UNIVERSIDADE ESTADUAL DE CAMPINAS

FLÁVIA NOGUEIRA DE SÁ

INFLUÊNCIA DA INTERAÇÃO COM PLANTAS HOSPEDEIRAS (ASTERACEAE) E INIMIGOS NATURAIS NA ABUNDÂNCIA DE TRÊS ESPÉCIES DE CASSIDINAE (COLEOPTERA CHRYSOMELIDAE) NA SERRA DO JAPI, SP.

Este exemplar corresponde à redação final
da tese defendida pelo (a) candidato (a)
Flávia Nogueira de Sá
e aprovada pela Comissão Julgadora.
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"Well life has a funny way of sneaking up on you
When you think everything's okay and everything's going right
And life has a funny way of helping you out when
You think everything's gone wrong and everything blows up
In your face."

Alanis Morissete

Dedico este trabalho aos meus pais,
Antonio e Neli, ao meu irmão, Bio
e aos meus amigos de verdade.

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Resumo

Neste trabalho foram estudadas populações de Cassidinae na Serra do Japi (SP) com os objetivos pricipais de determinar quais as espécies de planta da família Asteraceae são utilizadas por estes besouros, descrever a abundância dos Cassidinae (nas fases de ovo, larva e pupa) consumidores de Asteraceae ao longo do ano e determinar a influência de fatores abióticos e bióticos nas populações estudadas. Nosso estudo enfatizou as três espécies de Chrysomelidae consumidoras de Asteraceae mais abundantes na Serra do Japi, Stolas chalybea, S. areolata e Anacassis phaeopoda, todas especialistas em suas hospedeiras, Bidens segetum, Mikania cordifolia e Baccharis trimera, respectivamente. Apesar de muitas espécies de Asteraceae terem sido observadas nas trilhas estudadas (n=15), poucas foram utilizadas como hospedeiras (n=5). Desovas e larvas dos primeiros estádios de S. chalybea e S. areoloata foram mais frequentemente encontradas na superfície abaxial das folhas de suas plantas hospedeiras, enquanto que larvas mais desenvolvidas e adultos foram mais frequentemente encontrados na superfície adaxial da folhas. As frequências de Cassidinae em plantas hospedeiras foram significativamente relacionadas a características como altura, número de folhas e ramos nas plantas. O período de atividade das três espécies estudadas foi durante os nove meses mais chuvosos e quentes, e a diapausa ocorreu durante os meses de inverno (período de seca). A reprodução ocorreu principalmente durante os meses de verão. Além de fatores climáticos como chuva e temperatura, a presença de determinadas feno-fases nas plantas influenciaram a abundância e atividade reprodutiva dos Cassidinae. Também foram encontrados inimigos naturais das três espécies de Cassidinae, sendo a maioria parasitóides de ovos e larvas pertencentes às ordens Hymenoptera e Diptera. Um mermitídeo (Nematoda) parasita de adultos de S. chalybea e alguns hemípteros e aranhas predadando larvas foram também coletados. Censos de potenciais predadores de Cassidinae nas plantas hospedeiras dos mesmos durante o ciclo de 1997-1998 revelaram a sincronia das abundâncias dos predadores e suas presas ao longo do ano. Experimentos de exclusão indicam que predadores invertebrados voadores tendem a ser os maiores responsáveis pela mortalidade de larvas de S. chalybea. Nós sugerimos a influência de inimigos naturais como um dos principais fatores determinando o tamanho das populações dos Cassidinae estudados.

Abstract

We studied Cassidinae populations at Serra do Japi (SP) with the objectives of recognizing the species of Asteraceae that were consumed by these beetles, describing the abundance of Cassidinae (during egg, larva and adult stages) for a year and knowing the influence of abiotic and biotic factors on the study populations. We focused our investigation on the three commonest species of Chrysomelidae that consumed Asteraceae at Serra do Japi: *Stolas chalybea*, *S. areolata* and *Anacassis phaeopoda*. We observed that few species were consumed by those beetles, despite the close taxonomic relation of plants. These species were specialists to their host plants, *Bidens segetum*, *Mikania cordifolia* and *Baccharis trimera*, respectively. We observed that egg clusters and larvae of *S. chalybea* and *S. areolata* were more frequently localized on the underside of plant leaves, whereas older larvae and adult were more frequently found on the upper surface of leaves. The frequency of Cassidinae on plants were significantly related to traits like height and number of leaves and branches. The three species were active for about nine months per year, and had an arrest of development and activity during the winter/dry season. Reproductive activities occurred mainly during summer months. Besides climatic factors like rain and temperature, the presence of some pheno-phases of the host plants also exerted influences on the abundance and reproduction of Cassidinae. We also found natural enemies of the three species of Cassidinae. They were mostly hymenopteran and dipteran egg and larva parasitoids. A mermithida (Nematoda) parasiting adults and some heteropteran and spider predators were observed too. One year census on studied Cassidinae host plants revealed that the abundance of potential predators are synchronous to abundance of the beetles. Exclusion experiments suggested that flying invertebrate predators might be the main responsible for mortality of *S. chalybea* larvae. We suggest the importance of natural enemies of Cassidinae as one of the main factors in determining the size of the populations of tortoise beetles.

Introdução Geral

Insetos fitófagos representam mais do que um quarto de todos os organismos macroscópicos(Bernays e Chapman, 1994). Como consequência desta riqueza e abundância, estes são os principais elementos de ligação entre produtores primários, as plantas, e os animais de níveis tróficos superiores. Insetos fitófagos podem ter desempenhado um papel importante na evolução de plantas, selecionando diversos tipos de defesas químicas ou físicas. Características físicas e químicas das plantas podem afetar sua aceitação por insetos fitófagos ou influenciar a seleção de hospedeiro (Bernays e Chapman, 1994). Plantas hospedeiras também podem afetar o herbívoro associado através da sua fenologia, distribuição e presença de compostos secundários que podem alterar a sobrevivência e/ou performance do herbívoro (Bernays e Chapman, 1994). Tem-se observado fortes correlações entre a ocorrência de partes da planta preferidas pelos insetos e taxas de herbivoria, sugerindo que as plantas não só influenciam no número de insetos como também nas dinâmicas das populações dos mesmos, devido à sincronia entre o recurso e o consumidor. Tal sincronia já foi detectada por diversos autores (ex.: Campbell e McCaffrey, 1991; Clark e Clark, 1991; Vasconcellos-Neto, 1991; Medeiros, 1991; Crowe, 1995) e é determinada, na maioria dos casos, pela ocorrência de folhas jovens na planta hospedeira. A sincronia do ciclo biológico de herbívoros com o da planta hospedeira é proveniente da pressão de especialização dos mesmos (Verdyck, 1998).

O grau de especialização de insetos herbívoros pode ser influenciado pela disponibilidade da planta hospedeira. Competição, limitações sensoriais na localização de alimento, a presença de estruturas de defesa na planta hospedeira (sejam elas físicas ou químicas, na forma dos compostos secundários), também foram mencionados por Bernays e Chapman (1994) como fatores que podem levar à especialização de insetos herbívoros. Para cada espécie de inseto, esses fatores, entre outros, podem influenciar a amplitude de sua dieta. Entretanto nenhum destes é considerado como de maior importância.

A distribuição espacial e temporal de plantas hospedeiras também apresenta uma estreita relação com o comportamento, taxas de colonização, reprodução e densidade de populações de insetos herbívoros (Garcia e Altieri, 1992). A dispersão e composição de espécies vegetais afetam também os parasitóides, influenciando a localização do habitat e, consequentemente do hospedeiro também. Tal localização pode ser feita através de sinais químicos ou visuais; portanto, quanto maior a mancha de plantas hospedeiras, em muitos casos, mais parasitóides sejam atraídos (Queiroz, 1996; Meiners e Hilker, 1997). Entretanto o oposto também já foi observado por alguns autores (ex.: Thomas, 1989; Janzen, 1970), indicando que nem sempre mais parasitóides são atraídos onde a mancha da planta de seu hospedeiro é maior. A química dos compostos secundários foi durante muito tempo o tema principal nos estudos sobre interação herbívoro-planta e a evolução de sua especialização (Keese, 1997); apesar que, para insetos fitófagos, o uso do olfato na localização de plantas hospedeiras ainda é bastante discutido (Jermy et al., 1988). Por outro lado, como o uso de pistas visuais já foi comprovado(Jermy et al., 1988), especialmente através de marcas de herbivoria (ver Heinrich e Collins, 1983; Chaves, 1998 por exemplo), corrobora-se assim a importância da distribuição das plantas hospedeiras.

Enfim, é provável que a interação e organização dos organismos de níveis tróficos superiores possam ser influenciadas pelo seu recurso, no caso as plantas, para os insetos fitófagos (Price, 1992). Price (1992) discute também que as plantas podem ditar a dinâmica populacional de herbívoros, a importância de competição intraespecífica e a probabilidade de ataques por inimigos naturais. Além da influência pelas plantas hospedeiras, muitas populações de insetos tropicais apresentam uma sazonalidade marcada por estações secas ou chuvosas. Variações populacionais são determinadas por flutuações climáticas (Vasconcellos-Neto, 1980), sendo a pluviosidade, umidade e fotoperíodo os principais fatores que influenciam a dinâmica dos mesmos.

Juntamente com a ação de fatores abióticos e relação com a planta hospedeira, outros fatores importantes que poderiam influenciar a dinâmica populacional de herbívoros são competição e predação. Entretanto, a determinação dos principais fatores responsáveis pela estruturação não só de herbívoros, bem como de comunidades vegetais e animais tem sido alvo de constantes discussões há vários anos. Inicialmente, Darwin propôs a competição como o fator principal para explicar a ausência e presença de espécies em uma comunidade (Seifert, 1984). Durante a década de 40, as opiniões eram dividas entre a importância da competição ou de uma série de fatores, como fontes de energia, mobilidade da espécie, chance de ataque por predadores, estrutura e crescimento populacional, que deveriam atuar em conjunto, estruturando assim as diversas populações dentro de uma comunidade (May, 1984). Na década de 50, a principal controvérsia era sobre a importância de efeitos dependentes de densidade provenientes de interações biológicas ou causados pelo clima ou outros fatores ambientais, na determinação de tamanhos populacionais (May, 1984). Finalmente na década de 60, Hairston et al. (1960) introduziram a hipótese em que a influência da predação nas populações de herbívoros seria mais efetiva, à ponto de reduzir suas densidades a níveis inferiores aos requeridos para que houvesse competição.

Insetos fitófagos foram utilizados com sucesso para elucidar processos ecológicos e evolutivos exceto para o fenômeno da competição. Autores como Rathcke (1976), Lawton e Strong (1981), Strong et al. (1984), Den Boer (1985), Jermy (1985) e Forrester (1993), concluíram através de experimentos em campo ou através de revisões da literatura, que a competição é muito rara ou pouco significante para estruturar comunidades de insetos fitófagos. Além disso, nos casos onde esta foi detectada era relativamente assimétrica, levando ao questionamento sobre sua importância na organização dessas comunidades. Rathcke (1976) e Forrester (1993) sugeriram que a ausência de competição nessas comunidades possa ser explicado pelo fato dos insetos serem espécies fugitivas, ou devido ao parasitismo, qualidade do alimento, ou pelas suas características de seleção r, como tamanho pequeno, baixa longevidade, e serem altamente sensíveis às variações temporais e espaciais do ambiente. Entretanto, Rathcke (1976) mencionou alguns autores que utilizaram a competição para explicar a diversificação de insetos em novos habitats ou em novas plantas hospedeiras, ou para explicar as várias diferenças de espécies proximamente relacionadas. Tendo-se visto a pequena importância da competição, atualmente a predação tem recebido mais atenção nos estudos sobre a especialização de insetos fitófagos; uma vez que se tem observado que este tipo de interação, juntamente com o parasitismo, têm provocado alta mortalidade em insetos fitófagos (Keese, 1997).

Price et al. (1980) apoiam a hipótese de Hairston et al. (1960) enfatizando a importância dos organismos do terceiro nível trófico, particularmente predadores e parasitóides, nas interações inseto-planta, devendo inclusive ser considerados como parte da bateria de defesas contra herbívoros. Messina (1981) demonstrou que Solidago altissima (Asteraceae) apresentava maior crescimento e produção de sementes na presença de espécies de Formica (Hymenoptera: Formicidae). Essas formigas ofereciam proteção a um herbívoro da planta, o membracídeo Publilia concava, e também expulsava ou predava os principais herbívoros de S. altissima, os crisomelídeos Trirhabda virgata e T. borealis. Assim sendo, o predador atraído à planta pela exsudação de homópteros exerce um efeito positivo sobre a aptidão da planta hospedeira, devido à pressão de predação exercida sobre os herbívoros mastigadores. Price et al. (1980) afirmam ainda que a interação herbívoro planta, não pode ser compreendida sem considerar o papel do inimigos naturais, assim como a relação entre predador e presa também não pode ser entendida sem se considerar o papel das plantas.

De acordo com Bernays e Graham (1988) e Keese (1997) predadores e parasitóides são considerados os principais fatores na evolução de uma dieta especializada. Inimigos naturais podem interferir na dieta de um herbívoro, influenciando o grau de especialização ou preferência dos insetos fitófagos, dependendo da efetividade dos predadores e parasitóides associados a cada item alimentar. Isto acontece porque plantas podem atrair mais ou menos inimigos de acordo com sua estrutura ou composição química (Price et al., 1980). Parasitóides por exemplo, usam inúmeras pistas químicas e físicas para localizar seus hospedeiros (Meiners e Hilker, 1997). Estes autores demonstraram a importância de pistas químicas provenientes das fezes e ovos de Xanthogaleruca luteola (Chrysomelidae) na sua aceitação e reconhecimento pelo parasitóide de ovo Oomyzus gallerucae (Hymenoptera: Eulophidae). A importância de predadores e parasitóides na manutenção de populações de insetos fitófagos em baixos níveis tem sido detectada com bastante frequência. Por esta razão, esses inimigos naturais têm recebido destaque na área de controle biológico (veja Cox, 1996 para exemplos com Chrysomelidae).

A família Chrysomelidae é uma das maiores e mais diversas dentro da ordem Coleoptera e é exclusivamente herbívora (Hsiao, 1986). Membros das sub-famílias Criocerinae, Eumolpinae, Galerucinae, Alticinae, Hispinae e Chrysomelinae podem ser mencionados como de interesse econômico, por serem pragas agrícolas ou de espécies florestais (Jolivet et al., 1988). Mesmo assim, poucos estudos têm sido realizados sobre os Chrysomelidae e a maioria deles se refere a espécies de interesse econômico na região temperada, especialmente o besouro da batata do Colorado, Leptinotarsa decemlineata (ex.: Groden e Casagrande, 1986 e Cappaert et al. 1991).

Besouros crisomelídeos apresentam algumas características que os diferenciam dos demais insetos e que poderiam levar a resultados positivos sobre a presença da interação competitiva não só interespecífica, como também intraespecífica. A primeira seria a baixa dispersão. Dados sobre o comportamento de dispersão desses besouros indicam que os mesmos não se deslocam por grandes distâncias durante seu ciclo de vida, levando as larvas a se desenvolverem a alguns metros das plantas onde se desenvolveram seus pais (Strong, 1982). A segunda seria sobre o consumo em excesso da planta hospedeira, que indica que o recurso alimentar pode ser limitante para alguns crisomelídeos, levando-os assim à competição (Strauss, 1988). Entretanto, não são frequentes situações em que muitos indivíduos da mesma espécies são encontrados na mesma planta e casos de co-ocorrência de mais de uma espécie de Chrysomelidae numa mesma planta hospedeira não são comuns. Além disso, a literatura atual tem registrado mais frequentemente a influência de predadores e parasitóides na manutenção das populações desses besouros, destacando-se assim a importância desses organismos. Olmstead (1996) e Cox (1996) publicaram boas revisões sobre inimigos naturais de Chrysomelidae, discutindo a grande importância de predadores e parasitóides para esses insetos. Os inimigos naturais de Chrysomelidae são representados por organismos de diversos grupos taxonômicos. Apesar dos parasitóides das ordens Hymenoptera e Diptera, e artrópodes predadores serem os inimigos naturais de Chrysomelidae mais mencionados na literatura, parasitas intracelulares e aves predadoras também podem ser encontrados afetando esses besouros (veja Olmstead, 1996).

Os crisomelídeos estão associados a um grande número de plantas hospedeiras e apresentam uma tendência à especialização (Jolivet, 1988). Segundo Hsiao (1986) muitas famílias de plantas podem ser utilizadas, porém a maioria destes besouros é herbívora adaptada a utilização de poucas plantas taxonomicamente relacionadas. Mesmo nas subfamílias de Chrysomelidae com maior amplitude de plantas hospedeiras, existem muitas espécies monófagas (Jolivet, 1988).

Os Cassidíneos, besouros da subfamília investigada neste trabalho, são muito seletivos em relação à sua planta hospedeira, sendo geralmente oligófagos ou monófagos (Jolivet, 1988). Em um trabalho onde a ocorrência de 170 espécies de Cassidinae Neotropicais foi descrita, Buzzi (1994) observou que 49,4% das espécies são monófagas, 24,15% das espécies se alimentam de duas plantas, 10,6% se alimentam de quatro plantas, 8,2% se alimentam de três plantas, 2,3% de cinco plantas, 1,2% de seis plantas e 1,2% de sete plantas. Algumas espécies se alimentam de duas ou mais espécies da mesma família e outras se alimentam de duas ou mais espécies de plantas de famílias diferentes. Buzzi (1994) também verificou que entre as plantas hospedeiras, a família Convolvulaceae é a mais frequente (48,3 %), seguida por Asteraceae (14,7 %), Bignoniaceae (8,8%), Solanaceae (8,3 %), Arecaceae (7,6 %) e Lamiaceae (3,5 %).

Ao contrário de muitos outros insetos monófagos, os Chrysomelidae adultos utilizam recursos iguais aos das larvas (Olmstead, 1996), o que os tornam bastante adequados para estudos ecológicos.

As espécies de Cassidinae estudadas neste trabalho, <u>Stolas</u> <u>chalybea</u> (Germar, 1824), <u>S. areolata</u> (Germar, 1824), and <u>Anacassis</u> <u>phaeopoda</u> Buzzi, 1976 (Fig. 1), seguem o hábito de monofagia sugerido por Buzzi (1994) e a família Asteraceae é a segunda família mais frequente de hospedeiras da tribo Stolaini (a qual pertencem os gêneros Stolas e Anacassis). Dos 66 registros sobre o uso de plantas hospedeiras de 37 espécies deste grupo, 41 eram de plantas da família Convolvulaceae, 21 eram de Asteraceae e os demais eram de outras famílias de dicotiledôneas (Buzzi, 1994).

