

Population dynamics of *Coccus viridis*, a ubiquitous ant-tended agricultural pest, assessed by a new photographic method

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Abstract

Ant and homopteran mutualisms are complex because they depend on the behavior, abundance, and predation levels of both insect species. Because homopteran populations are critically impacted by these seasonally varying factors, it is important to measure the population dynamics of ant-tended homopterans outside of the laboratory. In this study, we develop a new method for documenting the population dynamics of sessile ant-tended homopterans in the field, using digital photography. We monitored the population dynamics of *Coccus viridis* (Green) (Hemiptera Coccidae), the green coffee scale, over a two year period, with and without the protection of the ant, *Azteca instabilis* (Smith) (Hymenoptera Formicidae). Our results reveal that *C. viridis* birth is episodic over the course of a single day, and that long term population dynamics depend critically on seasonality. We found that parasitization, chewing insect predation, and fungal infection are distinctive between *C. viridis* life-stages and that all three are critical factors governing *C. viridis* population growth and survivorship.

Key words: scale, insect, coffee, Mexico, natural enemies.

Introduction

Habitat selection is an essential task for all organisms; it mediates the availability of food, mates, nesting sites and refugia from natural enemies. For phytophagous insects, population growth and survivorship depend critically on the availability of plant resources (Zamora *et al.*, 1999; reviewed in Peeters, 2002) and the plants' structural benefits in affording predator protection (Willmer, 1982; Larsson *et al.*, 1997; Sebolt and Landis, 2002). Insects that feed directly on plant phloem, like many within the Rhynchota or Hemiptera order (e.g. aphids, scale insects, mealy-bugs), often prefer feeding at sites with the greatest access to phloem, like primary or secondary leaf veins, where greater resource acquisition is believed to confer fitness benefits for some plant juice sucking insects (Gibson, 1972; Hubbard and Potter, 2005). A number of studies have additionally shown that, by engaging in a mutualism with ants, homopterans gain access to food resources while gaining enemy-free space. In these mutualistic interactions, phloem-feeding homopterans provide a carbohydrate rich exudate to ants in exchange for protection from predators (Way, 1963; Buckley, 1987a; Hanks and Sadof, 1990; Holldobler and Wilson, 1990; Oliver *et al.*, 2008).

However, ant-homopteran mutualisms are inherently complex, and the growth and survivorship of homopteran populations are dependent on a number of factors, such as ant and homopteran behavior, the abundance of ants and homopterans and the abundance of ant and homopteran predators across seasons (Buckley, 1987b; Harmon and Andow, 2007; Majerus *et al.*, 2007; Styrsky and Eubanks, 2007; Grover *et al.*, 2008; Livingston *et al.*, 2008). Homopteran growth and predation rates, with and without ant attendance, have historically been measured in highly simplified greenhouse or laboratory settings, and thus have often failed to incorporate the effects of seasonality and natural predator and prey dy-

namics in their estimation. Scale insects, phloem-feeding homopterans that are only mobile during their first life-stage, are especially dependent on site-specific resource availability and predator protection for sustained growth. For these immobile insects, the seasonal variation in ant protection and homopteran predation are critical and can only be accurately outside of the laboratory. In this paper, we develop a new methodology for assessing scale population dynamics in the field.

One sessile homopteran that is ubiquitous in natural and agricultural ecosystems is *Coccus viridis* (Green) (Hemiptera Coccidae), commonly known as the 'green coffee scale'. A common coffee and citrus pest, *C. viridis* can exhibit rapid population growth and has plagued coffee and citrus plantations across the globe for decades (Frederick, 1943). Homopteran-tending ants, like *Azteca instabilis* (Smith) (Hymenoptera Formicidae) (Vandermeer *et al.*, 2002; Vandermeer and Perfecto, 2006), and *Camponotus* and *Crematogaster* spp. (Hanks and Sadof, 1990) are known to actively patrol colonies of *C. viridis*, providing them with protection from predators and parasitoids. This mutualistic interaction is believed to contribute to rapid *C. viridis* population growth and resulting infestation of agroecosystems (Hanks and Sadof, 1990), yet studies have not explicitly examined *C. viridis* population dynamics with and without ant protection. Moreover, no studies have examined fine-scale growth and predation patterns for ant-tended homopterans across microhabitat types in the field.

