This "anomalous" phenological stage was triggered by accidental irrigation from a nearby canal. The plant was carefully examined, and 17 eggs were found. This fact is suggesting an opportunistic behavior of the females, which would be able to find plants in the adequate phenological stage for egg laying. Unfortunately, phenological studies on the host plant in the western slopes of northern Chile were not found in literature. However, considering that precipitation is not always evenly distributed in space, under natural conditions, some plants could be expected to be out of the general phenological pattern, offering egg laying and food substrate for the butterfly throughout the year.

Based on the available data, it is evident that the most important proximate cause, as defined by van Schaik *et al* (1993), for the temporal pattern of egg abundance of *T. zelia andina* in the western slopes of the northern Chilean Andes is the availability of substrate for egg laying and larval feeding. Thus, it is expected that any factor modifying the plant phenology will be reflected in the butterfly phenology.

Besides the geographical variation, precipitation may also vary year after year, with the subsequent effect on plant phenology. Thus, future studies should assess the possible variation in phenology of the host plant–butterfly system at geographic and interannual scale.

Acknowledgments The authors thank Fiorella Vallebuona and Manuel Rodríguez for their help with good advice in the statistical part of the manuscript; Manuel Ayres for information about BioEstat 5.0; Paulo S. Oliveira, Universidade Estadual de Campinas, Brazil, for kindly sending literature; Valeska González, INIA-URURI, Arica, Chile, for making available climatic data; and M. R. Lazo de la Vega, for checking the English version. Financial support was obtained from Project DIEXA-UTA 9710–10, from Universidad de Tarapacá.

References

Aguirre LF, Anderson EP, Brehm G, Herzog SK, Jørgensen PM, Kattan GH, Maldonado M, Martínez R, Mena JL, Pabón JD, Seimon A, Toledo C (2011) Phenology and specific ecological interactions of Andean biota in the face of climate change. In: Herzog SK, Martinez R, Jørgensen PM, Tiessen H (eds) Climate change and biodiversity in the Tropical Andes. Inter-American Institute for Global Change Research (IAI) and Scientific Committee on Problems of the Environment (SCOPE), pp 68–92

Ayres M, Ayres Jr M, Ayres DL, Santos AS (2007) BioEstat Versão 5.0. Sociedade Civil Mamirauá, MCT-CNPq, Belém, Pará, Brasil

Bendicho-Lopez A, Diniz IR, Hay JD (2003) Abundance of *Clamydastis platyspora* (Elachistidae) on its host plant *Roupala montana* (Proteaceae) in relation to leaf phenology. J Lepid Soc 57:291–294

Braby MJ (1995) Reproductive seasonality in tropical satyrine butterflies: strategies for the dry season. Ecol Entomol 20:5–17

Canzano AA, Jones RE, Seymour JE (2003) Diapause termination in two species of tropical butterfly, *Euploea core* (Cramer) and *Euploea sylvester* (Fabricius) (Lepidoptera: Nymphalidae). Aust J Entomol 42:352–356

DeVries PJ, Murray D, Lande R (1997) Species diversity in vertical, horizontal, and temporal dimensions of a fruit-feeding butterfly community in an Ecuadorian rainforest. Biol J Linn Soc 32:343–364

Diniz IR, Bernardes C, Rodovalho S, Morais HC (2007) Biology and occurrence of *Inga* Busk species (Lepidoptera: Oecophoridae) on Cerrado host plants. Neotrop Entomol 36:489–494

Hilt N, Brehm G, Fiedler K (2007) Temporal dynamics of rich moth ensembles in the montane forest zone in southern Ecuador. Biotropica 39:94–104

Hodkinson ID, Bird JM, Hill JK, Baxter R (2001) Host plant growth characteristics as determinants of abundance and phenology in jumping-plant lice on downy willow. Ecol Entomol 26:376–387

Hudson IL (2010) Interdisciplinary approaches: towards new statistical methods for phenological studies. Climatic Change 100:143–171

Kitching RL, Orr AG, Thalib L, Mitchell H, Hopkins MS, Graham AW (2000) Moth assemblages as indicators of environmental quality in remnants of upland Australian rainforest. J Appl Ecol 37: 284–297

Luebert F, Pliscoff P (2006) Sinopsis bioclimática y vegetacional de Chile. Editorial Universitaria, Santiago, p 316

Mopper S (2005) Phenology—how time created spatial structure in endophagous insect populations. Ann Zool Fenn 42:327–333

Morais HC, Diniz IR, Silva DMS (1999) Caterpillar seasonality in a central Brazilian cerrado. Rev Biol Trop 47:1025–1033

Morellato LPC, Talora DC, Takahasi A, Romera EC, Zipparro VB (2000) Phenology of Atlantic rain forest trees: a comparative study. Biotropica 32:811–823

Muniz DG, Freitas AVL, Oliveira PS (2012) Phenological relationships of *Eunica bechina* (Lepidoptera: Nymphalidae) and its host plant, *Caryocar brasiliense* (Caryocaraceae), in a Neotropical savana. Stud Neotrop Fauna E 47:111–118

Muñoz AE, Bonacic C (2006) Variación estacional de la flora y vegetación en la precordillera andina de la comuna de Putre (I Región de Tarapacá, Chile) durante el período 2002–2003. Gayana Bot 63:75–92

Peña LE, Ugarte AJ (1996) Las mariposas de Chile. Editorial Universitaria, Santiago, Chile, p 359

Pessoa-Queiroz R, Morais HC, Diniz IR (2008) Abundance and temporal distribution of *Gonioterma exquisita* Duckworth (Lepidoptera, Elachistidae, Stenomatinae) on *Byrsonima pachyphylla* Griseb. (Malpighiaceae) in the Brazilian Cerrado. Rev Bras Entomol 52:62–67

Pieloor MJ, Seymour JE (2001) Factors affecting adult diapause initiation in the tropical butterfly *Hypolimnas bolina* L. (Lepidoptera: Nymphalidae). Aust J Entomol 40:376–379

Pinheiro F, Diniz IR, Coelho D, Bandeira MPS (2002) Seasonal pattern of insect abundance in the Brazilian cerrado. Austral Ecol 27:132–137

Primack RB, Ibáñez I, Higuchi H, Lee SD, Miller-Rushing AJ, Wilson AM, Silander JA Jr (2009) Spatial and interspecific variability in phenological responses to warming temperatures. Biol Cons 142:2569–2577

Ribeiro DB, Prado PI, Brown KS Jr, Freitas AVL (2010) Temporal diversity patterns and phenology in fruit-feeding butterflies in the Atlantic forest. Biotropica 42:710–716

Silva NAP, Frizzas MR, Oliveira CM (2011) Seasonality in insect abundance in "Cerrado" of Goiás State, Brazil. Rev Bras Entomol 55:79–87

VanSchaik CP, Terborgh JW, Wright SJ (1993) The phenology of tropical forests: adaptive significance and consequences for primary consumers. Annu Rev Ecol Syst 24:353–377

Vargas HA (2012) First host plant record for *Teriocolias zelia andina* Forbes (Lepidoptera: Pieridae) and evidences for local specialization. Neotrop Entomol 41:524–525

Visser ME, Both C (2005) Shifts in phenology due to global climate change: the need for yardstick. Proc R Soc B 272:2561–2569

Wolda H (1988) Insect seasonality: why? Annu Rev Ecol Syst 19:1–18

Wood TK, Olmstead KL, Guttman SI (1990) Insect phenology mediated by host-plant water relations. Evolution 44:629–636