Tendo-se em vista a importância de plantas hospedeiras e dos inimigos naturais em populações de insetos fitófagos, este trabalho foi desenvolvido com o objetivo principal de caracterizar a interação de Cassidinae da tribo Stolaini com plantas da família Asteraceae e seus inimigos naturais.

O estudo está organizado em três capítulos, onde o primeiro trata do reconhecimento das asteráceas e cassidíneos na área de estudo e da utilização dessas plantas pelas espécies estudadas; o segundo trata da descrição da flutuação das populações de Cassidinae ao longo de um ano e o terceiro da influência dos inimigos naturais nos besouros estudados.

Objetivos

1 - Determinar quais as espécies de planta da família Asteraceae são utilizadas por Cassidinae da tribo Stolaini na Serra do Japi.

2 - Descrever a abundância dos Cassidinae (nas fases de ovo, larva e pupa) consumidores de Asteraceae ao longo de um ano.

3 - Determinar a influência de fatores climáticos e das plantas hospedeiras na flutuação das populações de Cassidinae estudadas.

4 – Reconhecer a influência dos inimigos naturais na mortalidade de ovos, larvas e adultos das espécies estudadas.

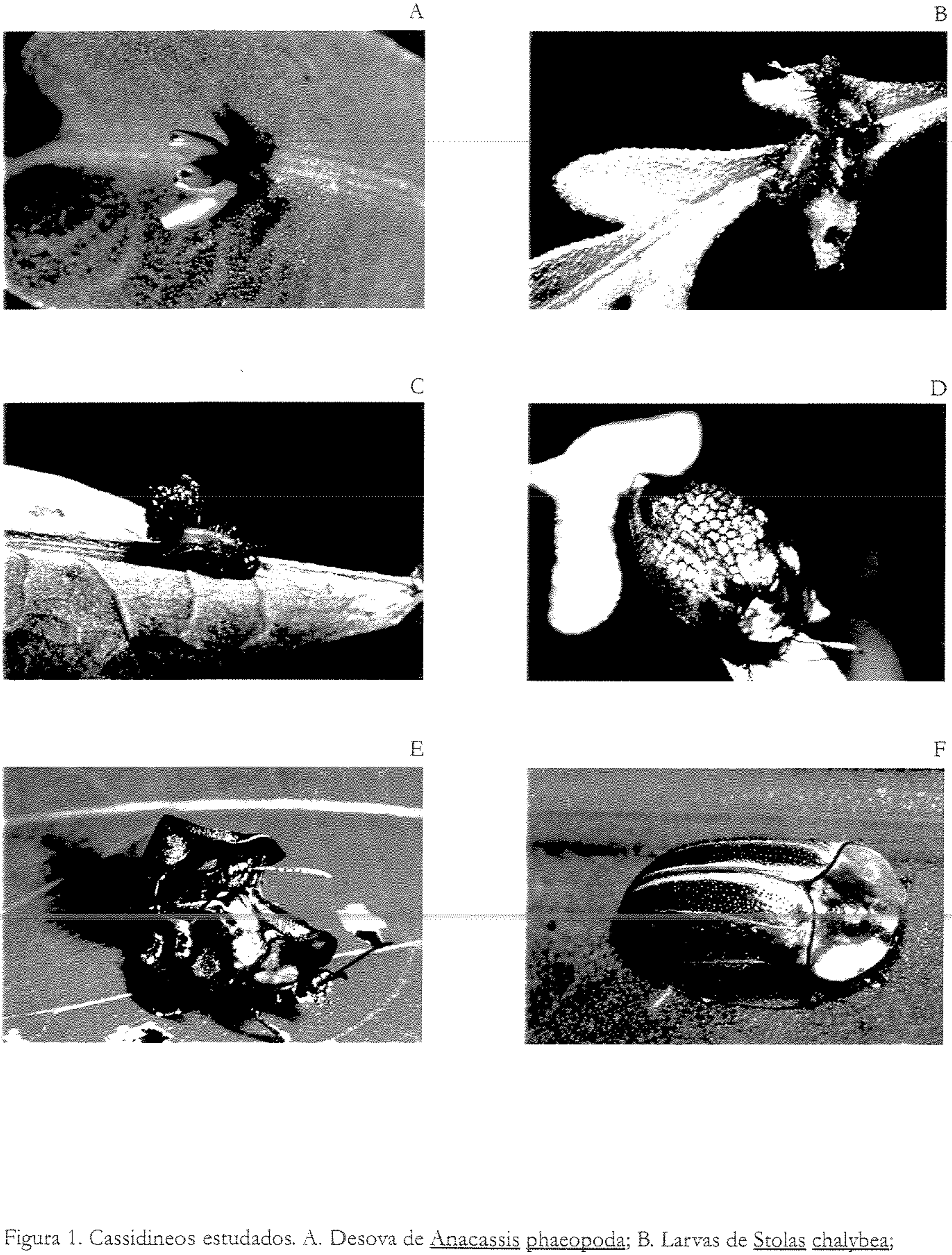

Figura 1. Cassidineos estudados. A. Desova de Anacassis phaeopoda; B. Larvas de Stolas chalybea; C. Larva de Anacassis phaeopoda; D. Adulto de Stolas chalybea; E. Casal de Stolas areolata; F. Adulto de Anacassis phaeopoda. Fotos: Cláudio E. G. Patto.

Área de Estudo:

O trabalho foi desenvolvido na Serra do Japi, Jundiaí, São Paulo (23° 11'S/46° 52' W), no limite sul da zona tropical. Esta serra (700 – 1300 m. de altitude) é formada por um maciço que se estende de sudoeste para nordeste, entre o Porto do Japi, no rio Tietê (município de Cabreúva, SP) e a Fazenda do Japi, situada a seis quilômetros da cidade de Jundiaí, SP (Fig. 2) (Pinto, 1992).

Segundo Pinto (1992), a Serra do Japi reúne uma rara e excepcional conjunção de fatores bióticos e abióticos que condicionam uma vegetação rica e diversificada.

Pode-se observar um gradiente negativo acentuado de precipitação anual à medida que se desloca no sentido São Paulo - Campinas. As chuvas concentradas principalmente no período de primavera - verão. Nos meses de maior índice pluviométrico chega-se a atingir 250 milímetros ao mês, enquanto que na estiagem (de junho à agosto) os níveis são inferiores a 41 milímetros. O mês mais frio é julho é o mais quente é janeiro. Desta forma pode-se reconhecer o clima como sazonal com duas estações: uma quente e chuvosa (de setembro à maio) e outra mais fria e seca (de junho à agosto) (Pinto, 1992). O clima na região é classificado como Cwa, de acordo com o sistema de Koppen (Medeiros e Vasconcellos-Neto, 1994).

A Serra do Japi ocupa uma posição geográfica muito peculiar no estado de São Paulo, estando situada em uma região de interface entre duas fisionomias de vegetação distintas: a Mata Atlântica e as florestas mesófilas semidecíduas do planalto. Ao longo de toda sua área, a influência de elementos atlânticos e de planalto se faz sentir, ora com predominância de um, ora com predominância de outro (Leitão-Filho, 1992).

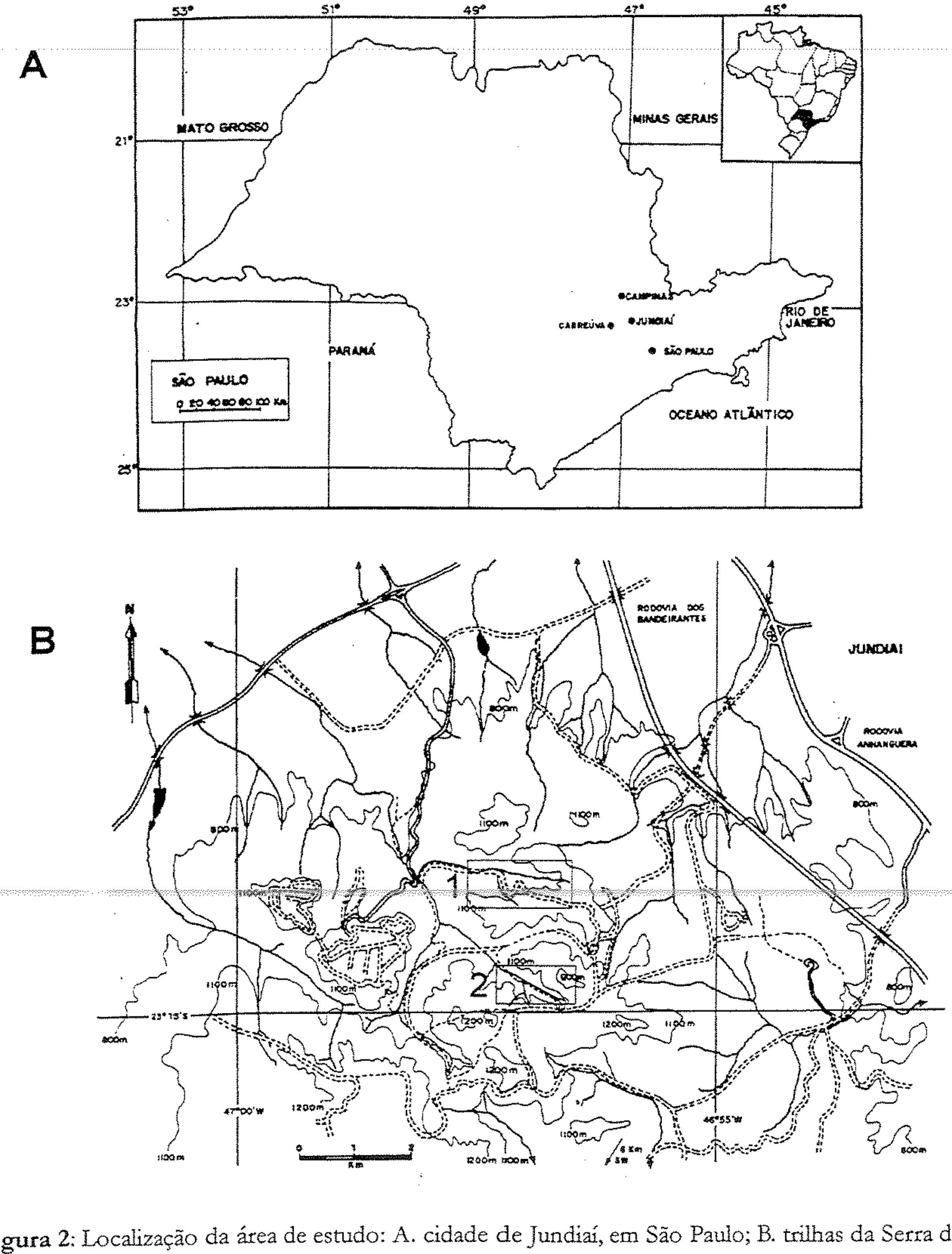

Figura 2: Localização da área de estudo: A. cidade de Jundiaí, em São Paulo; B. trilhas da Serra do Japi (adapatado de Brown-Jr., 1992). O retângulo número 1 indica a trilha do Mirante e o retângulo número 2 indica a trilha do Paraíso III.

Formações florestais arbóreas predominam. Porém, as partes baixas e as partes altas da serra apresentam características fisionômicas distintas quando comparadas. Nas partes baixas, em altitude de 750 à 870 metros, a fisionomia é típica de mata de planalto, com árvores espaçadas de diâmetro elevado, com copas não formando um dossel contínuo. Já nas partes altas a fisionomia é de uma mata cuja as árvores estão adensadas, de diâmetro e porte nitidamente menores do que o observado nas partes baixas e com dossel contínuo (Rodrigues et al., 1989 e Leitão-Filho, 1992).

A atividade reprodutiva e de brotamento das plantas ocorre principalmente a partir de agosto e setembro, declinando à partir de dezembro. Isto ocorre concomitantemente ao maior acúmulo de nutrientes na serrapilheira, seguido à estação seca. Durante esta estação a pequena disponibilidade hídrica e as baixas temperaturas influenciam as espécies vegetais, estimulando a queda de folhas e a deiscência e ou dispersão de diásporos anemocóricos, aumentando assim a serrapilheira e logo, a concentração de nutrientes (Rodrigues e Shepherd, 1992).

Este trabalho foi realizado em duas trilhas já demarcadas na Serra, a trilha do Mirante e a do Paraíso III (Fig. 3). A primeira trilha é localizada a 1170 metros de altitude e pode ser caracterizada como um ambiente bastante sombreado pela cobertura das árvores da mata adjacente; enquanto que o Paraíso III, é localizada a aproximadamente 1070 metros de altitude, apresentando uma área aberta onde pode ser observada uma grande mancha de Baccharis trimera (Asteraceae) e uma área sombreada, como na trilha do Mirante (Fig.3).

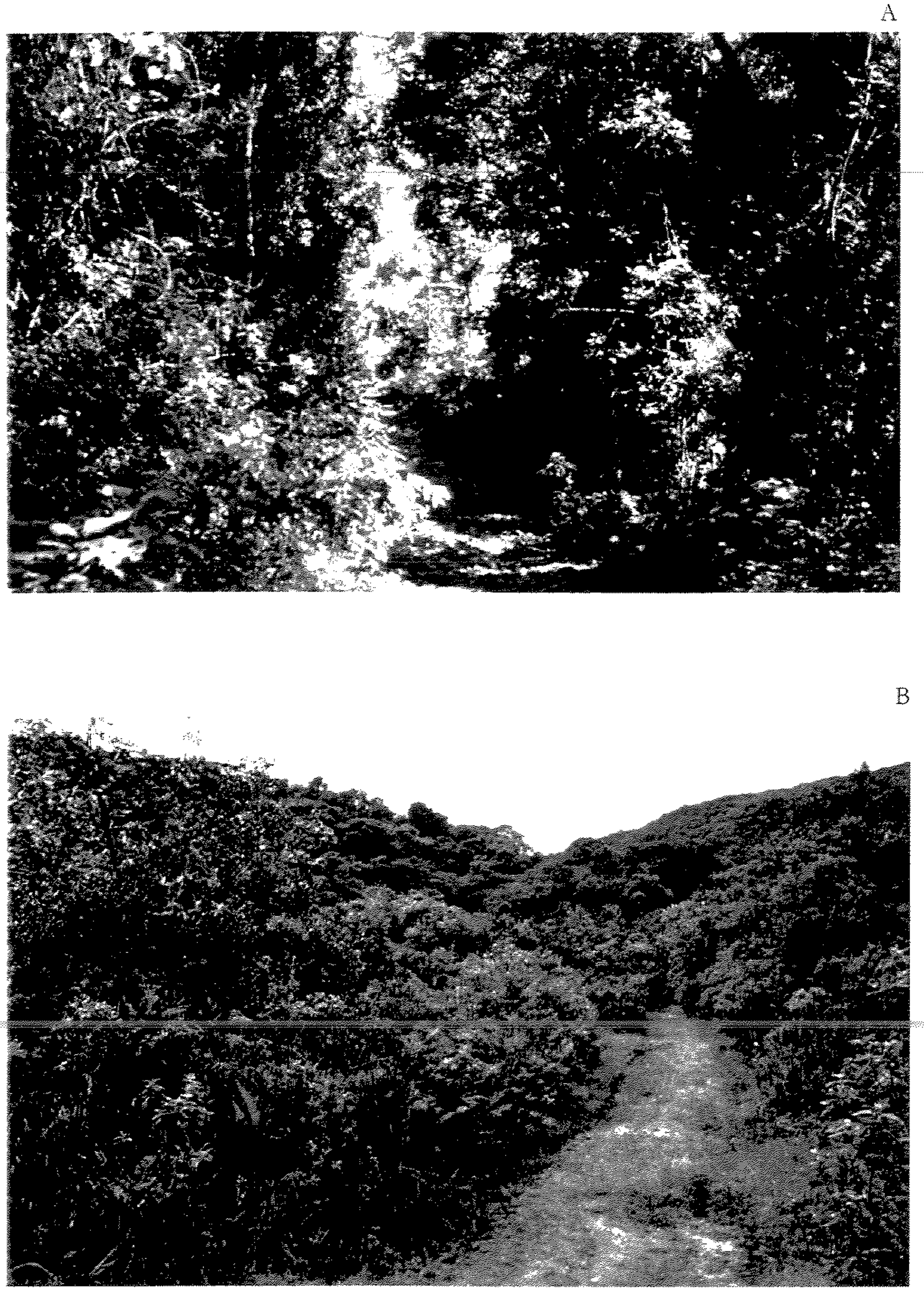

Figura 3. Vista das trilhas estudadas na Serra do Japi. A. Trilha do "Mirante"; foto da autora B. Trilha "Paraiso III", foto de Cláudio Patto.

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Capítulo 1

ASTERACEAE UTILIZATION BY TROPICAL STOLAINI BEETLES (COLEOPTERA: CHRYSOMELIDAE: CASSIDINAE).

Abstract: Plants of the family Asteraceae are one of the most consumed hosts by Cassidinae beetles. In this work, we investigated the utilization of these plants by Cassidinae at Serra do Japi (SP), and after inspecting several Asteraceae at the study site, we observed that, despite the close taxonomic relationship of the plants, few species were consumed by these beetles. We focused our investigation on the three most common species: Stolas chalybea, S. areolata and Anacassis phaeopoda. These species were specialists to their host plants, Bidens segetum, Mikania cordifolia and Baccharis trimera, respectively. We observed that egg clusters and larvae of Stolas spp. were more frequently located on the underside of plant leaves, whereas older larvae and adult were more frequently found on the upper surface of leaves. The frequency of Cassidinae on plants was significantly related to traits like height and the number of leaves and branches. Our results confirm previous observations on Chrysomelidae specialization and localization on host plants.

Introduction

A continuous spectrum exists between insect species that will only feed on one plant species and others that feed on a very wide range of plants in many different families (Bernays and Chapman, 1994). Therefore, it is usual to separate the insects into categories depending on their host plant ranges. The categories are: monophagous, for insects that feed on only one species of plant or plants within the same genus; oligophagous, for those that feed on a number of plants in different genera but within one plant family and polyphagous, when feeding is on plants from different families (Bernays and Chapman, 1994). Except for Orthoptera, in all other phytophagous insect orders, the ranges of plants eaten by individual species are very limited, and 70% or more of the species are oligophagous or monophagous (Bernays and Chapman, 1994).