Interestingly, the predominantly sessile life style of *C. viridis* also permits one major unexplored avenue for ecological research in the field: the detailed monitoring of population dynamics via digital photography. While plant demographers are capable of reporting large recapture rates, a large literature in insect ecology is devoted to the statistical analysis of recapture data which is almost always incomplete (e.g., Wileyto *et al.*, 1994; 2000; Alpizar-Jara and Smith, 2008). Insect ecologists

working with effectively sessile insects, such as scale insects, can utilize the advantages of a system with nearly complete recapture via digital photography.

In this study, we establish a new method for documenting scale insect growth and predation rates in the field, using digital photography. This methodology can be utilized for many sessile insects and we provide a detailed summary of our technique and analyses. Using this method, we document the first in-situ study of *C. viridis* population dynamics across multiple leaf microhabitats, in the presence and absence of ants, in a shade coffee system in southern Mexico.

Materials and methods

Study area

The study was conducted in the Soconusco, a coffee growing region in the highlands of Chiapas, Mexico. The farm examined, Finca Irlanda, is an organic shaded coffee farm (commercial polyculture, sensu Moguel and Toledo, 1999) where *Coffea arabica* L. (Rubiaceae) is grown under a canopy of diverse shade trees. Between 2006 and 2008, ten one ha plots were monitored within the shade coffee farm in order to record the population dynamics of *C. viridis* on coffee bushes located in each plot. Six of the ten sites were established in locations inhabited and patrolled by a large colony of the homopteran-tending ant, *A. instabilis*, while the four remaining sites were located far from ant colonies (minimum of 200m). In each site, 6-10 branches were selected for the study, each on separate coffee bushes, located more than two meters away from other sampled branches. In each branch, the abaxial surface of one leaf was photographed for a continued population survey.

Population survey

Two different photographing schedules were employed in order to examine *C. viridis* population dynamics over long and short time intervals, respectively:

Three years, weekly study:

Each population was photographed during the morning (between 0700 and 1100), once every 5-7 days from June to July of 2006, February to August of 2007, and July to August of 2008.

Ten hours study:

One population was photographed every hour in a single day, from 0700 to 1700.

Digital photographs of marked leaves were visually analyzed to document the presence and transition of *C. viridis* through three life stages: crawlers, nymphs, and adults. Since this species exhibits direct metamorphosis, stage categories are mainly based on size, although life history information suggests a minimum of three stages (Frederick, 1943); crawlers are not yet permanently fixed to the plant, nymphs are fixed to the plant but do not produce offspring, and adults are fixed to the plant and produce offspring. Because adults and nymphs are non-mobile, we define a single leaf as a single 'population'.

Using the digital photographs, we estimated the numbers of individuals of *C. viridis* in each life-history stage for each time-period. Analyses of the photos were conducted by importing the images into Serif PhotoPlus 6.0 (Serif Inc., 2002), a photo-processing software that allowed us to document mortality type and *C. viridis* population numbers using magnification and enhanced-contrast features. To aid in the counting and localization of all individuals across photo-dates for a single leaf, we delineated leaf sections based on primary and secondary leaf venation (figure 1). Based on the basic leaf structure, we examined three microhabitat types that decrease in vein accessibility: 1) the primary vein, 2) the 5 pairs of narrow basal secondary veins (starting from the pedicel, on both sides of the primary vein), and 3) the leaf area between the secondary veins. Data collection was not continued past the 5th secondary vein because the vast majority of leaves had no *C. viridis* individuals beyond this portion of the leaf.

Individual scales of all life stages were noted and marked within the photo-software and photos were compared with previous dates in order to assess life stage type and mortality for each individual scale. *C. viridis* suffers from three visibly diagnosable mortality types, 1) attack from hymenopteran parasitoids, 2) chewing insect predation, caused largely by coleopterans, such as *Azya orbiger* Mulsant (Coleoptera Coccinellidae) (Liere and Perfecto, 2008) and 3) fungal attack by the 'white halo fungus', *Verticillium lecanii* (Zimmerman) (Frederick, 1943).