A large variety of factors have been proposed as determinants of diet breath in phytophagous insects (Jaenike, 1990). The evolution of plant-insect associations has been guided to a large extent by plant chemistry, but ecological factors and the insect genetics have also received much attention. Sá and Macedo (1998) mentioned that the adaptation of insect populations to their host plants involves two major characters: behavior that influences the choice of a plant for feeding and reproduction or physiologicals traits that affect growth and reproduction on a particular host plants. Jaenike (1990) believes that the optimal diet breath represents a trade-off between realized adult fecundity and mean offspring fitness. There are many factors involved in the evolution and maintenance of specialization also, and none of them can be singled out as predominant According to Bernays and Chapman (1994), when a host plant is extremely abundant, encounter rates of insects will be relatively high, and a higher number of insects could feed upon them, which may lead to narrow diets. Like it is considered for diet breath determination, host plant availability, the presence of deterrent chemical compounds or physical defense attributes like trichomes and spines of the plant were also cited by Bernays and Chapman (1994) and Jermy (1994) as enabling factors for specialization. Limitations in sensory processing to locate food and interspecific competition (which is a very questioned factor) can also be mentioned. Predators of the herbivores were suggested by Bernays and Graham (1988) and Keese (1997) to be the dominant factor in the evolution of narrow host range. The influence of this factor is supported by "the enemy-free-space" hypothesis.

Once an insect has arrived on a plant it is faced with the decision of whether or not to accept it. It has been shown that physical properties of the host plant, like its height, diameter and orientation, play a role in the decision-making behavior of insects(Bernays and Chapman, 1994). It means that most phytophagous insect species tend not only to specialize with respect to the plant taxa they feed on, but they also tend to be associated with particular parts of plants (Bernays and Chapman, 1994). So, they may not be so specific in relation to their food, but may have preferences. Preferred plant traits have been correlated with higher herbivory rates, because they not only influence the number of insects on a given plant (depending on the amount of the preferred resource on that), but they also influence their population dynamics because of the synchrony between insect and resource. Young leaves are a commonly preferred plant part. Feeding on young foliage may allow insects to grow quickly and reach bigger body size. Those leaves are less tough and fibrous, and usually contain more nutrients and water (Quintero, 1997). Young leaf preference, among other effects, may cause herbivory rates to be related with plant size parameters (e.g.: Solomon, 1981 and Macêdo et al., 1994).

Chrysomelidae beetles are associated with a large number of host plants; however, most of the species are specialized feeders adapted to one or a few host plant species that are taxonomically related (Hsiao, 1986; Jolivet et al.,1988). Nevertheless, some chrysomelid subfamilies have a broad host plant choice even though the species themselves are monophagous (Jolivet, 1988).

According to Jolivet (1988), the subfamily Cassidinae is the most specialized within the Chrysomelidae. Buzzi (1994) revised host plant records for 170 species of Neotropical Cassidinae and observed that 49.4% were monophagous, 24.1% fed on two host plant species, 10.6% fed on four host species, 8.2% fed on three host plants and 4.7% fed on five, six or seven host plant species.

The greatest number of Cassidinae records was on Convolvulaceae (48.3%), followed by Asteraceae (14.7%), Bignoniaceae (8.8%), Solanaceae (8.3%), Arecaceae (7.6%) and Lamiaceae (3.5%).

According to Buzzi (1994), Convolvulaceae is also the most frequent host plant family of the Stolaini, the tribe of Cassidinae of thestudied genera. Considering a list of 66 host plant records of Stolaini, 62.12% of them were on Convolvulaceae, 31.82% were on Asteraceae and 6.06% were distributed among Borraginaceae, Cucurbitaceae, Sterculiaceae and Myrtaceae. Few Cassidinae species feeding on more than two species were recorded.

Considering the importance of Asteraceae for Stolaini cassidines, in the present work, we listed which species of plants were available at Serra do Japi for potential use by these beetles and we also checked their relative abundance at two study sites in the same area.

Our main objective was to know which Asteraceae plants were consumed by Stolaini beetles how they were utilized by them. We also investigated which plant traits were the most frequent oviposition sites and which plant traits were related to the frequency of cassidines on the plant.

Material and Methods

Study area

This work was conducted in southeastern Brazil, at Serra do Japi (23° 11'S/46° 52' W), a mountain ridge located at the southern limit of the tropical zone in São Paulo state, Brazil.

The climate at the area corresponds to the subtropical moist type, being classified as Cwa, according to the Koppen system (Medeiros and Vasconcellos-Neto, 1994). Two distinct seasons can be observed: warm and rainy summer and cold and dry winter. Because of the large range of altitude (700 m to 1300 m), mean temperature may vary from 11.8° to 18.4°C in July (the coldest month) and from 18.4 to 22.2°C in January (the warmest month) and precipitation varies from 41 mm to 250 mm in the dry and rainy seasons respectively (Pinto, 1992).

Semideciduous mesophytic forests of altitude are predominant at Serra do Japi. The forest is seasonal with the period of leaf fall from April to September, approximately (Leitão-Filho, 1992).

We concentrated our study along two trails: "Mirante" trail (1170 m altitude) and "Paraíso III" trail (1070 m altitude). Both trails can be characterized by the shade provided by the trees located along their edge. "Paraíso III" also presents a open swampy area, where a large patch of Baccharis trimera (Less.) D.C. (Asteraceae) was observed.

Asteraceae at the study trails

Since Asteraceae family is quite diverse and not all species are used as host by Cassidinae, we selected, after a survey in the literature (e.g.: Costa Lima, 1955; Buzzi, 1988; Buzzi, 1994), some tribes to work on. Heliantheae, Astereae and Eupatorieae were selected because all host plant records for the Stolainii Cassidinae were on species of one of these tribes.

In order to characterize study trails by the composition and abundance of Asteraceae species, we established an area of 1400 square meters (700 m X 2 m) at each of the sides of "Mirante" and "Paraíso III" trails where we counted the number of individuals of the tribes Eupatorieae, Astereae and Heliantheae and calculated the relative abundance of each species.

Thirty individuals of each species belonging to the studied tribes, located in the study area were tagged. We marked every individual of those species which less than 30 individuals were found.

To determine which plant species were utilized by Stolaini, we checked all tagged Asteraceae and have registered the presence of Cassidinae (in any stage of their life cycle). This same procedure was repeated twice during the period of peak of abundance of Chrysomelidae: in December, 1997 and in February, 1998.

Host plant utilization

In order to check host plant acceptance and development of two Cassidinae species that feed on Asteraceae, Stolas chalybea and S. areolata , we experimentally reared 90 larvae of each species (obtained from eggs at the time they hatched) on Mikania 'sp.2', M. cordifolia and Bidens segetum (30 larvae at each plant species) in plastic boxes in the laboratory. Larvae were obtained from eggs at the time they hatched We provided fresh leaves to the larvae every other day, and maintained them at room temperature and photoperiod.

To recognize patterns of host plant utilization by the Cassidinae S. chalybea, S. areolata and Anacassis phaeopoda, we visually checked tagged individuals of their host plants every fifteen days and recorded the position of egg clusters, larval groups and adults on host plant. We also considered records of Cassidinae localization on non tagged plant individuals observed throughout study period.

Position was defined as location on the stem or on the upper or underside of host plant leaves. These observations were made considering the following larval classification: small larvae- 1st and 2nd instars, medium larvae- 3rd and 4th instars and mature larvae-5th instar.

Data collected at "Paraíso III" and "Mirante" populations were summed and analyzed together because too few individuals were observed on each trail.

Plant traits utilized by Cassidinae

To find out if some plant traits were related to the presence of the herbivore beetles, we measured the height of each of their host plants tagged at "Mirante" and "Paraíso III" trails, counted the numbers of branches and leaves originating from a randomly chosen 30 centimeter stem segment, and the number of new branches at the entire plant individual. We also measured the length of each new branch and calculated the mean length for each plant individual. All these measures and counts were made in December, 1997. Relation between beetle presence and plant trait was checked by multiple regression analysis. We considered the number of Cassidinae on a given host plant individual from December, 1997 to May, 1998 as dependent variables and their normality was tested by Shapiro Wilk test. We used Neperian logarithm and square root transformation on independent variables of non normal distribution and non homoscedastic variances. We used logarithm transformation on egg cluster frequency of A. phaeopoda. We did not analyze data on eggs and larvae frequency of S. areolata and larvae frequency of A. phaeopoda because there were too few observations on them.

In the cases where at least one of the variables (independent or dependent) did not follow the premises of the multiple regression analysis, graphic analysis of residuals was considered (using student residue on the Y-axis and estimated values on the X-axis). Results of multiple regression were only considered when it was demonstrated that the presence of variables of non-Normal distribution did not induce error in the analysis conducted.

We used the Mann-Whitney – U test to test for differences in the frequency of eggs, larvae and adults found on host plants in the sun or shade.

Results

Potencial Asteraceae species present at the studied trails

In two different censuses at each study trail, we checked 120 individuals of Mikania cordifolia, 90 individuals of Mikania 'sp.2', 67 Mikania 'sp.3', 60 Baccharis trimera, 30 Baccharis 'sp.2', six Baccharis 'sp.3', 105 Trichogoniopsis adenantha, six Eupatorium 'sp.1', 73 Eupatorium 'sp.2', 120 Bidens segetum, plus nine individuals of three non identified species (all Asteraceae). We observed that only Bidens segetum Mart. Ex. Colla, 1834, Mikania cordifolia (L.f.) Willd., Baccharis trimera, Eupatorium sp. and Mikania sp., were consumed by Stolaini tortoise beetles. Those plant species were utilized by Stolas chalybea (Germar, 1824), Stolas areolata (Germar, 1824), Anacassis phaeopoda Buzzi, 1976 (Table 1.1), Stolas cf. implexa (Boheman, 1850), and S. cf. turrita respectively.

Stolas chalybea, S. areolata, S. cf. implexa, and S. cf. turrita all feed on leaves of their host plants, whereas Anacassis phaeopoda feeds on the alate stem of their host plant.

Because we observed S. cf. implexa, and S. cf. turrita very rarely, we decided to focus our work on the three more abundant species S. chalybea, S. areolata and Anacassis phaeopoda.

We also observed that S. chalybea and S. areolata were strictly monophagous. Both species only developed until pupal stage when they were fed by their natural host plants. None of the larvae reared on non host plant could develop further the first instar (Table 1.2). The same pattern was observed in the field, so eggs, larvae and adults of S. chalybea, S. areolata and A. phaeopoda were not observed on any plant but their host species (Table 1.3). Exceptions for this pattern of host plant utilization (see tables 1.1 and 1.3) were eggs or beetles found on neighboring plants of their host which had direct contact to them.

Host plant utilization

We found 56.67 % of egg clusters of S. chalybea on old leaves, 40 % on young leaves and 3.33 % on the stem (n= 30). Egg clusters of the two Stolas species had a tendency to be found on the underside of leaves of their host plants (Table 1.4). Egg clusters of S. areolata were sometimes found on the leaves or stem of a plant just beside their host (from now on referred to as neighboring plants) (Table 1.4). We also observed one pupa and one fifth instar larva on neighboring plant leaves.

Larvae until the fourth instar of the two studied Stolas species were frequently found on the underside of leaves. This pattern was also observed for fifth instar larvae of S. chalybea, whereas fifth instar larvae of S. areolata were more frequently observed on the upperside of leaves (Fig. 1.1).

Larvae of both Stolas species seemed to pupate on leaves of their host plant; however, pupae of either species were not frequently observed.

Adults of the three species were always isolated and, following the tendency of older larvae of Stolas areolata, they were more frequently found on the upper side of leaves (Table 1.4). Some of them were also found on neighboring plants of their hosts (Table 1.4).

Host plant selection

The number of new branches on Cassidinae host plants was the most common trait that remained in multiple regression models after backward steps, suggesting their relation to Cassidinae presence. It was positively related to the frequency of egg clusters and larvae groups of S. chalybea on B. segetum and the frequency of adults of S. areolata on M. cordifolia. However, it was negatively related to the frequency of egg clusters and larvae groups of A. phaeopoda on B. trimera (Table 1.5). Host plant height also influenced positively larval and adult of S. chalybea frequencies on their host plant, as well as A. phaeopoda larvae and S. areolata adult frequencies(Table 1.5). Other traits, like number of branches, the number of leaves or mean length of new branches also were related to Cassidinae abundance as egg, larva or adult. Nonetheless, not all models were significant at the 5% level (Table 1.5).

Non significant results of Mann-Whitney tests revealed that egg clusters, larval groups and adults of S. chalybea, S. areolata or A. phaeopoda were equally frequent on plants in or out of shade (during the most of the day (Table 1.6). No tests were conducted for the number of egg clusters of A. phaeopoda because they were only found on sun plants and for the frequency of larval aggregation because only one of them was found on a shade plant.

Discussion

Although Astereae and Eupatorieae are very taxonomically close tribes, and Eupatorieae and Heliantheae are tribes with many parallelisms (Bremer, 1987), each of the three Stolaini species studied in this work used only one host plant species. Therefore, monophagy among tortoise beetles is in accordance with Jolivet (1988a) who considered Cassidinae one of the most specialized Chrysomelidae subfamilies. However, observations of Buzzi (1994) of S. chalybea on some Mikania species (including M. cordifolia) and A. phaeopoda on some other Baccharis species let us believe that these beetles are probably able to feed on different host plant species, if different populations (perhaps more geographically separated) were to be taken into account. Thus, we can consider them as functional monophages, which concentrate on one species in a particular habitat, but take different plant foods in different places (Crawley, 1983). According to Köpf et al. (1996) there are many cases that apparently polyphagous insect species actually consist of specialized populations.

The preference for oviposition on the under side of leaves seems to be a common pattern among Chrysomelidae considering data in the literature (see Jolivet, 1988b, Flowers, 1991; Charlet, 1992, Frieiro-Costa, 1995). This pattern was also observed for the larviposition of the Chrysomelidae Gonioctena sibirica (Kudo et al., 1995). Ovipositing on the underside of leaves may suggest the selection of sites by females; and we believe this behavior is very important for the survival of eggs and immatures because it provides protection against unfavorable abiotic conditions (Frieiro-Costa, 1995; Chaves, 1998) and natural enemies (Heinrich, 1979). Nevertheless, Chaves (1998) using caterpillar models to quantify predation on the underside or upperside of leaves, observed that the location of the prey on the leaf did not influence on the probability of being predated. This author suggested that the great number of Lepidoptera that can be frequently found on the underside of leaves might adopt this behavior for other reasons but predation.

Because location on the underside of leaves was frequent for larvae as well, we believe that it may play a role in their protection against abiotic factors and natural enemies. This pattern was clearer to young larvae because they might be more vulnerable to those factors. This vulnerability may not be the same for mature larvae and adults, as larvae of S. areolata and adults of both Stolas species that more frequently found on the upper side of leaves. Mature larvae and adults of both species of Stolas showed intense reflex bleeding, which may act as defense against attacks by predators. Sá and Macêdo (1999) also observed this same pattern of larvae location for the Cassidinae Plagiometriona flavescens and also suggested that it might be explained by the decrease of vulnerability at later instars and adulthood. Finding Stolas eggs and adults on neighbor plants of their hosts may also be considered another strategy to avoid encounters with natural enemies that are visually attracted to preys.

Frequency of eggs and larvae of S. chalybea were positively related to the abundance of new branches on their host plants probably because new leaves may be the preferred resource for them.

Freitas et al. (1999) investigating the selection of oviposition sites by a lepidopteran community observed that most of the records of egg and larva were on new leaves, indicating an apparent preference compared to mature leaves. Damman (1987) presented an example which young leaves of Asimina spp. were nutritionally superior to larvae of the pyralid moth (Omphlalocera munroei). Furthermore, we can suggest that the same relationship was not found for adult because they may feed on different plants, avoiding intraspecific competition with immatures. Negative relationship between the frequency of adults and number of new branches may confirm our hypothesis.

The positive relationship between the number of leaves on the host plant and larvae of S. chalybea showed that the beetles were more frequently found on plant individuals where leaves, the food resource, were more abundant and may make the plant more apparent. Many studies have indicated that plants that stand out from surrounding vegetation seem to be oviposited more frequently (see Rausher et al., 1981; Courtney, 1982). Plant apparency is an important trait for visually oriented insects. Wiklund (1984) studying 51 species of butterflies in Sweden showed that species that used visually apparent host plants found them more easily. Conversely, negative relationship between the number of leaves or branches and adults of S. areolata, eggs and larvae of A. phaeopoda suggest that beetles were not more frequently found where their food resource was more abundant.

Plant height was another parameter that was positively related to the frequency of larvae S. chalybea and A. phaeopoda and adults of S. chalybea and S.areolata. Positive relationship between chrysomelids and larger plants were also detected by Windig (1993) and Macêdo et al. (1994). The former author observed that when situated in dense vegetation, larger plants were more frequently eaten by Longitarsus jacobaeae (Chrysomelidae).

Higher frequencies of Cassidinae on plants where new branches were more abundant (so more vigorous growth is expected) or on larger individuals support the Plant Vigor Hypothesis proposed by Price (1991) and follow the pattern already observed for many phytophagous insects of different orders (Price, 1991).

A. phaeopoda was more frequently found on plant at sunny sites where its host plant, B. trimera, was found more frequently. The other two Cassidinae species did not show any difference in frequency of occurrence on plants, what may suggest that sun incidence did not affect Stolas beetles..

This work supported previous knowledge about the high specialization of phytophagous insects, especially Cassidinae. It also showed that besides the host plant species itself, some plant traits, especially those related to their size and presence of preferred resources, were important in determining the frequency of Cassidinae.

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Tables and figures

Table 1.1 Relative abundance of Asteraceae species of the tribes Eupatorieae, Astereae and Heliantheae found at "Mirante" and "Paraíso III" trails, at Serra do Japi, and absolute abundance of Cassidinae beetles (as egg, larval or adult stages) observed on each of those species during two census of plants at the same trails.

	Abundance							
ASTERACEAE	**"Mirante" Trail**				**"Paraíso III" Trail**			
	Plants	**Sc**	**Sa**	**Ap**	**Plants**	**Sc**	**Sa**	**Ap**
Tribe Eupatorieae						0	0	0
Eupatorium 'sp.1'	0	0	0	0	11.5	0	0	0
Eupatorium 'sp.2'	1.1	0	0	0	12.1	0	0	0
Eupatorium 'sp.3'	0	0	0	0	0.42	0	0	0
Eupatorium 'sp.4'	0.06	0	0	0	0	0	0	0
Eupatorium 'sp.5'	0.06	0	0	0	0.63	0	0	0
Eupatorium 'sp.6'	0.25	0	0	0	0	0	0	0
Mikania cordifolia	7.9	0	48	0	7.4	0	1	0
Mikania 'sp.2'	6.0	0	0	0	6.8	0	0	0
Mikania 'sp.3'	7.0	0	0	0	2.2	0	0	0
T. adenantha	62	0	0	0	23	0	0	0
Tribe Astereae								
Baccharis trimera	0	0	0	0	15	0	0	5
Baccharis 'sp.2'	0,.37	0	0	0	1.33	1	0	0
Baccharis 'sp.3	0	0	0	0	0.84	0	0	0
Baccharis 'sp.4'	0	0	0	0	7.3	0	0	0
Baccharis 'sp.5'	0	0	0	0	1.6	0	0	0
Tribe Heliantheae								
Bidens segetum	6	28	0	0	9.7	15	0	0
Total n	1626	28	48	0	517	16	1	5

Abbreviations: Sc= Stolas chalybea, Sa= Stolas areolata and Ap= Anacassis phaeopoda

Table 1.2 Number of larvae of Stolas chalybea and S. areolata that survived until the pupal stage after been reared on Mikania 'sp.2', M. cordifolia and Bidens segetum in the laboratory. Thirty first instar larvae of each species fed on leaves of each plant species.

Plant species	Number of larvae that reached pupal stage	
	Stolas chalybea	Stolas areolata
Mikania 'sp.2'	0	0
M. cordifolia	0	30
B. segetum	30	0

Table 1.3 Number of egg clusters (E), larvae (L) and adults (A) found on each host plant from October, 1997 until October, 1998 at two study trails in Serra do Japi.

	"Mirante" trail			"Paraíso III" trail		
	S. chalybea	S. areolata	A. phaeopoda	S. chalybea	S. areolata	A. phaeopoda
	E/L/A	E/L/A	E/L/A	E/L/A	E/L/A	E/L/A
B. segetum	7/30/9	0/0/0	0/0/0	24/21/19	0/0/0	0/0/0
M. cordifolia	0/0/0	6/13/21	0/0/0	0/0/1	0/3/4	0/0/0
B. trimera	*	*	*	0/0/0	0/0/0	7/12/20

* Plant species not observed at that trail.

Table 1.4 Localization of egg clusters and adults of Stolas chalybea and Stolas areolata on their host plants, Bidens segetum and Mikania cordifolia respectively.

Cassidinae species	Developmental stage	Localization			
		Underside of leaves	Upperside of leaves	Stem	Neighbor plant
Stolas chalybea (N= 59)	Egg	83.05 %	6.78 %	10.17 %	0
Stolas chalybea (N= 31)	Adult	12.90 %	51.62 %	12.90 %	22.58 %
Stolas areolata (N= 14)	Egg	50 %	0	28.57 %	21.43 %
Stolas areolata (N=35)	Adult	22.86 %	60 %	11.43 %	5.71 %

Table 1.5 Results of Stepwise, backward multiple regressions testing for relationships between host plant size parameters and number of S. chalybea, S. areolata and A. phaeopoda individuals (as egg, larva or adult) on a given plant.

Cassidinae Species	Dependent Variable	Number of Steps	Model	R^2	p	n
S. chalybea	Egg cluster frequency	3	Y= -1.309 + 1.580 Ln (D+1)	0.502	0.002	16
A. phaeopoda	Egg cluster frequency*	2	Y: 1.903-0.263 Square root D- 0.153 B	0.599	0.102^{ns}	8
S. chalybea	Larva frequency	3	Y: -1.328+2.847 Ln (A+1) + 0.155 Square root (C+0.5) + 0.030 D	0.607	0	26
A. phaeopoda	Larva frequency	1	Y: 2.017 + 4.384 A - 0.261D + 3.492 Square root E	0.897	0.007	9
S. chalybea	Adult frequency	2	Y: 1.585 - 0.269 Square root(D+0.5) + 0.658 A	0.187	0.126^{ns}	23
S. areolata	Adult frequency	2	Y: 2.679-0.087 C+ 0.013A + 0.057D	0.579	0.043	13
A. phaeopoda	Adult frequency	2	Y: 2.969 - 0.294 B	0.393	0.071^{ns}	9

*Variable of Normal distribution.

Abbreviations: A- plant height, B- number of branches on 30 cm. of stem, C- number of leaves on 30 cm. of stem, D- number of new branches, E- mean length of new branches, Ln- Neperian logarithm, ns- non significant relation at 5% level.

Table 1.6 Mann-Whitney tests results for the frequency of S. chalybea , S. areolata and A. phaeopoda egg clusters, larval groups and adults on their host plants individuals in the sun or in the shade.

Cassidinae species	Developmental stage	Rank Sum		Valid N for		U	z	p
		Shade plants	Sun plants	Shade plants	Sun plants			
S.chalybea	Egg	77.0	59	9	7	31.0	-0.529	0.958
	Larva	147.0	84.0	14	7	42.0	-0.535	0.601
	Adults	182.0	28.0	16	4	18.0	-1.320	0.186
S. areolata	Larva	18.50	26.50	4	5	3.50	-1.592	0.111
	Adults	45.0	46.0	7	6	17.0	-0.571	0.568
A. phaeopoda	Adults	9.00	36.00	2	7	6.00	-0.29	0.77

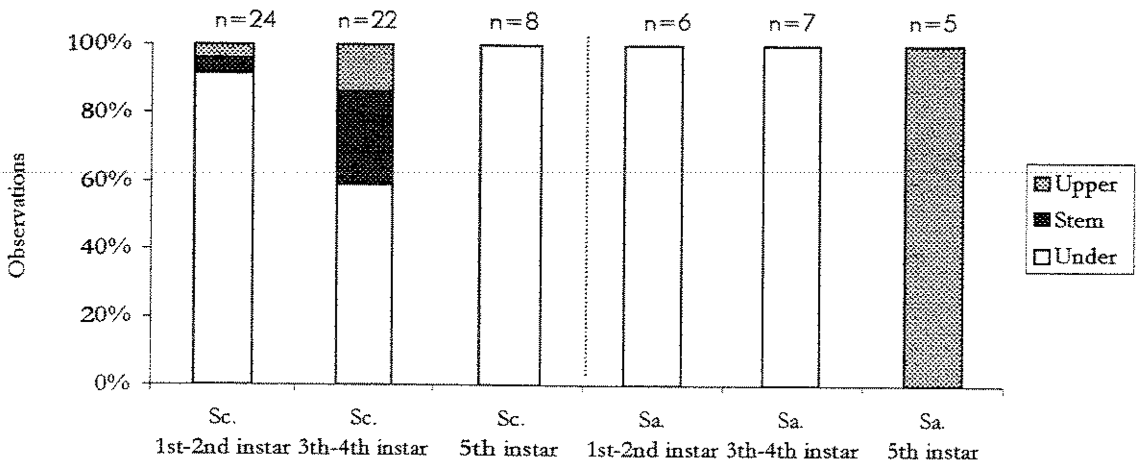


Figure 1.1 Localization of Stolas chalybea and S. areolata larvae on their host plants at "Mirante" and "Paraíso III" trails, at Serra do Japi, SP.

Abbreviations: Sc= Stolas chalybea, Sa= Stolas areolata.

Capítulo 2

BIOLOGICAL DATA AND POPULATION ABUNDANCE OF THREE SPECIES OF STOLAINI BEETLES (COLEOPTERA: CHRYSOMELIDAE: CASSIDINAE) IN A TROPICAL FOREST AREA.

Abstract: Some life history traits and the fluctuation of abundance of two populations of Stolas beetles, S. chalybea, and Stolas areolata and one population of Anacassis phaeopoda were investigated at an Atlantic Forest area in São Paulo state during the cycle of 1997-1998. The three species were active for about nine months per year, and had an arrest of development and activity during the winter/dry season. It was observed that temperature can exert influences on Stolas populations, whereas no effect was detected for rainfall. Because preferred resources (like young leaf) were related to temperature and rainfall, we suggest the importance of climate on Cassidinae populations by indirect effects. That was confirmed by their synchrony with the presence of some pheno-phases of their host plants.

Introduction

Very little is known about the population dynamics of tropical insect populations, and most knowledge is based on populations of temperate region (Wolda, 1992). For this reason, little theory exists and few hypothesis have been formed (Medeiros and Vasconcellos-Neto, 1994). Even the studies on this subject for temperate species concerns few species of economic interest. These facts reinforce the urgent needs to obtain information about the population dynamics of tropical insects, so theory about the structure and functioning of communities may be improved.

Some studies dealing with tropical population of Chrysomelidae beetles showed that they are active throughout the year (Nakamura et al., 1989; Macêdo et al. 1994; Sá and Macêdo ,1999). Nonetheless, other authors studying other Chrysomelidae species in subtropical areas or areas close by showed that they may undergo a winter diapause and reproduce in the summer (see Medeiros and Vasconcellos-Neto, 1994; Frieiro-Costa, 1995; Becker and Freire, 1996; Vasconcellos-Neto and Jolivet, 1998). This same seasonality was observed for other insect groups like butterflies (Vasconcellos-Neto, 1991; Brown, 1992) and Tettigoniidae orthopterans (Del-Claro, 1991a) and Alticinae chrysomelids (Del-Claro, 1991b) in this same region.

After studying tropical insects for 14 years, Wolda (1992) observed that long-term abundance of most populations presented a large fluctuation in numbers along time. The author believes that environmental changes cannot explain such variation since very little information about the life history of these animals are available. This pattern questions the effectiveness of regulation of such populations. However, short-term variation of insect occurrence may be caused by different factors. For instance, diapause may be caused by many abiotic or biotic factors and provides a seasonal pattern on insect occurrence. Temperature, rainfall, and photoperiod describe only a portion of tropical seasonality; probably because of lower variation of these factors in this region. Nonetheless, Cornell et al. (1998) compiling life table studies from several herbivorous insects species concluded that weather kills more temperate-zone immatures than tropical/subtropical ones. Much of the selective pressure for insect seasonality in the tropics is likely to come from biotic sources. However, there are some evidences of abiotic parameters influencing dynamics of tropical insect populations, as well. For instance, rain may exert influence on the reproduction, growth and many other vital activities of the host plant and insect (Delinger, 1986). This might help explain why much of the seasonality of tropical insect populations is determined by dry/wet periods. Besides this, humidity can also be cited as one of the most important diapause-inducers in the Neotropical region (Hsiao, 1986). Day length may also be considered an important seasonal indicator in tropical regions of higher latitudes. In any case, little evidence exist to confirm the role of these climatic factors on diapause induction (Delinger, 1986). Furthermore, detecting the effects of climate on tropical herbivorous insects is not common (Sá and Macêdo, 1999) and sometimes those effects do not exert direct influence on a given population.

Biotic factors can also strongly influence dynamics of populations of tropical insects. For instance, food clearly plays a role on their seasonality (Wolda, 1988) exerting important short or long-term effects. Considering only those short-term effects, the influence of host plants on their associated phytophagous insects may be due to their local abundance, distribution, chemistry, morphology and/or phenology (Strong et al., 1984). The phenology of a given plant can also influence populations of herbivorous insects because it can vary in their suitability during development. This variation can be not only in foliage phytochemistry (providing change in quality), but also on the plant availability. Some cases of temporal limitation in specific food availability have been studied, and synchrony of insects with their host plant traits, like egg hatching with bud burst (Bernays and Chapman, 1994) is quite common (e.g.: Campbell and McCaffrey, 1991; Clark and Clark, 1991; Vasconcellos-Neto, 1991; Medeiros, 1991; Crowe, 1995). Other insect species may not be so specific in relation to their food, but may have preferences. Preferred plant traits have been correlated with higher occurrence of herbivore and with their population dynamics.

Spatial and temporal distribution of host plant may influence behavior, reproduction, colonization and density of populations of herbivorous insects and their parasitoids (Garcia and Altieri, 1992). If host localization by parasitoids can be provided by chemical or visual signs of the plant, then the larger the patch of the host plant, the more parasitoids will be attracted (Queiroz, 1996; Meiners and Hilker, 1997).

Wolda (1988) emphasized the importance of investigations into the seasonal patterns of insect populations in the tropics; especially if gathered information is on both adult and immature stages and on the basic life history of the species concerned. Furthermore, detailed studies on demographic characteristics between closely related species or between populations within the same species are better for understanding population dynamics and the evolution of life history traits (Nakamura, 1983).

In this work, we investigated some aspects of the biology of three species of Cassidinae, Stolas chalybea (Germar, 1824), S. areolata (Germar, 1824), and Anacassis phaeopoda Buzzi, 1976 and studied their population dynamics at Serra do Japi, at two sites at different altitude. We also had the objective of trying to detect if plant phenology and/or climatic parameters influenced the number of Cassidinae during a year.

Material and Methods

Study area

This work was carried out at Serra do Japi (23° 11'S/46° 52' W), a montane area located at the southern limit of the tropical zone in São Paulo state, Brazil.

The climate in the area is classified as Cwa, according to the Koppen system, corresponding to the subtropical moist type (Medeiros e Vasconcellos-Neto, 1994). Because of the large range of altitude (700 m to 1300 m above sea level), mean temperature varies from 11.8° to 18.4°C in July (the coldest month) to 18.4° to 22.2°C in January (the warmest month) (Pinto, 1992). Rainfall is higher in December and January (precipitation can reach 250 mm in one month), but from October to March it is also much higher than in the colder months (mean precipitation in the months in this season is 41 mm). These climatic conditions result in two distinct seasons during the year: warm and rainy summer and a cold and dry winter (Pinto, 1992) (Fig. 2.1).

Semideciduous mesophytic forests of altitude are predominant at Serra do Japi (Leitão-Filho, 1992). Reproductive activity and plant sprouting occur mainly from August to December in Serra do Japi (Morellato, 1992), coinciding to the increase in temperature and rainfall. At study sites, vegetation was basically composed of short trees of low stem diameter

We concentrated our study along two trails: "Mirante" trail (1170 m altitude) and "Paraíso III" trail (1070 m altitude). Both trails can be mainly characterized by the shade provided by the trees located along their edges. "Paraíso III" also presents a open swampy area, where an large Baccharis trimera (Asteraceae) patch was observed.

Life history

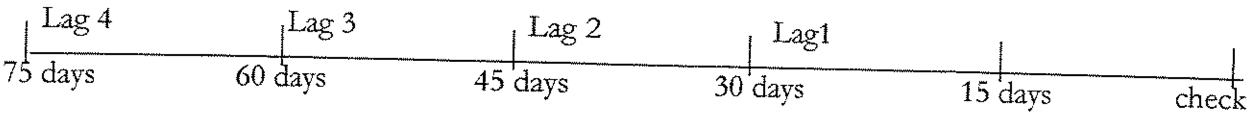
In order to obtain data on the life history of Stolas chalybea, S. areolata and Anacassis phaeopoda, we tagged 30 individuals of their respective host plants, Bidens segetum, Mikania cordifolia at "Mirante" and "Paraíso III" trails and Baccharis trimera at the latter trail. We visually checked tagged plants every 15 days for the numbers of egg clusters, larvae, and adult from July, 1997 until September-October, 1998. We also considered data obtained on records of Cassidinae on non tagged plant individuals observed throughout study period. Time of the day when this protocol was conducted was very variable (always during day). We also recorded the sizes of egg clusters and larval groups, and determined medians, standard error and deviation for each of them. The difference on size of egg clusters of each of the studied Cassidinae species by was tested using the Kruskall-Wallis test (after checking non-Normality of data). When the test was significant, we used Dunn Q-test to determine differences between samples. When it was not possible to include one of the species in the analysis, we used Mann-Whitney U-test instead of Kruskall-Wallis test.

Fluctuation of populations

During the conduction of the same protocol for plant checking described above we also recorded the number of egg clusters, larval groups and adults on the tagged host plants. Considering the number of cassidines per plant on each census, we estimated population sizes at each study trail by a projection of the number of beetle individuals per plant on the total number of host plant present along the 700 meters of "Paraíso III" and "Mirante" trails.

In an attempt to recognize the factors that influence the abundance of egg deposition, larva and adult, we considered the relative abundance of different pheno-phases monthly recorded for each checked individual. We also considered maximum, minimum and mean (mean of maximum and minimum) temperatures and rainfall for each period of time between the censuses. Temperature data were calculated by an arithmetic mean of daily temperatures from the day following the last check until the present check day. Rainfall was calculated by summing all data in the same period. The number of rainy days was also considered for each interval. Egg, larva and adult abundance were tested for relation with weather parameters using Spearman correlation indexes. This index was selected after testing the distribution of Cassidinae numbers and weather data and realizing that most of them were not normal, even after transformation. Shapiro Wilk test was used to test for Normality of these data.

In an attempt to detect the influence of weather on reproduction and on the number of individuals at the following developmental stage, we also correlated egg, larva and adult abundance with climatic parameters in 30 (Lag1), 45 (Lag2), 60 (Lag3) and 75 (Lag4) days previous to a given check . Each 'Lag' period corresponded to 15 previous days (see illustration bellow).



Asteraceae species composition, relative and absolute abundance of host plant species were also investigated to verify the influence of plant density on Cassidinae .

Results

Life history

Stolas chalybea, Stolas areolata and Anacassis phaeopoda deposited their eggs in clusters. Egg cluster sizes of S. areolata and S. chalybea were significantly larger than A. phaeopoda (Table 2.1).

After emerging from the eggs, larvae of S. chalybea and S. areolata remained aggregated. Nevertheless, the size of the groups decreased as the larvae developed (Table 2.1). A. phaeopoda larvae also remained aggregated after emerging from the eggs, and the size of the groups also seemed to decrease as they developed. However, we observed A. phaeopoda larval groups of 3rd - 4th and 5th instars only once (each) during the entire study period, and both of them were isolated (Table 2.1).

Population fluctuation

The general pattern of the populations of S. chalybea, S. areolata and A. phaeopoda was that adults were first observed active from September-October, when they left diapause, and started their reproductive activities in the spring, reaching higher levels in the summer. During the fall reproduction lowered and was completely interrupted in the middle of May. Because of the disappearance of Cassidinae, we believe that diapause period was during dry-winter season.

During the studied life cycle (1997-1998) we observed S. chalybea in the field from the beginning of October, until the beginning of June as adult. Eggs and larvae were observed from the beginning of November until in the end of April (egg) and May (larva), with one peak of abundance a few days after adult diapause and another during the warmest months. After overwintering, S. chalybea was observed in the field again in the end of September as larvae and adults and in the end of October as eggs. This pattern was very similar to populations at "Mirante" and "Paraíso III" trails (Fig. 2.2).

In the same cycle, S. areolata was observed from the end of September until the end of May as adult, eggs were observed from the beginning of November until the end of May, and larvae from the end of November, until the end of April. Two peaks of abundance of eggs were also observed in a similar time as observed for S. chalybea. After overwintering, an adult was observed in the end of September (Fig. 2.3).

We observed that abundance of population of S. areolata at "Mirante" trail seemed to be larger than at the other trail, whereas populations of S. chalybea did not differ much in both trails.

The first observation of A. phaeopoda was observed in the field after overwintering (in 1997) was in the middle of September, as adult and larva. First eggs were observed in the end of the same month. Its population was also small, even in the summer, as were S. chalybea and S. areolata (Fig. 2.4). The population of A. phaeopoda was active as adults until the middle of June (when it was observed for the last time before diapause) and eggs until the beginning of June. After overwintering in 1998, we only observed one adult in the end of September.