Statistical analysis

We compared growth rates and birth rates across the three microhabitats for each of the life stages and for two treatment types, with ants and without ants. Growth rates for nymphs and adults were calculated as the proportion of individuals that move from one life stage to the next divided by the total number of individuals in the previous life stage at the previous time step. Given that crawler migration onto a new leaf is relatively low, the birth rates for crawlers were calculated as the number of new crawlers divided by the total number of adults in the previous time step. We calculated predation rates as the proportion of individuals predated at each time step. Because of the extreme variation and non-linear dynamics evident in *C. viridis* population growth and predation over time, we have devoted another paper to the modelling of these data using maximum likelihood via iterated filtering (MIF, e.g. Ionides *et al.*, 2006) and thus we do not examine the significance of explanatory variables in *C. viridis* population growth and predation in this paper.

Results

C. viridis crawlers are born and emerge from beneath their mothers over 2-3 hours across a single day (figure 2). The increase in crawler numbers is periodic, exhibiting a wavelike pattern, with multiple bursts of crawler emergence in a single day, each followed by a slow tapering until the following burst. Long term *C. viridis*

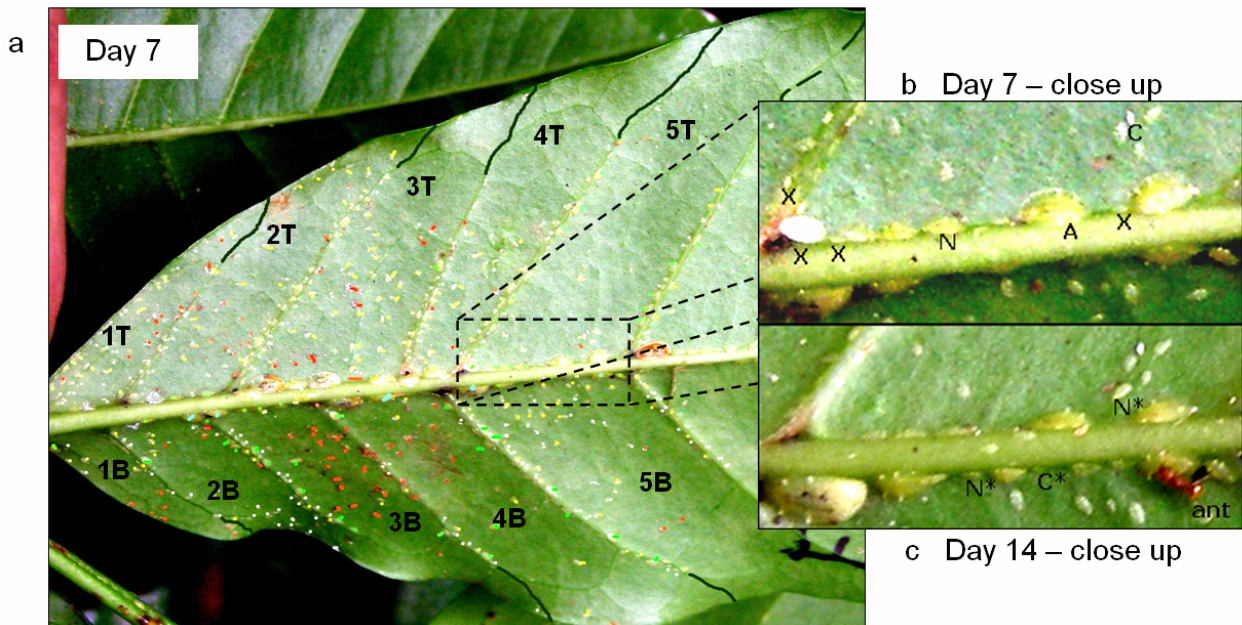


Figure 1. Example of digital photograph-based estimate of *C. viridis* population parameters. (a) The area of census includes the leaf area (labeled) and all veins, starting from the pedicel till the 5th secondary vein for both the top (T) and bottom (B) of the abaxial surface of the leaf. *C. viridis* individuals are labeled with colored marks which designate life stage and predation events since the previous photo. (b) Close up of leaf on day 7, where a crawler (C), nymph (N) and adult (A) are marked, and crosses (X) indicate individuals that are missing on the following photo, taken on day 14. (c) Close up of day 14, where new crawlers and nymphs are labeled with an asterisk (C* and N*) and *A. instabilis* is seen patrolling (ant). (In colour at www.bulletinofinsectology.org)