We observed mating activity of S. areolata in March, 1999, and A. phaeopoda in January, February and in March, 1998.

No significant correlation was detected between the abundance of studied populations of S. chalybea or S. areolata and mean rainfall during the periods between each census. Nonetheless, we observed a strong influence of rain (controlling dry and humid seasons) on the regulation of diapause and reproduction of Cassidinae. Significant correlations were found between the abundance of Cassidinae populations and temperature with a lag period (Table 2.2).

Cassidinae were more abundant during the occurrence of plants bearing young or mature leaves pheno-phase and this abundance decreased when plants bore senescent leaves. Although the studied populations of S. chalybea and S. areolata were quite small, we observed that they responded to the phenology of their host plants (see figures 2.5 and 2.6). S. chalybea and S. areolata decreased their population when plants with young and mature leaves were less frequent and when individuals with few or without leaves became more frequent. The populations of S. areolata became active again (after diapause) almost at the same time that M. cordifolia with young leaves began to appear, whereas S. chalybea was observed about one month after the sprouting of new leaves of B. segetum.

The population of A. phaeopoda was more abundant from the middle of the fructification period (see figure 2.7 for illustration of B. trimera phenology) until the beginning of the dry season (when the stem became drier).

The rate of S. chalybea per host plant individual at "Paraíso III" was higher than at "Mirante", although B. segetum was 1.77 times more abundant at the latter trail (Table 2.3). However, relative abundance of B. segetum at "Paraíso III" was higher than at "Mirante" trail (Table 2.3). On the other side, the rate of number of S. areolata per host plant individual was almost 5.5 times higher at "Mirante" trail, although M. cordifolia was only 1.22 times more abundant at that trail and its relative abundance was also lower (Table 2.3). Nothing could be concluded about the relation of A phaeopoda with the abundance of its host plant and localization because it was only found at "Paraíso III".

S. chalybea and S. areolata were more abundant on their host plants in the life cycle previous to the one investigated in this work. This can be observed by the number of Cassidinae per host plant during preliminary census conducted by the authors at Serra do Japi (Table 2.4).

Discussion

Egg cluster sizes of the three species, although significantly different from each other, were quite distinct from data in the literature. Authors, like Buzzi (1994) and Frieiro-Costa (1995), observed larger egg cluster sizes of some other Stolaini species in comparison to the size of the two Stolas species studied. On the other hand, in the case of A. phaeopoda, it is the first record of egg clustering in the literature. Buzzi (1994) revising systematic aspects of some Anacassis species only found isolated eggs of A. phaeopoda.

S. chalybea and S. areolata seemed to have the same pattern of occurrence throughout the year: they became active with the end of the dry season, reproduced more intensely during the rainy season (summer) and reduced their activity (entering in diapause) during the period of lower temperature and less rainfall. A. phaeopoda showed a similar pattern, although it could be observed at the field earlier at the end of the dry season, and its reproduction was displaced to the end of the super-humid season. The periods of activity of these species seem to be longer than other Chrysomelidae species that diapause during the winter/drought (about two to three months longer). Nonetheless, this must have been because most of the studies were conducted in temperate or subtropical regions (e.g.: Hare, 1983; Naranjo and Sawyer, 1989; Pucci et al., 1991; Cappaert et al., 1991). Diapause was known to occur in the eggs, late instar larvae, pre-pupae and adults of Chrysomelidae (Cox, 1994). However, for the Cassidinae genera that diapause has already been recorded it occurs only in adults, as we do believe that happen with the three species in this work (see Cox, 1994 and Takizawa, 1994).

Because eggs were observed so early after the end of diapause of adults, and when their numbers were still too low (first peak of abundance of eggs), we suggest that females of the three studied species overwinter already fertilized. No mating couples were observed during the entire study time for S. chalybea, copula was observed only during the summer season for the other two studied species. However, we believe that females use stocked sperm from the previous season rather than finding mates when their populations are so low. Stevens and McCauley (1989) observed that females of the chrysomelid Plagiodera versicolora overwinter inseminated, and use sperm from more than one male. These authors believe that these females have the advantage of not needing to find mates prior to laying eggs in the spring. On the other hand, they believe that there is a risk that the sperm can become inviable during the winter, and males that mate prior to diapause have the chance of mating with females that would not survive to the winter.

There seems to be a difference in size of populations of S. areolata in "Mirante" and "Paraíso III" trails, with larger population at the former trail. Although the species richness and physical structure of plant community can exert a profound effect upon the rate at which a given plant is attacked by invertebrate herbivores (Crawley, 1983), we believe that the difference between the numbers of S. areolata at both trails was not due to higher abundance of their host plant at "Mirante". Further more, M. cordifolia relative abundance at that trail was lower than at "Paraíso III" trail. Thus, we suggest that there might be less pressure by natural enemies at that "Mirante" or there might be some abiotic influence of the 100 meter altitude difference between them. Nevertheless, detecting abiotic effects (by climatic parameters) on herbivorous insect populations is not very common (see for example Nakamura and Abbas, 1989; Macêdo et al., 1994; Rodriguez-Del Bosque and Magallanes-Estala, 1994 and Sá and Macêdo, 1999). However, some significant relation were detected for the studied populations and temperature, specially at the areas where they were more abundant. As the interaction between host plant density and relative plant density allows a vast range of responses (Crawley, 1983), an opposite situation was observed for S. chalybea: the rate of cassidine per host plant was higher at "Paraíso III" trail, where its host plant was less abundant than at the other study trail, but its relative abundance was higher.

Higher temperatures seem to increase ovipositing activity of S. chalybea populations, thus explaining, the higher numbers of observed eggs at higher temperatures. This also explains the peak of abundance of eggs of the three Cassidinae species in the warmest months of the year. A similar situation was observed for Galerucella calmariensis and G. pusilla, for which drop in fertility coincided with decreased daily temperatures (Blossey, 1995). Furthermore, the positive relationship between larvae and temperature at lag 3 reinforces this hypothesis.

The observed negative relationship between temperature and egg numbers of S. areolata at "Mirante" trail can be better explained indirectly by biotic factors. Because the positive relationship between lag 3 larvae and lag 4 adults is so strong, we believe that higher temperature might increase mating activities resulting in higher numbers of larvae and adults later. Reduced numbers of eggs of S. areolata could be explained if the same positive relationship with temperature also happened for predators of eggs. We evidenced it as potential predators of S. areolata were more abundant during the months of higher temperature, especially at "Mirante" trail (Sá and Vasconcellos Neto, in preparation – see chapter three of this thesis). We also believe that temperature exerts a stronger influence on S. chalybea, S. areolata and A. phaeopoda than correlation tests detected. We suggest that higher temperatures in the spring and summer may influence the appearance of Cassidinae in the field after diapause and their reproduction respectively.

Tanaka and Tanaka (1982) observed a significant (although not strong) positive relationship between arthropod abundance and rainfall after a three week lag. Caldas (1995) observed that rainfall was one of the main factors responsible for the low level of first instar larvae of Anaea ryphea (Lepidoptera: Nymphalidae) recruitment. In contrast to these results, no effect of rainfall on the studied population was observed. However the period of absence of rain has lowered all the studied populutions. This effect, also observed for other Chrysomelidae populations (see Medeiros, 1991), may be caused by the association of the dry season with lowering of activities, which may be reinforced by a shortage of food caused by the fall of host plant leaves. Brown (1992) reported this same situation for butterflies in Serra do Japi that showed synchronized abundance with the increase of temperature, rainfall and plant sprouting around September, and decreased their populations at the end of the fall when pressure by natural enemies was more intense, senescent host plants were more frequent and lower temperature and rainfall were observed.

According to Crawley (1983) breeding success of many herbivore species depends critically on the synchrony between reproduction and the availability of high-quality food. Courtney and Duggan (1983) observed considerable death of some late instar larvae and pupae of the butterfly Anthocharis cardamines caused by bacterial attack upon animals under stress or feeding on less suitable foodplants. On this study, we observed that not only reproduction but also the abundance of the three studied species of Cassidinae also showed synchrony with plant phenology. This is surely because feeding resources (leaves or alate stem) are crucial for these beetles and their occurrence varies along the year. Del-Claro (1991b) also observed the chrysomelid Homophoeta octoguttata was more abundant when individuals of its host plant, Peltodon radicans(Labiatae), bearing leaves were more abundant too. Many authors have also detected synchrony of herbivorous occurrence with the abundance of preferred food (see Campbell and McCaffrey, 1991; Clark and Clark, 1991; Vasconcellos-Neto, 1991; Medeiros, 1991 and Crowe, 1995 for examples) and some explain this by the seasonal change in nutritional quality of leaves, making young and tender leaves mostly often the less deterrent resource (Feeny, 1970; Crawley, 1983).

As could be observed from earlier observations of the previous life cycle (1996-1997), Cassidinae seemed to be less abundant during the study period. We believe that this decrease in population size is probably due to the phenomenon called "El Niño" which occurred during the period of the 1997-1998 life cycle. This is a cyclic phenomenon that provokes a decrease in rainfall and increase in temperature (see figure 2.1 for effects on rainfall and temperature in the 1998 summer). This might have influenced the studied populations, reducing their mating activity. We suggest that S. chalybea, S. areolata and A. phaeopoda populations should be investigated during years when the influence of the climatic phenomenon is absent to confirm the patterns of occurrence and abundance presented in this work. However this work provides valuable information about some populations of Cassidinae being influenced by an atypical climatic situation.

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Tables and Figures

Table 2.1: Size of egg clusters and larval aggregations (at different life stages) and results of Kruskal-Wallis or Mann-Whitney tests testing for differences among S. chalybea, S. areolata and A. phaeopoda observed in the field during 1997-1998 life cycle at Serra do Japi.

		Cassidinae species			
Developmental stage	Parameter	S. chalybea	S. areolata	A phaeopoda	Statistical Test
Egg cluster	Median	6 ^{a}	10 ^{a}	3 ^{b}	H= 18.75
	Min. – 25th Perc.	2 - 4	2 - 4	1 - 2	p= 0.001
	Max. – 75th Perc.	21 - 9	22 - 16	7 - 5	DF= 2
	n	80	14	17	
1st, 2nd instar larvae	Median	3 ^{a}	1 ^{a,b}	1± ^{b}	H= 13.44
	Min. – 25th Perc.	1 - 1	1 - 1	1 – 1	p= 0.001
	Max. – 75th Perc.	5 - 4	4 - 3	3 - 1	DF= 2
	n	24	6	15	
3rd, 4th instar larvae	Median	1 ^{a}	1 ^{a}	1 ^{a}	U= 88
	Min. – 25th Perc.	1 - 1	1 - 1	1 – *	p= 0.368
	Max. – 75th Perc.	4 - 2	4 - 4	1 - *	DF= 1
	n	21	7	1	
5th instar larvae	Median	1 ^{a}	1 ^{a}	1 ^{a}	U= 6
	Min. – 25th Perc.	1 – 1	1 - 1	1 – *	p= 0.6214
	Max. – 75th Perc.	2 - 1	3 – 1	1 - *	DF= 1
	n	8	17	1	

* Parameter not calculated because of low sample size.

Abbreviations: Min.- Minimum value, Max.- Maximum value, 25th Perc.- 25th percentile, 75th Perc.- 75th percentile, DF- degrees of freedom.

Medians with different letters were significantly different from each other at 5% level.

Table 2.2: Spearman correlation indexes between Cassidinae abundance (as egg, larva or adult) and temperature at Serra do Japi during study period (1997-1998 cycle).

Species	Trail	Lag period X Developmental stage**	Temperature		
			Maximum	Minimum	Mean
S. chalybea	"Paraíso III"	Lag 3 X eggs	0.641*	0.615*	0.677*
		Lag 3 X larvae	0.701*	0.726*	0.773*
S. areolata	"Mirante"	Lag 1 X eggs	-0.738*	-0.679*	-0.768*
		Lag 3 X larvae	0.608*	0.657*	0.663*
		Lag 4 X adults	0.881*	0.811*	0.854*

* Index was significant at 5% level of significance.

ns.- non significant

** For every development phase, we also tested the correlation between the abundance of Cassidinae with 1, 2 , 3 and 4 Lag periods and minimum, maximum and mean temperatures, rainfall (in mm.) and number of rainy days. Results not mentioned in the upper table were not significant.

Table 2.3 Abundance and richness of species of the tribes Heliantheae, Astereae and Eupatorieae (Asteraceae) at "Mirante" and "Paraíso III" trails at Serra do Japi, SP, with emphasis to Bidens segetum, Mikania cordifolia and Baccharis trimera and the abundance of Cassidinae* on them.

	"Mirante" trail	"Paraíso III" trail
Total number of plant individuals	1626	517
Total number of species of Asteraceae	12	15
Number of B. segetum	245 (77%)**	138
Number of M. cordifolia	128 (22%)**	105
Relative abundance of B. segetum	15.07%	26.70%
Relative abundance of M. cordifolia	8.0%	20.3%
Number of B. trimera	0	220
Number of S. chalybea* (Cassidinae/host plant)	376 (1.53)	294 (2.13)
Number of S. areolata* (Cassidinae/host plant)	171 (1.33)	25 (0.24)
Number of A. phaeopoda* (Cassidinae/host plant)	0 (0)	286 (1.33)

* Number of Cassidinae corresponds to the projection of summed numbers of eggs, larvae and adults during the 1997-1998 cycle considering total number of their respective host plant at each trail.

** Number in parentheses mean percentage of exceeding individuals of B. segetum or M. cordifolia at "Mirante" trail in relation to "Paraíso III" trail.

Table 2.4 Abundance of Stolas chalybea and S. areolata (as, larvae, pupae oand adults) observed on Bidens segetum and Mikania cordifolia during preliminary observations (from March until June,1997) at Serra do Japi SP. Numbers between parentheses are the absolute abundance of Cassidinae and host plants censused.

	Larvae/Plant	Pupae/Plant	Adults/Plants	Larvae+Pupae+Adults/ Plants
Stolas areolata	2 (14/7)	1.33 (8/6)	1.26 (29/23)	1.42
Stolas chalybea	9.5 (19/2)	1 (2/2)	1.25 (14/12)	2.19

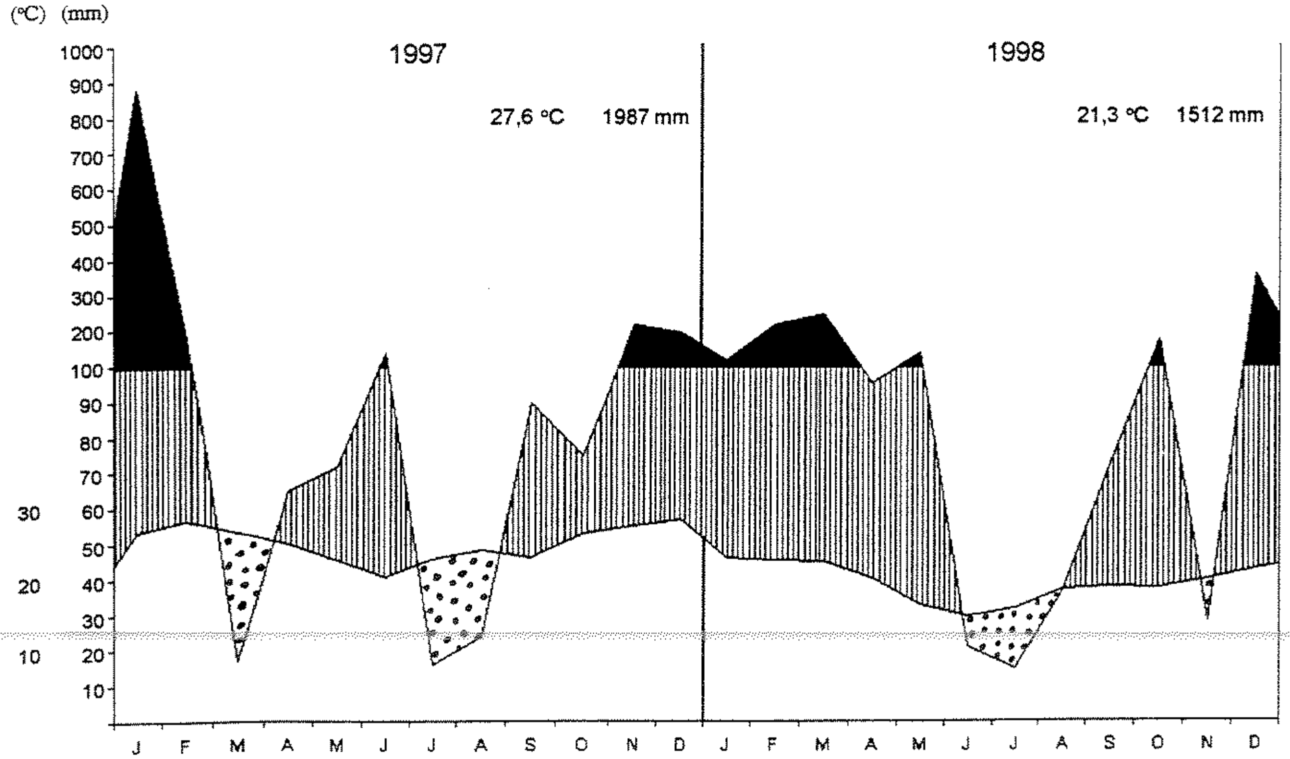

Figure 2.1: Climatic diagram of Jundiaí (where Serra do Japi is located), in São Paulo state, during the years 1997 and 1998 (according to Walter and Lieth, 1960). Original data was collected in a station at 715 m and temperature data was corrected to 1170m (Data provided by Instituto Agronômico de Campinas). Dotted region represents dry periods, and dark region represents super-humid periods.