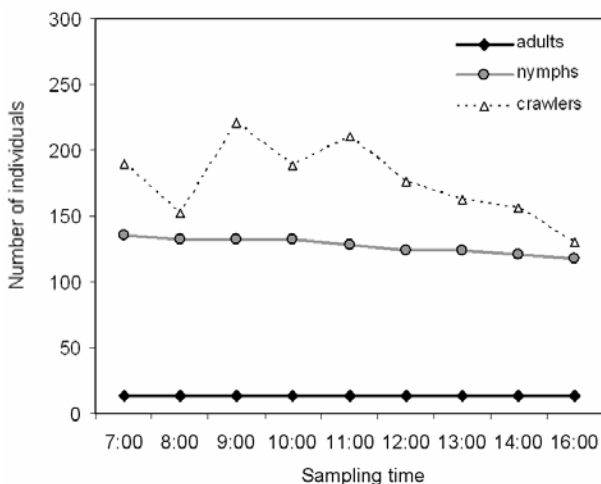


Figure 2. Population dynamics of *C. viridis* across a single day.

dynamics are extremely variable between populations and the growth trajectory of each life-stage is unpredictable (figure 3). In general, *C. viridis* populations reach higher densities and persist for longer periods of time in the presence of ants (figure 3). *C. viridis* population dynamics across microhabitats are also distinct (figure 4). The growth rates of *C. viridis* are similar across microhabitats, with slightly greater adult growth rates along the primary vein. Predation rates for adults and nymphs also appear lower in primary vein and sec-

ondary vein microhabitats than in leaf areas, while crawler predation is similar across within-leaf microhabitats (figure 5).

Monthly *C. viridis* adult and nymph parasitism rates decrease with the onset of the summer rainy season, while crawler parasitism rates do not increase or decrease seasonally. Monthly *C. viridis* adult and nymph chewing predation rates also decrease with the onset of the summer rainy season, while crawler chewing predation rates do not increase or decrease seasonally. Monthly *C. viridis* adult fungal rates appear bimodal, with the greatest fungal attack rates in May and August, while nymph and crawler fungal attack rates do not increase or decrease seasonally (figure 6).

Discussion

Short term crawler birth and migration

Our study is the first to document scale insect birth in the field based on direct observation, and our results indicate that *C. viridis* crawler emergence is continuous across a single day. Crawlers emerge from underneath their mothers in a wavelike pattern, with multiple bursts of crawler emergence over the course of a single day. The pattern suggests that sharp increases in *C. viridis* crawler emergence are followed by sharp decreases in crawler presence, which could be the result of immediate crawler migration to other leaves, or immediate predation of crawlers by natural enemies.

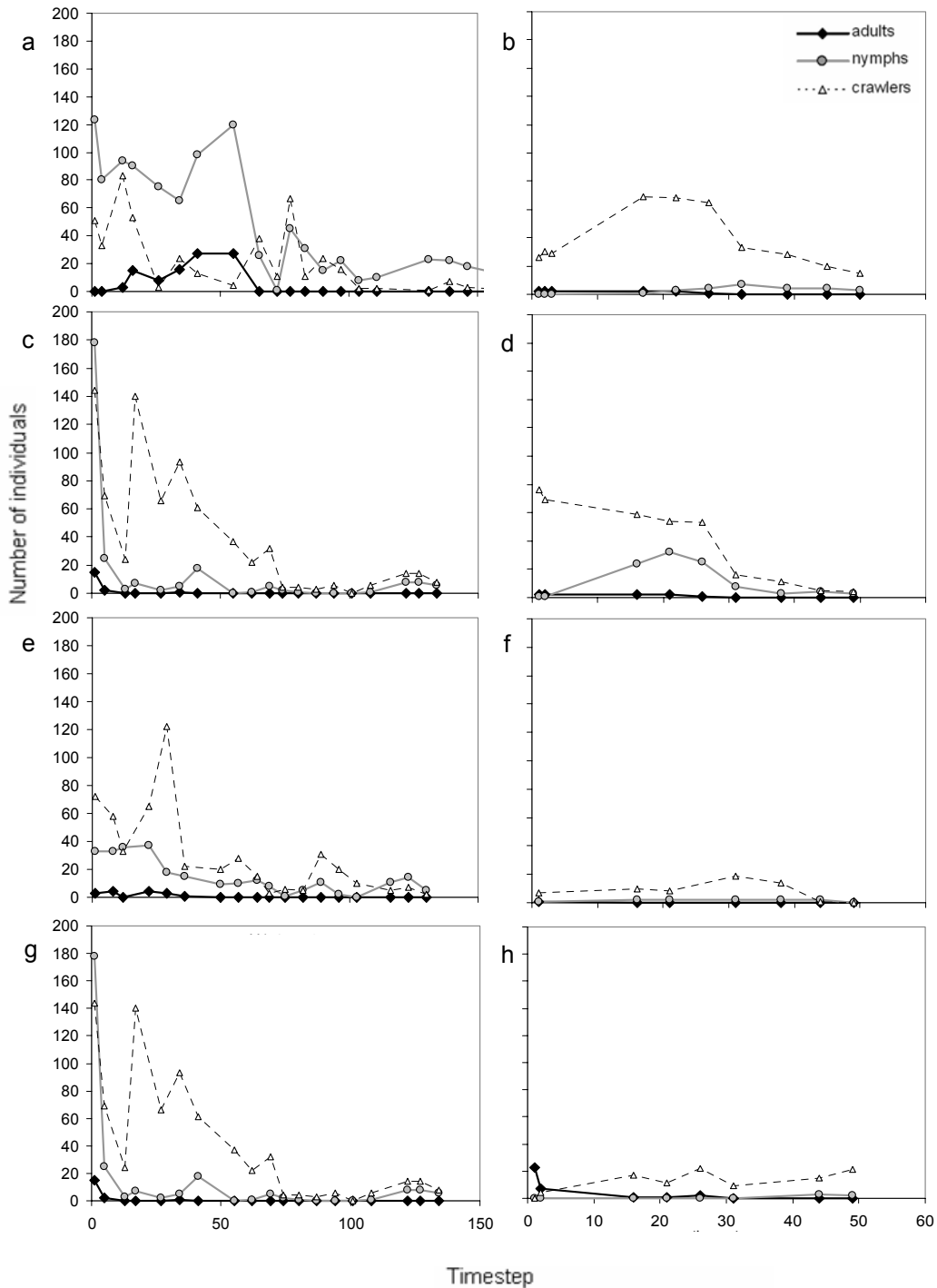


Figure 3. Population dynamics for four leaves with ants (a, c, e, g), four leaves without ants (b, d, f, h).

Long term growth and predation rates

C. viridis populations exhibit greater population densities and longer population persistence in the presence of *A. instabilis* ants, as expected from the basic mutualism involved (Way, 1963; Gaume *et al.*, 1998). Ants provide protection against predators and parasitoids, leading to increased consumption of resources by *C. viridis* (Van Der Goot, 1916). Furthermore, the removal of the honeydew produced by the scales reduces the proliferation of sooty mold, which can reduce *C. viridis* growth and survivorship (Bess, 1958).

Within-leaf microhabitat appeared to have little influence on *C. viridis* growth rates, but had a slight impact on adult and nymph predation levels, with primary vein microhabitats providing the most protection from chewing insect predation. Chewing insect predation of *C. viridis* in primary vein microhabitats may be limited due to the presence of the abutting vein wall, in addition to the more consistent presence of patrolling ants, since ants are known to reduce patrolling at the population periphery, especially if homopteran populations are large (Harmon and Andow, 2007). Better ability to attach to