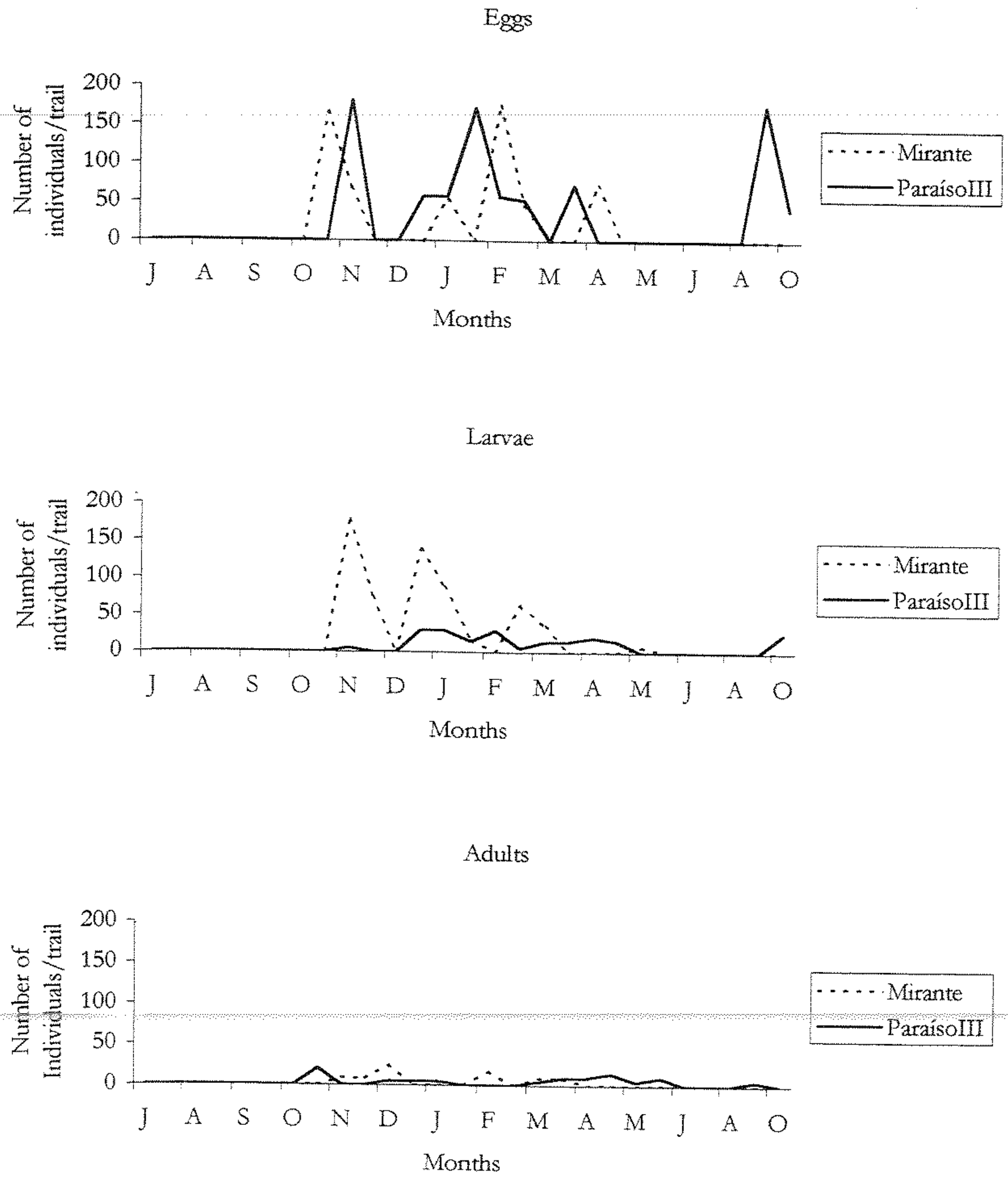

Figure 2.2 Population abundance of Stolas chalybea at different life stages at "Mirante" and "Paraíso III" trails at Serra do Japi, SP.

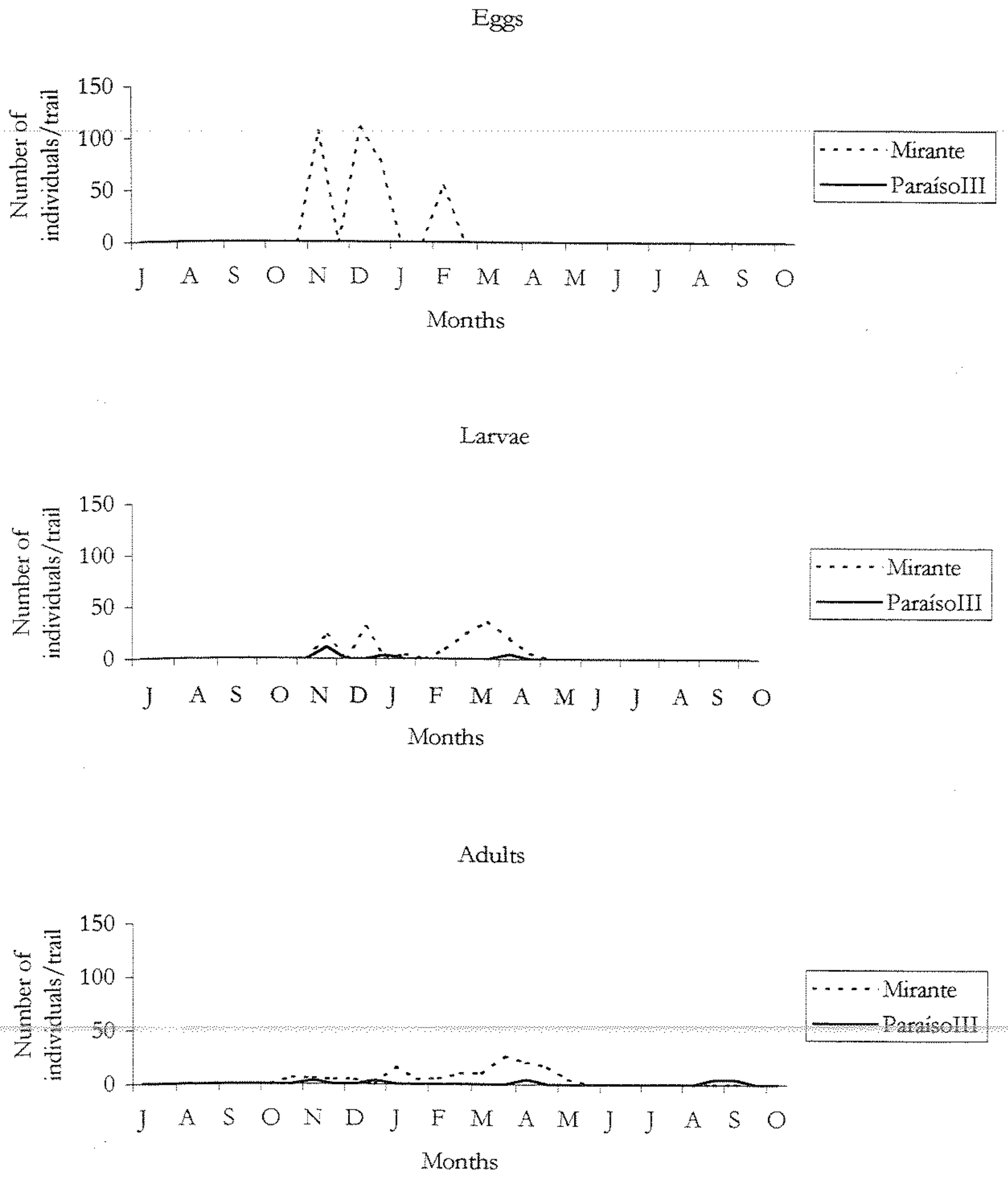

Figure 2.3 Population abundance of Stolas areolata at different life stages at "Mirante" and "Paraíso III" trails at Serra do Japi, SP.

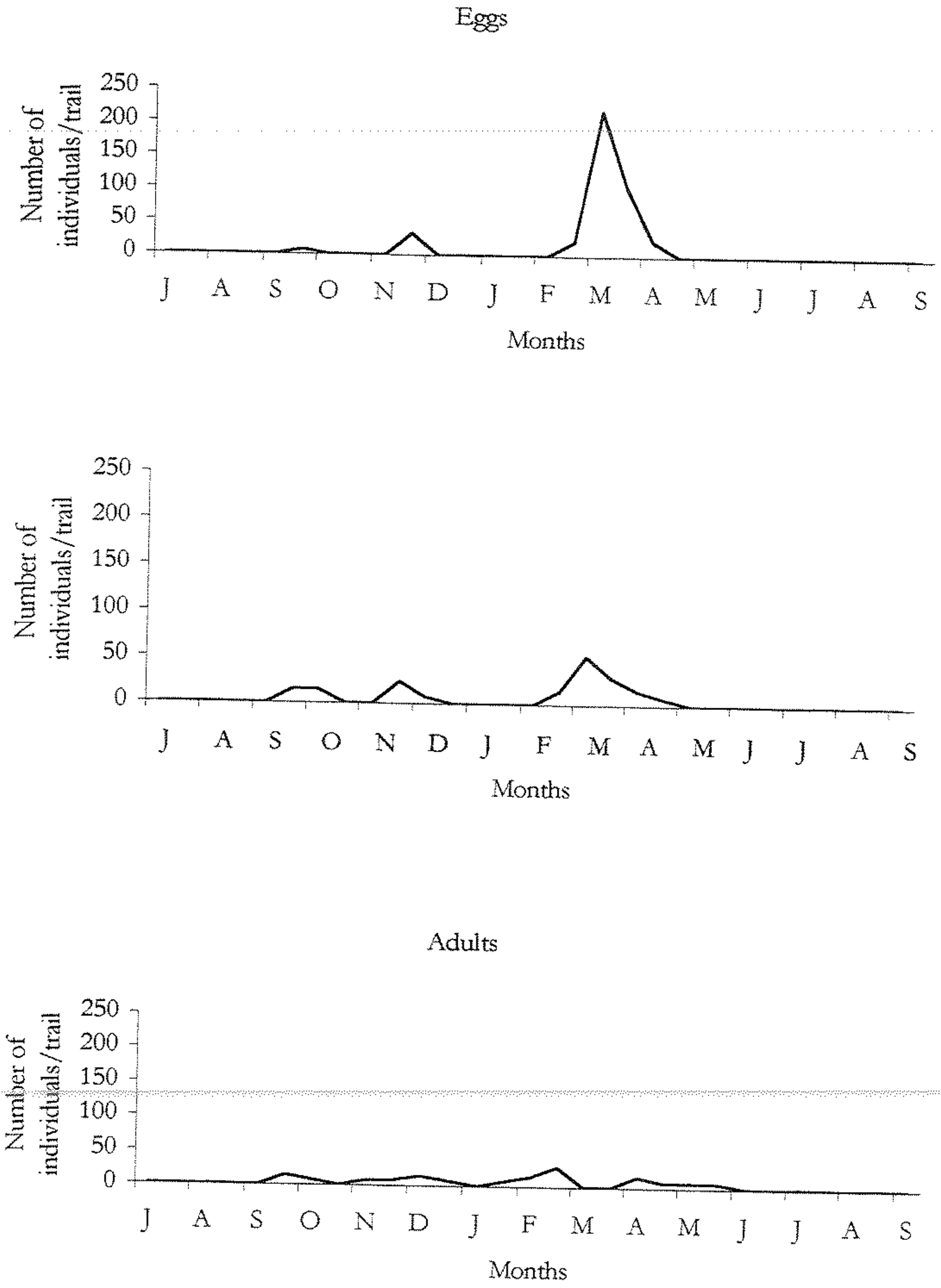

Figure 2.4 Population abundance of Anacassis phaeopoda at different life stages at and "Paraíso III" trail at Serra do Japi, SP.

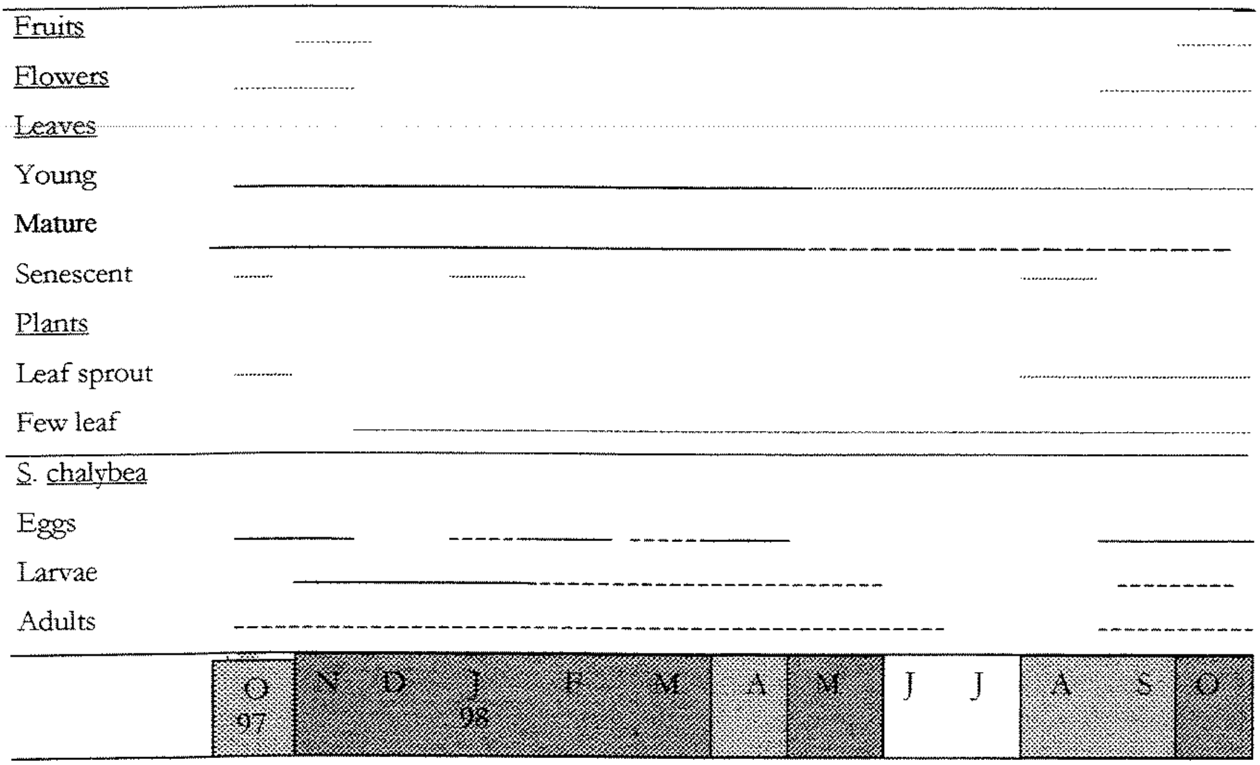

Figure 2.5 Frequency of main phenophases of Bidens segetum and Stolas chalybea observed during 1997-1998 study period. Frequency of phenophases was respresented in four categories: absent ( ), rare (------), intermediate (---) and abundant (—). We observed that some plants completely lost their leaves from October (1997) until January (1998) and from April to October (1998). Frequency of the beetle was represented as frequent (—), rare (---) and absent ( ). Months painted in white represent dry period, and painted in light and dark gray represent humid and super humid periods respectively.

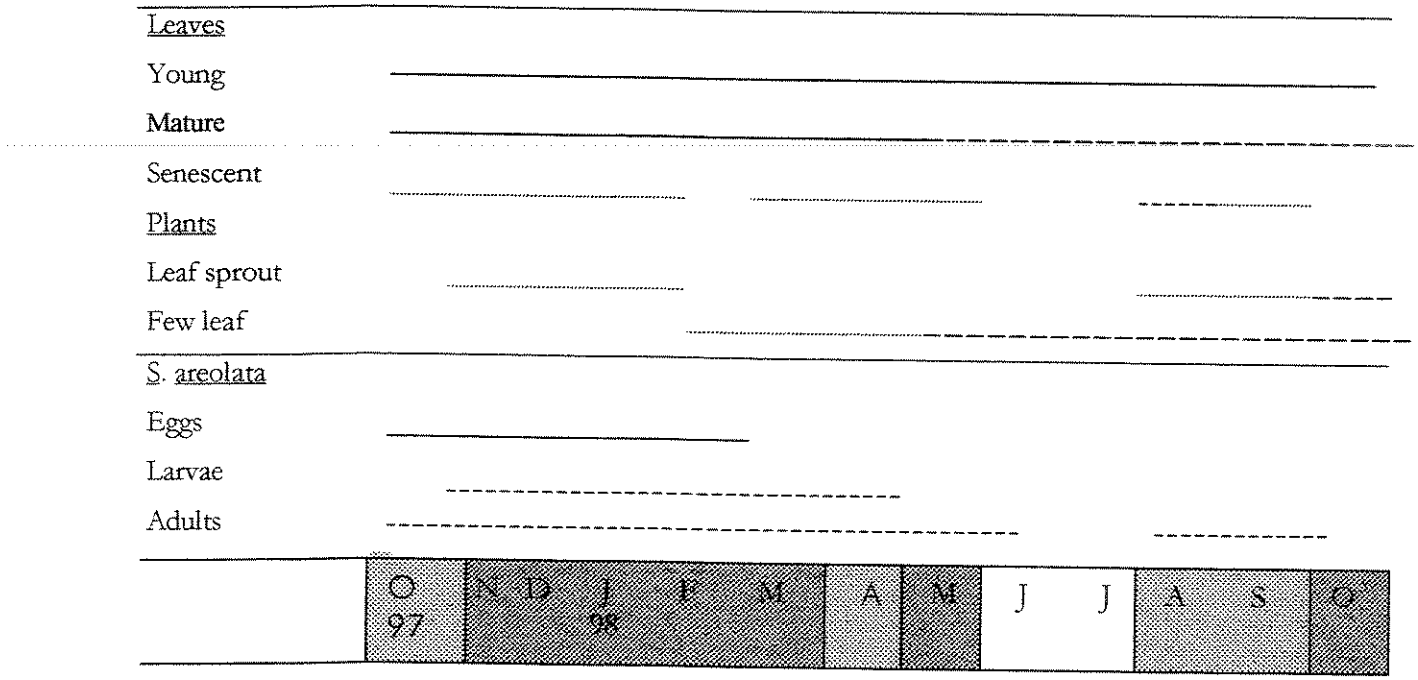

Figure 2.6 Frequency of main phenophases of Mikania cordifolia and Stolas areolata observed during 1997-1998 study period. Frequency of phenophases was respresented in four categories: absent ( ), rare (┄┄), intermediate (---) and abundant (——). We observed that some plants completely lost their leaves from February until October (1998). Frequency of the beetle was represented as frequent (——), rare (---) and absent ( ). Months painted in white represent dry period, and painted in light and dark gray represent humid and super humid periods respectively.