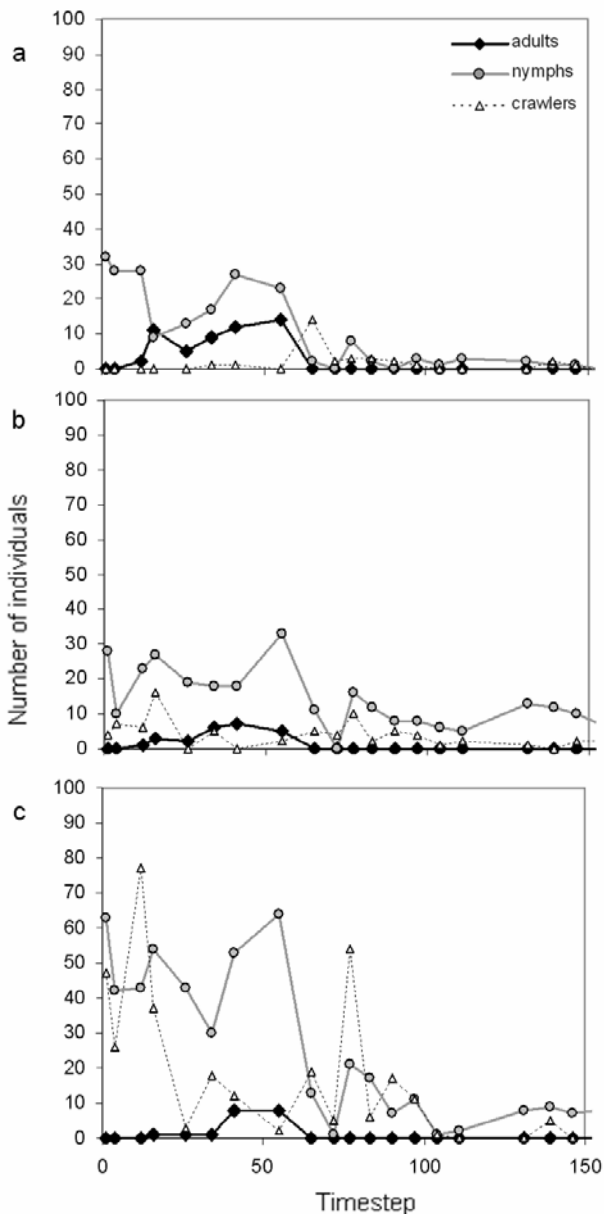


Figure 4. Population dynamics on a single leaf (with ants) across three leaf microhabitats, (a) primary vein, (b) secondary veins, (c) leaf area (between veins).

the primary vein may also provide *C. viridis* nymphs protection against the whiplash caused by host plant movement, and thus may reduce mortality levels (Southwood, 1973; Bernays, 1991).

Results also indicate that the parasitization, chewing insect predation, and fungal attack rates of *C. viridis* are distinctive between life-stages and depend critically on seasonality. Monthly *C. viridis* adult and nymph parasitism rates decrease with the onset of the summer rainy season, with the highest levels of parasitization recorded during the dry season, between the months of February and May. Similarly, patterns of adult and nymph chewing predation rates also decrease with the onset of the summer rainy season, with the highest levels of parasitization recorded during the dry season. These patterns may be due to 1) greater densities of general *C. viridis*

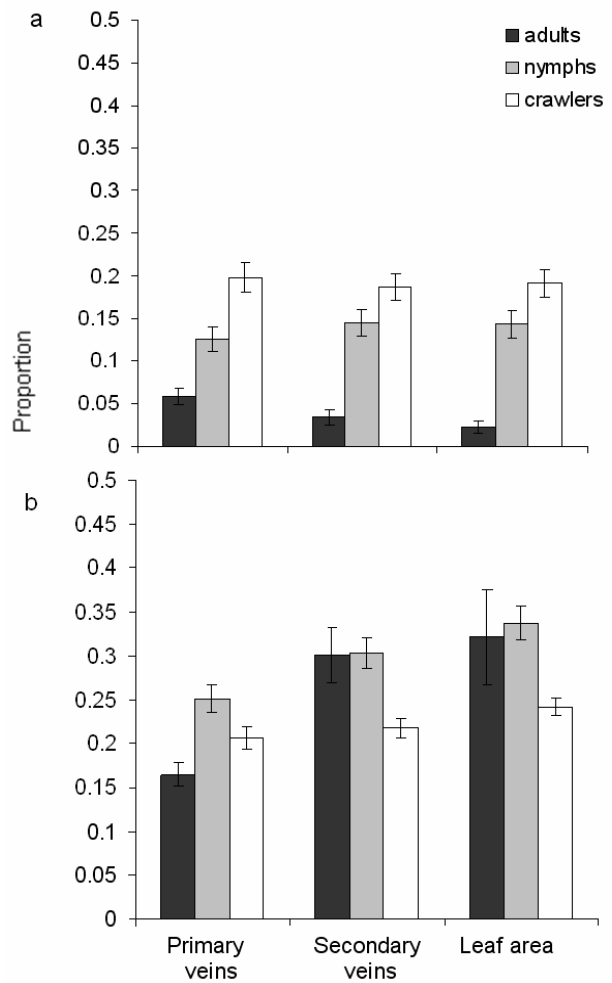


Figure 5. *C. viridis* weekly (a) growth rates and (b) predation rates across three within-leaf microhabitats.

predators, 2) greater densities of *C. viridis* predators which are resistant to ant-attack or 3) weaker ant-protection during the dry season.