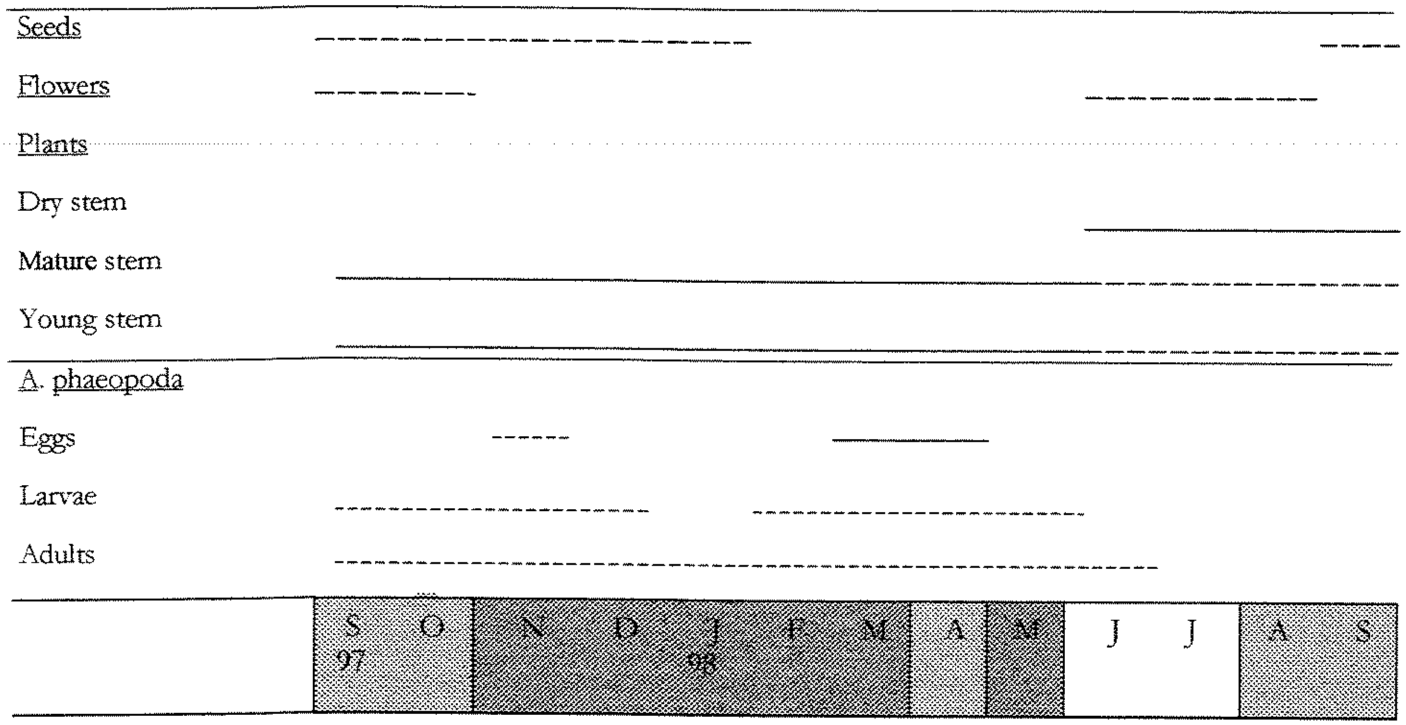

Figure 2.7 Frequency of main phenophases of Baccharis trimera and A. phaeopoda. observeduring 1997-1998 study period. Frequency of phenophases was respresented in four categories: absent (   ), rare (┈┈), intermediate (---) and abundant (——), frequency of the beetle was represented as frequent (——), rare (---) and absent (   ). Months painted in white represent dry period, and painted in light and dark gray represent humid and super humid periods respectively.

Capítulo 3

NATURAL ENEMIES OF THREE SPECIES OF CASSIDINAE (COLEOPTERA: CHRYSOMELIDAE) AND EXPERIMENTAL EVALUATION OF THE INFLUENCE OF PREDATORS ON STOLAS CHALYBEA.

Abstract: Three species of Cassidinae, Stolas chalybea, Stolas areolata and Anacassis phaeopoda, were investigated in a tropical forest to understand how they are influenced by natural enemies. The majority of parasitic organisms obtained from these cassidines were Hymenoptera, but we have also observed Diptera on Stolas larvae. The only parasite observed in adult was a Mermithida; nevertheless, some heteropteran and spider predators were observed. A one-year census on the studied Cassidinae host plants revealed that the abundance of potential predators is synchronous with the abundance of the beetles, sometimes showing a small lag in response. This may suggest the influence that the size of Cassidinae populations may have on the regulation of invertebrate predators. Exclusion experiments suggested that flying invertebrate predators were the main factor responsible for mortality of S. chalybea larvae. Mortality, however, were not significant between different areas, host plant individuals, between rainy and less rainy experiments and between experimental treatments.

Introduction

The importance of natural enemies in controlling herbivore populations was first mentioned by Hairston et al. (1960). According to these authors, populations of herbivores are not limited by their food supply, and the alternative hypothesis of control by weather would lead to false implications to populations of herbivores. Recent work still recognizes the importance of predators in the suppression of many phytophagous insects. Parasitoids have also been considered as an important cause of death for these insects, especially those that are endophytic (Hawkins et al., 1997). Cox (1996) mentioned that predators and parasitoids are not mutually exclusive, although some authors have observed that sometimes the importance of predation and parasitism are inverse (see Keese, 1997; Monteiro, 1981 for examples). Many programs for biological control of phytophagous insects, including chrysomelids, have been successful due to the impact of predators and parasitoids on their populations (Cox, 1996).

Selective pressure imposed on Cassidinae by natural enemies has likely resulted in the diversity of defensive attributes like physical, behavioral and possibly chemical adaptations. Despite these attributes, natural enemies could heavily impact tortoise beetle populations in the field (Olmstead, 1996). Life tables and population studies of various Cassidinae species indicate that most enemy-induced mortality takes place in the egg or larval stage. Roughly 65% of the reported enemy-cassidine interactions involve larvae and pupae, whereas there have been few records for adults (Olmstead, 1996). This might be because adults appear to be well defended against attacks by enemies.

Natural enemies of Chrysomelidae are represented by organisms of many different taxa. They vary from intracellular parasites to larger predators like birds. The former group is represented by viruses, that may cause pathological damage or significant mortality (Selman, 1988); microsporidia (Protozoa), that can also be extremely pathogenic to leaf beetles (Toguebaye et al., 1988) and gregarines, another Protozoan group (Theodoridès, 1988). There are also some non-intracellular parasites, like Laboulbeliales (Fungi: Ascoycetes) (Balazuc, 1988) and nematodes (Poinar-Jr., 1988). There are representatives of three groups of Nematoda that naturally parasitize Chrysomelidae (Poinar-Jr., 1988). Most case reports are for the Mermithidae (order Mermithida) group, and its occurrence on Chrysomelidae have involved immature nematodes (Poinar-Jr., 1988). Because of this, very few parasites were described or identified (Poinar-Jr., 1988). Immature mermithid leaves the body of its host before adult transformation; and because most researchers do not have this information, they collect immature nematodes, which are not identifiable (L.C. Ferraz, personal communication).

Cassidinae is the most frequently parasitized subfamily among Chrysomelidae (Olmstead, 1996). Cox (1994) attributed this to the behavior of larvae that are fairly sedentary and, therefore, predictable on their host plants. Enemies of Cassidinae eggs appear mainly to be ants and hymenopterous parasitoids, the latter being more frequent (Olmstead, 1996). Of the parasitic Hymenoptera observed attacking Cassidinae eggs, the Eulophidae and Tetracampidae are the most diverse and have been noted as heavily impacting populations. Dipteran and hymenopteran parasitoids together are the most frequent enemies of larvae and pupae of Cassidinae (Olmstead, 1996). Nevertheless, their frequency is very much reduced among shield protected larvae (Olmstead and Denno, 1993). There are only occasional reports of parasitism by adult Cassidinae. This is more frequently caused by dipterans, nematodes and fungi (Olmstead, 1996).

Most of the records for insect predators of Chrysomelidae are for the Holartic, with few in the Neotropics, Afrotropics, Oriental and Australasian regions (Cox, 1996). Records that concern Cassidinae show that immature and adult stages are preyed upon by a range of insects. Asopines (Heteroptera) is among the most frequent predators, as it preys on larvae, pupae and adults (Olmstead, 1996). About 72% of published accounts of predation on Cassidinae are by insects with piercing/sucking mouth parts. Mandibulate insects are considered ineffective predators of larvae but those that were observed attacking cassidines include vespid wasps that circumvent the dorsal shields of tortoise beetles (Olmstead and Denno, 1993; Olmstead, 1996).

Besides richness of potential predators of Cassidinae, their abundance is also an important parameter that should be taken into account. There is much evidence that predation and parasitism are density dependent interactions and populations of natural enemies are well synchronized to their preys. Charlet (1992) has observed larva parasitoid of Zygogramma exclamationis (Chrysomelidae) showed good synchrony with its host and showed a functional response to larval populations, maintaining parasitism rates constant. Cappaert et al. (1991) detected in a population of Leptinotarsa decemlineata significant correlation between total eggs and total predators or group of predators, suggesting synchrony between predators and eggs. They have, indeed, observed that peak predator abundance follows peak oviposition, sometimes with a small lag. A significant relationship was also detected between egg density and the rate of egg damage. According to the authors, these evidences indicate the density dependence of L. decemlineata predation.

We can expect Chrysomelidae populations to disperse their spatial or feeding range searching for enemy free space, because of the intense influence of natural enemies on them. Therefore, occupation of different habitat, occurrence in different seasons and utilization of different behavior would be observed as traits that reduce their vulnerability (Paleari, 1997).

In this work, we investigated the natural enemies (parasitoids, parasites and predators) of the Cassidinae species, Stolas chalybea, S. areolata and Anacassis phaeopoda in an Atlantic forest area in São Paulo state, with the purposes of identifying the parasites and predators of these cassidines at the studied area and checking if the populations of natural enemies were synchronized with the population of their hosts. We also quantified mortality of larvae of S. chalybea by the exclusion of different groups of predators, like ants, spides, flying invertebrates or vertebrates, in the field.

Material and Methods

Study area

This work was conducted at Serra do Japi (23° 11'S/46° 52' W), a mountain ridge located at the southern limit of the tropical zone in São Paulo state, Brazil.

The climate in the area corresponds to the subtropical moist type, being classified as Cwa, according to the Koppen system (Medeiros e Vasconcellos-Neto, 1994). Two distinct seasons can be observed: warm and rainy summer and cold and dry winter. Because of the large range of altitude (700 m to 1300 m above sea level), mean temperature vary from 11.8°C –18.4°C in July (the coldest month) to 18.4°C – 22.2°C in January (the warmest month) (Pinto, 1992).

Semideciduous mesophytic forests of altitude are predominant at Serra do Japi. The forest is seasonal with the period of leaf fall from April to September, approximately (Leitão-Filho, 1992). At study sites, vegetation was basically composed of short trees of low stem diameter.

We concentrated our study along two trails: Mirante and Paraíso III. Both trails can be characterized by the shade provided by the trees located along their edge. Paraíso III also presents an open swampy area where a large Baccharis trimera (Asteraceae) patch was observed.

Natural enemies

Mortality during each developmental stage was estimated by the mean number of individuals per egg cluster or larvae groups. To obtain these data, we censused every fifteen days 30 individuals of host plants of each Cassidinae species in each studied trail during the 1997-1998 cycle and recorded the number of individuals in the groups of eggs or larvae we found.

We collected egg clusters, larvae and adults of Stolas chalybea, S. areolata and Anacassis phaeopoda along different trails (but "Mirante" and "Paraíso III" trails) at Serra do Japi from October, 1999 until February, 1999. They were maintained in laboratory conditions (room temperature and photoperiod) to obtain their parasitoids. The beetles received leaves of their host plant every other day. They were also inspected every other day for parasitoid emergence. Eggs were maintained until their hatching, larvae were maintained until pupation and adults were reared for a month.

For each egg cluster of S. chalybea observed at the field, we recorded the number of eggs infected by fungi, parasitized, preyed upon or hatched (these three last conditions were evaluated by marks on the eggs).

Abundance of Natural Enemies on host plants

To evaluate the influence of natural enemies of S. chalybea, S. areolata and A. phaeopoda, we censused every fifteen days, 30 individuals of host plants of each Cassidinae species during the 1997-1998 cycle for their potential predators. Predator species found in less than 10% of the plant censuses were not considered. Plant individuals were randomly chosen. The same census procedure was carried out at two different trails for host plants of S. chalybea and S. areolata (therefore 60 plants of each species were checked).

Spearman correlation index was used to investigate the relation between the abundance of S. chalybea, S. areolata and A. phaeopoda and their potential predators. We tested Cassidinae abundance (using data collected by Sá and Vasconcellos-Neto, in preparation – see chapter 2) and the number of plants with predators and Cassidinae abundance and the number of predator morpho-species observed in each census. We also correlated abundance of S. chalybea, S. areolata and A. phaeopoda with their potential predators in 30 previous days(Lag1), 45 (Lag2), 60 (Lag3) and 75 (Lag4) days previous to a given check.

Exclusion experiment

In order to evaluate the role of different groups of predators on cassidine larvae, we chose Stolas chalybea and its host plant, Bidens segetum, as a model to study the impact of different groups of predators on Cassidinae due to their abundance in the study sites. We conducted a field experiment with exclusion of predators using a crossover Latin Square design (see table 3.1).

We introduced four 2nd or 3rd instar larvae on each of the 16 B. segetum experimental individuals. This number of larvae was chosen by the mean size of egg clusters observed in the field during the 1997 cycle and small larvae were used because larval mortality was higher in these instars (see figure 3.1). Experimental plants were distributed in four different squares, where four individuals of B. segetum were submitted to four exclusion treatments during each of the four different periods. The periods were of four days each and were undertaken on consecutive days. Plant size was standardized; therefore all the 16 experimental plants we chose were of similar size. In each square the four experimental plants were submitted to each treatment only once, thus every plant was submitted to the four treatments after the four periods. The four squares were located at four different trails: one at Mirante trail, two near Mirante trail and one at Paraíso III trail, in Serra do Japi. The four treatments were: plant covered by a coarse mesh cage (3.2 cm. of diameter mesh), plant covered by fine-mesh fabric, plant with resin barrier (we have applied Tangle foot®, Michigan, USA, on the main stem of the plant, just under the first ramification and cut neighboring plants which were in direct contact with the experimental plant) and control. Resin was applied on a masking tape attached on the plant stem, so that could be completely removed before the application of the following experimental treatment. We randomly chose the sequence of treatments for each plant at the four squares. Before applying the experimental treatments at each host plant, they were checked and every observed organism was taken off.

The number of preyed larvae was recorded at the end of each period, and at the beginning of the next treatment the group of four small larvae was recomposed.

The experiment was conducted twice: from December 7th to 23rd, during a rainy period, and from March 5th to 21st, during a period of less rain. Mortality rates of larvae during each experiment were compared by Mann-Whitney test.

Kruskal-Wallis tests evaluated differences between each of the experimental traits (plant individual, areas, period and exclusion treatment). For this analysis we have used the mortality rate for each trait. The Shapiro Wilk test and Bartlett test were conducted to test normality of data and homogeneity of variances, respectively, prior to using Kruskal-Wallis.

Results

Natural enemies

By the mean number of individuals in each life stage we estimated that the greatest mortality of S. chalybea, S. areolata and A. phaeopoda was from the egg phase to 1st and 2nd instar larvae. The mean number of individuals in groups of larvae at that stage decreased (see figure 3.1) being 66.98%, 87.5% and 65.65% lower than in egg clusters of S. chalybea, S. areolata and A. phaeopoda respectively. Decrease in the mean size of larval groups was also observed from 1st-2nd instar larvae to 3rd-4th instar larvae (42.46%,and 11.5% for S. chalybea and A. phaeopoda respectively) and from 3rd-4th to 5th instar larvae, it was of 20.20% and 39.91% for S. chalybea and S. areolata respectively (see figure 3.1).

Infection by fungi, parasitism and predation were the main causes of death of eggs of S. chalybea in the field and only 39.42% of them hatched (Total n= 319) (Fig. 3.2). These three mortality factors could be observed acting on the same egg cluster.

We obtained six different egg parasitoid species (all Microhymenoptera) and two other species were observed only as phoretic on S. chalybea elytrum (Table 3.3). Phoretic parasitoids were not very commonly observed. Only two species were obtained from the eggs of S. areolata (Table 3.3), and we observed that not all eggs in the same cluster were parasitized.

We obtained the same species of Tachinidae (Diptera) as a larvae parasitoid from S. chalybea and A. phaeopoda and a different one, also Tachinidae, from S. areolata. We observed that all larvae of the same group were infected when this parasitoid of S. areolata was obtained.

The nematode Hexamermis sp. (Mermithidae) was the only parasite obtained from adults. Its parasitism rate in S. chalybea was 3% (n=100 adults).

The pentatomids Stiretrus decemguttatus and Oplomus catena (Hemiptera: Asopinae), and some other Asopinae nimphs preyed upon S. chalybea larvae. Spiders preyed upon those larvae and adult Cassidinae too. An adult S. chalybea was observed attached to the web of Nephila clavipes (Tetragnathidae) (R. Xavier, personal communication). In laboratory conditions, mortality rates of larvae and adults of S. chalybea were 5.2% (n= 77) and 10% (n= 50) respectively; whereas natural mortality of larvae and adults of S. areolata reared in the laboratory were 16.67% (n= 12) and 10% (n= 30) respectively.

Abundance of potential predators on Cassidinae host plants

Ants (Formicidae) attending Aleyrodidae homopterans or foraging on plants, spiders and heteropterans (especially Pentatomidae) were the most frequent predators found on Cassidinae host plants, Bidens segetum, Mikania cordifolia and Baccharis trimera.

Census of potential predators on host of S. chalybea, S. areolata and A. phaeopoda over the course of a year showed that these organisms were not found all year round. The occurrence pattern of predators seemed to be very similar on the three host plant species (Figs. 3.3, 3.4 and 3.5). Abundance and occurrence of potential predators of S. chalybea and S. areolata on B. segetum and on M. cordifolia were positively correlated with the abundance of their preys (Table 3.4). No significant correlation was detected for the potential predators of A. phaeopoda on its host plant B. trimera.

Exclusion experiment

It rained on eight out of the seventeen days of the first experiment, totaling 224 millimeters of rainfall. In the second experiment, it rained on four out of the seventeen days of experiment, totaling 50.5 millimeters rainfall. Despite this difference in rainfall between the first and second experiments, Mann-Whitney-test comparing mortality rates in both experiments did not detect any significant difference among them (Table 3.5). Nevertheless, mortality rates of larvae submitted to each treatment on the second experiment tended to be lower (Fig. 3.6).

Kruskal-Wallis-tests revealed that there was no significant difference between mortality in the different areas, nor between different plant individuals. These results confirm the validity of these two experiments by the usage of homogenous areas and plants. Significantly different larvae mortality was detected between different periods; however, mortality did not differ between the different exclusion treatments (Table 3.5). S. chalybea larvae mortality was lower on plants protected by fine-mesh fabric in the two experiment periods (Fig. 3.5). However, this difference was not significant. Mortality on plants submitted any treatment did not differ statistically from mortality on control plants.