In the study region, insect densities, including predators and prey of *C. viridis*, are higher in the dry season (Williams-Guillen *et al.*, 2008). Predators of *C. viridis* include voracious coccinellid beetles, like the larvae of *A. orbigera*, which cannot be attacked by ants due to a protective coating of sticky waxy filaments (Liere and Perfecto, 2008). Predators that have protective physical, chemical, or behavioral mechanisms may be more effective predators of *C. viridis*, despite *A. instabilis* patrolling, when they are most abundant in the dry season. Unlike parasitism and chewing insect predation rates, fungal attack rates for *C. viridis* adults appear bimodal, with the greatest fungal attack rates in May and August. Thus, fungal attack may be triggered by rain, as previously hypothesized (Perfecto and Vandermeer, 2008). However, high attack rates are not sustained throughout the wet-season and are recorded again only when rains abate in the month of August, suggesting that the optimal environment for fungal attack may be moderate, but not intense, rainfall.

Only one other study has examined the role of tempo-

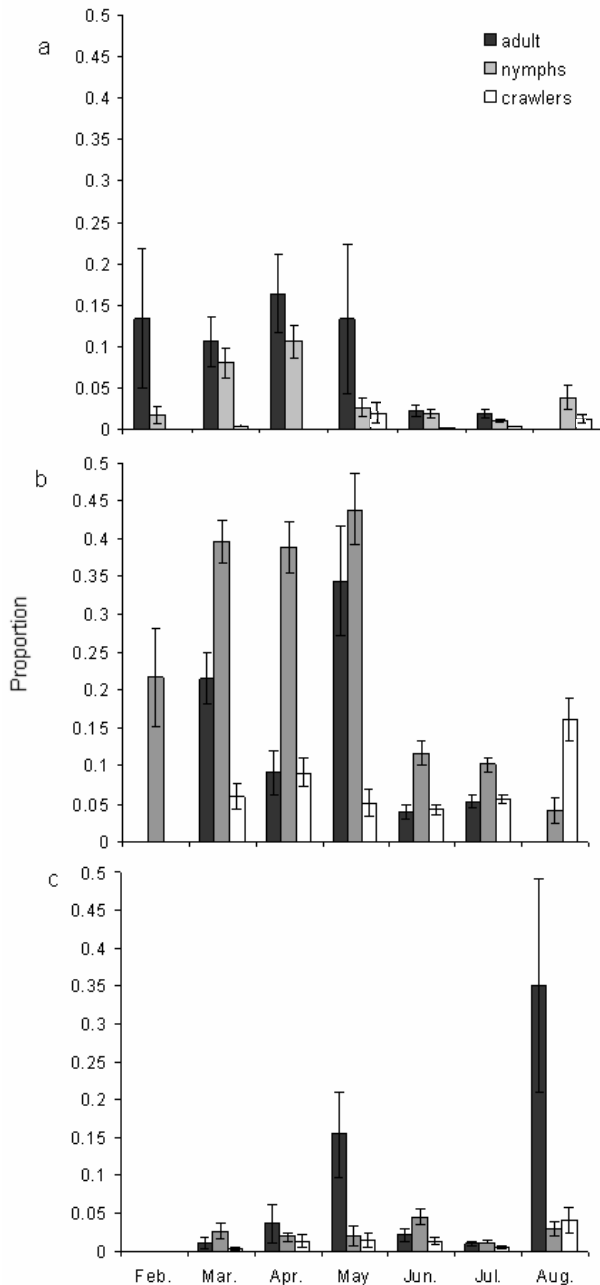


Figure 6. *C. viridis* monthly (a) parasitism, (b) chewing predation, and (c) fungal attack rates for adults, nymphs and crawlers.

ral variability in an ant-homopteran mutualisms in the field, and this study found significant differences in homopteran growth rates, with and without ants, over time (Billick and Tonkel, 2003). Like our study, Billick and Tonkel (2003) suggest that predator density and temporally variable ant foraging patterns are likely causes for the varying patterns seen in homopteran survivorship. Using uniquely fine-scale population monitoring methods across space and time, our results reveal that mutualisms between *C. viridis* and *A. instabilis* are complex and dynamic and depend distinctly on seasonality, likely due to seasonally increasing *C. viridis* predation pressure.

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