Discussion

Because host plants of Cassidinae are so abundant in the field, we believe that natural enemies are important factors regulating their populations, as its is believed for all phytophagous insects (see Hairston et al., 1960 and Cornell et al., 1998 for instance). Parasitism rates of the three species studied were high and quite similar in average. However, the calculation of rates of parasitism and influence of predators should be reinforced still, because due to the low abundance of S. areolata and A. phaeopoda in the field (see Sá and Vasconcellos Neto, in preparation- see chapter 2), few parasitoids species and predators were found.

Rate of parasitism for eggs of S. areolata observed at this study were lower than registered in the literature. We observed that 28.57% of the eggs were parasitised, whereas Carroll (1978) and Paleari (1997) found parasitism rates of eggs of other Stolaini species varying from 37.93 to 88% collecting fewer eggs. The low abundance of eggs of S. chalybea, S. areolata and A. phaeopoda in the field may explain low parasitism. Although both of the studies were conducted in the Amazon region, which is well known for its diversity, we observed a higher diversity of egg parasitoids parasiting Cassidinae.

Species of Emersonella have been frequently collected as egg parasitoids by many authors (e.g. Carroll, 1978; Nakamura and Abbas, 1987; 1988; Frieiro-Costa, 1995 and Paleari, 1997) causing high mortality of eggs in the field and reducing Cassidinae populations. In most of the records of Emersonella occurrence, hosts were Cassidinae species of the tribe Stolaini, the same of Stolas and Anacassis. According to Paleari (1997), similarity of body shape and size of the host and reproductive potential are characteristics that would explain the susceptibility of more than one Cassidinae species to the parasitism by Emersonella spp. The same traits would also be important for predators recognition. Parasitism of eggs of different species by the same parasitoid species is quite common (M. Tavares, personal communication).

Mortality during egg stage was the highest, probably due to the influence of the many taxonomically different groups of natural enemies. Parasitism, fungi infection and predation had similar impact on eggs of S. chalybea. According to the literature, the main Cassidinae egg predators are hymenopterans, hemipterans and arachnids. These three groups were very common on the studied host plants. Ant predation might have been the strongest factor for mortality because we observed many host plants with ants (especially Myrmicinae and Ponerinae) attending nymphs of Aleyrodidae homopterans. Although it was not tested, many works have already confirmed the protection of plants by these ants (see Buckley, 1987 for revision). Besides bug-attending ants, Solenopsis may be another ant group that can be considered a potential predator of eggs, as recorded by Paleari (1997), and occurs at Serra do Japi.

Stiretrus decemguttatus (Heteroptera: Asopine) preyed on larvae of S. chalybea on B. segetum. According to Paleari (1997) however, available information on this species in the literature suggests that it mostly preys on eggs and pupae, and is a predator restricted to Chrysomelidae.

S.chalybea and S. areolata layed their eggs attached to the underside of their host plant leaves very often (Sá and Vasconcellos-Neto, in preparation – see chapter one). Paleari (1997) also mentioned the importance of oviposition on less exposed places to avoid parasitism. Because leaves of B. trimera were very rare, eggs of A. phaeopodoa were always attached to the stem surface of their host plant, and consequently were more exposed to natural enemies. The filaments by which eggs of A. phaeopoda are attached to their host plants (see figure 1 at general introduction of this thesis) may play a role in their protection. A protective apparatus would be more important for A. phaeopoda since their location on host plants probably was not effective in reducing parasitism. Olmstead (1996), mentioned that the function of egg filaments is not known; however, it is believed that they may serve to reduce encounters with some predators and parasitoids. Nevertheless, it seemed that ovipositing on the under surface of leaves was not so effective for S. chalybea because it showed a higher parasitism rate in comparison to the other two species. The lower parasitism rate of eggs of S. areolata may be explained by their ovipositing on neighboring plants of their hosts (Sá and Vasconcellos-Neto, in preparation – see chapter one). This may reduce the likelihood that they will be found by parasitoids that use plant chemical cues to find their herbivorous hosts (Vinson, 1976; Rowell-Rahier and Pasteels, 1992; Köpf et al., 1997 and Meiners and Hilker, 1997). However, predation on S. areolata eggs of this species was not quantified.

Egg clustering was another possible strategy to avoid high rates of predation and parasitism employed by the three species of tortoise beetles. This strategy has been considered as defensive for eggs because it is possible that some eggs in the cluster would be shielded by others and, in the case that the reproductive capacity of enemies is limited, it would be observed that not all of the eggs in the same cluster would be attacked by the ovipositing females (Olmstead, 1996), as was observed for both Stolas species in this study.

According to the data in the literature, larval parasitism rates is very variable. Charlet (1992) has observed a Tachinidae, Doryphorophaga macella, parisitizing larvae of Zygogramma exclamationis at parasitism rates varying from 0 to 100% during different study years. Therefore, one year data, as we presented at this study, may not be considered a pattern for the parasitism for longer periods because they can change over a longer period of time.

The parasitism rate observed for larvae of S. areolata could have been over-estimated because too few larvae were collected and one of the aggregations collected was entirely parasitized. As 100% parasitism of larvae aggregations seem to be a frequent pattern for this species, we propose that a clearer estimate of larvae parasitism would be the number of parasitized aggregations, and not the number of larvae.

Low specificity of Tachinidae parasitoids can be mainly explained by their low numbers in the field. Keese (1997) observed that Ophraella slobodkini (Chrysomelidae) larvae were not common enough to support a population of its parasitoid. Monteiro (1981) also suggested that low availability of pupae of Mclungia salonina (Lepidoptera, Nymphalidae) in the field reduced the chances of finding a specialized parasitoid for this developmental stage. Another factor that could be responsible for the increase of host diversity is high parasitism rates. It was observed by Monteiro (1981), when parasitism rates are at their maximum, parasitoids end up using different and more abundant host species. However, this is not the case for the Cassidinae species studied.

Many authors consider predation the most important mortality factor for phytophagous insects (Bernays, 1997; Gomes-Filho, 1997 and Hawkins et al., 1997). There are some examples in which low parasitism is compensated by predation(see Monteiro, 1981and references therein). Invertebrate predators are often considered generalized feeders, and the presence of these generalist species in the field increases the chances of predation. Ants and pentatomid heteropterans were the most common invertebrate predators of Cassidinae; and because they were frequently found in the field, it would be expected that they caused high mortality. The impact of ants, that have been reported to attack larvae and pupae, on tropical populations of Cassidinae is substantial (Olmstead, 1996). However, Carroll (1978) has observed that only young larvae fall victim to foraging ants. Older larvae were preyed upon by common species of pentatomid bugs. The same situation was also observed by Paleari (1997) as most of the records of predation of Botanochara sedemcimpustulata, Zathrephina lineata and Chelymorpha aff. alternans (all Stolaini) by the pentatomid Stiretrus decemguttatus, was about larvae on fourth and fifth instars. This author suggested that the predator might easily obtain a larger amount of food by feeding on mature larvae. Besides, eggs and adults had strong external protection, whereas the fecal shield of larvae of those species did not cover their entire body. The fecal shield is recognized as an effective larval protection against parasites and predators by many authors (Olmstead and Denno, 1992; 1993; Olmstead, 1996 and Quintero, 1997), but it may fail when it leaves parts of the body exposed or when larvae are attacked by predator groups, like ants. As for the cassidine species studied by Paleari, fecal shield of both Stolas species and Anacassis investigated by us, did not cover the whole dorsal surface of larvae, which could have provided their vulnerability to natural enemies. Because of intense pressure and richness of predators, one kind of leaf beetle defense would not provide complete protection.

Potential predators of S. chalybea, S. areolata and A. phaeopoda had a very similar pattern of occurrence over a year compared to their cassidine preys. They were more abundant on host plants in the summer months, the same period of higher abundance of Cassidinae (Sá and Vasconcellos-Neto, in preparation – see chapter 2). Synchrony between preys and predators or parasitoids has been detected by other authors too (Charlet, 1992 and Cappaert et al., 1991). We believe that the same synchrony occurs with the populations of both Stolas species and Anacassis studied and their parasitoids, because not only Cassidinae but most phytophagous insects had lower populations during winter months in Serra do Japi (F.N. Sá and J. Vasconcellos Neto, personal observation). A low, but significant positive correlation between S. chalybea and S. areolata and the occurrence and richness of predators on their host plants suggest the influence of the abundance of potential predators on these organisms. Significant results with lag, may indicate that a population of Cassidinae not only was related to the abundance of their prey at the present time, but also may decrease their population as a response to higher abundance of predators in the past.

As recorded in the literature, few observations were made on natural enemies of adult Cassidinae. Potential predators, like spiders, passeriformes and heteropterans (including S. decemguttaus) were observed on their host plants and at the study area. Nevertheless, adult Cassidinae definitely did not seem to be the preferred preys for those predators, or they must be well protected against them. Thanatosis, dropping off their host plants and reflex bleeding were the possible defensive strategies observed in the field for the three studied species. The latter is quite commonly observed in the Stolaini beetles, as it was recorded in Botanochara impressa (J. Vasconcellos-Neto, personal communication), S. chalybea and S. areolata. Predation experiments on Chelymorpha cribraria adults (Cassidinae: Stolaini) showed that they were unpalatable to birds and spiders (Vasconcellos-Neto, 1988).

Despite non-significant difference among exclusion treatments, it is possible to suggest the importance of flying predators as mortality factors for S. chalybea larvae. For plants where heteropterans, wasps and spiders were excluded (by fine-mesh fabric barrier), mortality rate was the lowest compared to other treatments (see figure 3.6). We believe that the 20% rate of mortality of plants submitted the fine meshed tissue treatment may have been due to ants, although we expected to detect a greater importance of them since only small larvae were used in the experiment. The importance of invertebrate predators of Chrysomelidae may be confirmed by the data in the literature (see Cox, 1996 and Olmstead, 1996). Besides, the higher importance of invertabrate predators to Cassidinae larvae was confirmed in these experiments by the high mortality of larvae submitted to coarse mesh treatment.. As mentioned by Marquis and Whelan (1994), in this treatment it would be expected a greater spider population inside cages because that would provide an additional substrate for web construction and because of differential bird predation, which would also increase the populations of other invertebrate predators. Marquis and Whelan (1994) also detected the importance of avian predation upon leaf chewing insects on

Quercus alba also using predator-exclusion methods. They observed a higher number of insects inside cages, lower biomass production and reduction of leaf area on those plants. Quintero (1997) also suggested that predation by vertebrates upon Cassidinae may be higher than generally assumed and perhaps, of great impact. She also supposed that the disappearance of larvae of Eurypedus during her study was due to a lizard or an avian predation. However, we did not expect to detect higher importance of mortality caused by this group of animals because they are probably more efficient at preying on larger larvae. Gomes-Filho (1997), also investigating the role of different predator groups and using a very similar exclusion method, has found significant differences between mortality rates of Lepidoptera larvae of the control and of all the applied treatments. However, larvae of different sizes were used, which could have attracted a greater diversity of predators.

Our previous investigations on S. chalybea, S. areolata and A. phaeopoda, at the same site have detected some factors, like environmental temperature and host plant utilization, that weakly explained the low numbers of these populations in the field (Sá and Vasconcellos Neto, in preparation – see chapters one and two). In this case, natural enemies seem to play a more effective role in controlling populations of Cassidinae. Although more direct observations on the influence of predators and more intense collections of S. areolata and A. phaeopoda are still needed, this work can support the hypothesis of the control of herbivore populations by their natural enemies.

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Tables and Figures

Table 3.1 Example of Crossover Latin square design using Bidens segetum to test predation effect on Stolas chalybea larvae at Serra do Japi, SP.

Plant / Period	Bidens segetum			
	1	2	3	4
1	Control	Resin	Fine mesh	Coarse mesh
2	Resin	Fine mesh	Coarse mesh	Control
3	Fine mesh	Coarse mesh	Control	Resin
4	Coarse mesh	Control	Resin	Fine mesh

Table 3.2 List of groups of excluded potential predators on Bidens braziliensis during exclusion experiments.

Treatment	Excluded predators	Potential predators
Control	None	Ants, Birds, wasps, hemiptera and spiders
Resin	Ants and spiders	Birds, wasps, hemiptera and spiders
Fine mesh	Birds, wasps, hemiptera and spiders.	Ants
Cage	Birds	Ants, wasps, hemiptera and spiders

Table 3.3 Egg and larval parasitoids of Stolas chalybea, S. areolata and Anacassis phaeopoda found at Serra do Japi, SP and their rate of parasitism.

	S.chalybea		S. areolata		A. phaeopoda	
Parasitoid species	Egg	Larva	Egg	Larva	Egg	Larva
EULOPHIDAE (HYMENOPTERA)						
Emersonella sp.1	X		X			
Emersonella sp.2	X					
Emersonella sp.3*	X					
Emersonella sp.4	X					
Tetrastichus sp.	X		X			
Paracryas sp. 1	X					
Paracryas sp. 2*	X					
ENCYRTIDAE (HYMENOPTERA)						
Ooencyrtus sp.	X					
TACHINIDAE (DIPTERA).						
Eucelatoriopsis parkeri		X				X
Tachinidae sp.				X		
% of parasitism						
Egg parasitoids**	51.93 (n=181)		28.57 (n=84)		Not investigated	
Larva parasitoids**	19.39 (n=98)		46.15 (n=26)		20 (n=5)	
Adult parasitoids**	0 (n=100)		0 (n=30)		0 (n=7)	

* Phoretic species.

** Sample size (n) corresponds to the number of hosts examined.

Table 3.4 Significant Spearman correlation indexes for the relation between the abundance of Stolas chalybea and Stolas areolata and number of host plants with potential predators or the number of predators types throughout 1997-1998 cycle at Serra do Japi, Jundiaí, SP.

Species	Trail	Development phase**	Number of Plants with Predators X Cassidinae	Number of species of Predators X Cassidinae
S. chalybea	Mirante	Eggs	ns (n= 20)	0.459* (n=20)
	Paraíso III	Larvae	0.473* (n=20)	ns (n= 20)
	Mirante	Lag 2 larvae	ns (n= 18)	0.507* (n=18)
S. areolata	Mirante	Lag 2 eggs	0.651* (n=18)	0.547* (n=18)
	Mirante	Lag 4 eggs	0.611* (n=16)	0.613* (n=18)

* significant at $p < 0,05$.

ns.- non significant

** For every development phase, we also tested the correlation between Cassidinae with 1, 2 , 3 and 4 Lag periods and Number of plants with predators and between Cassidinae and number of species of predators. Results not mentioned in the table above were not significant on both correlations.

Table 3.5 Mann- Whitney and Kruskal-Wallis results for two experiments on mortality of experimental Stolas chalybea larvae submitted to four predator exclusion treatments during four days and four different periods on Bidens segetum undertaken at Serra do Japi, SP.

Variable	**U/H**	**p**	**DF**
Experiment	1930	0.46	1
Plant	35.94	0.25	31
Area	4.20	0.24	3
Period	23.36	0.001*	7
Treatment p 1	2.93	0.40	3
Treatment p 2	0.27	0.96	3
Treatment p 3	3.19	0.36	3
Treatment p 4	1.68	0.64	3
Treatment p 5	1.43	0.70	3
Treatment p 6	4.93	0.18	3
Treatment p 7	3.16	0.37	3
Treatment p 8	0.48	0.92	3

* Significant difference at the 5% level.

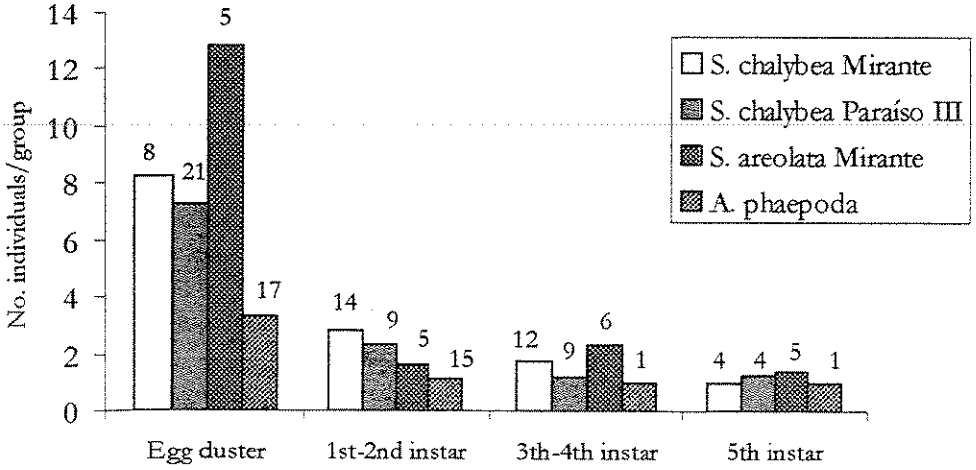


Figure 3.1 Mean number of individuals per group of each developmental stage during 1997-1998 cycle at studied trails in Serra do Japi, SP. Numbers over each bar corresponds to the number of groups observed in the studied period.

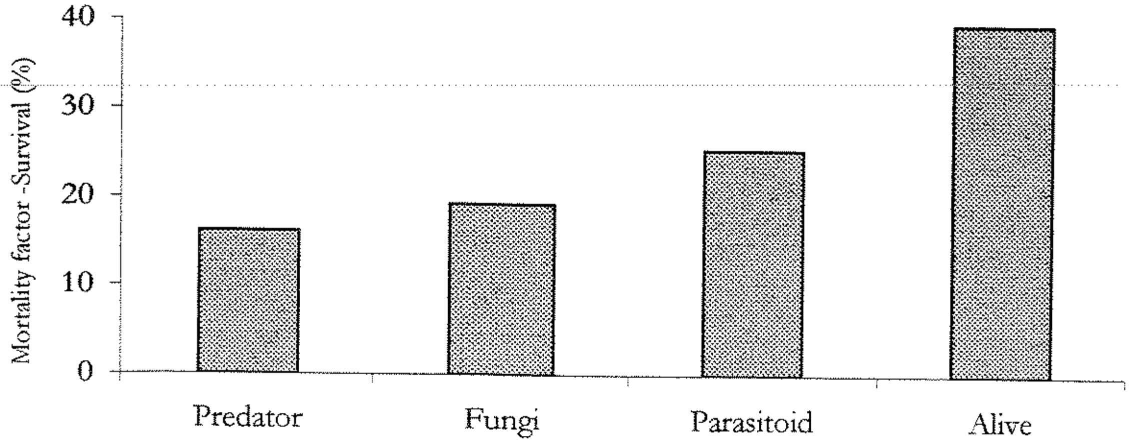


Figure 3.2 Percentage of eggs of S. chalybea atacked by natural enemies and alive in the field - Serra do Japi, SP, in 1998 (n= 319).

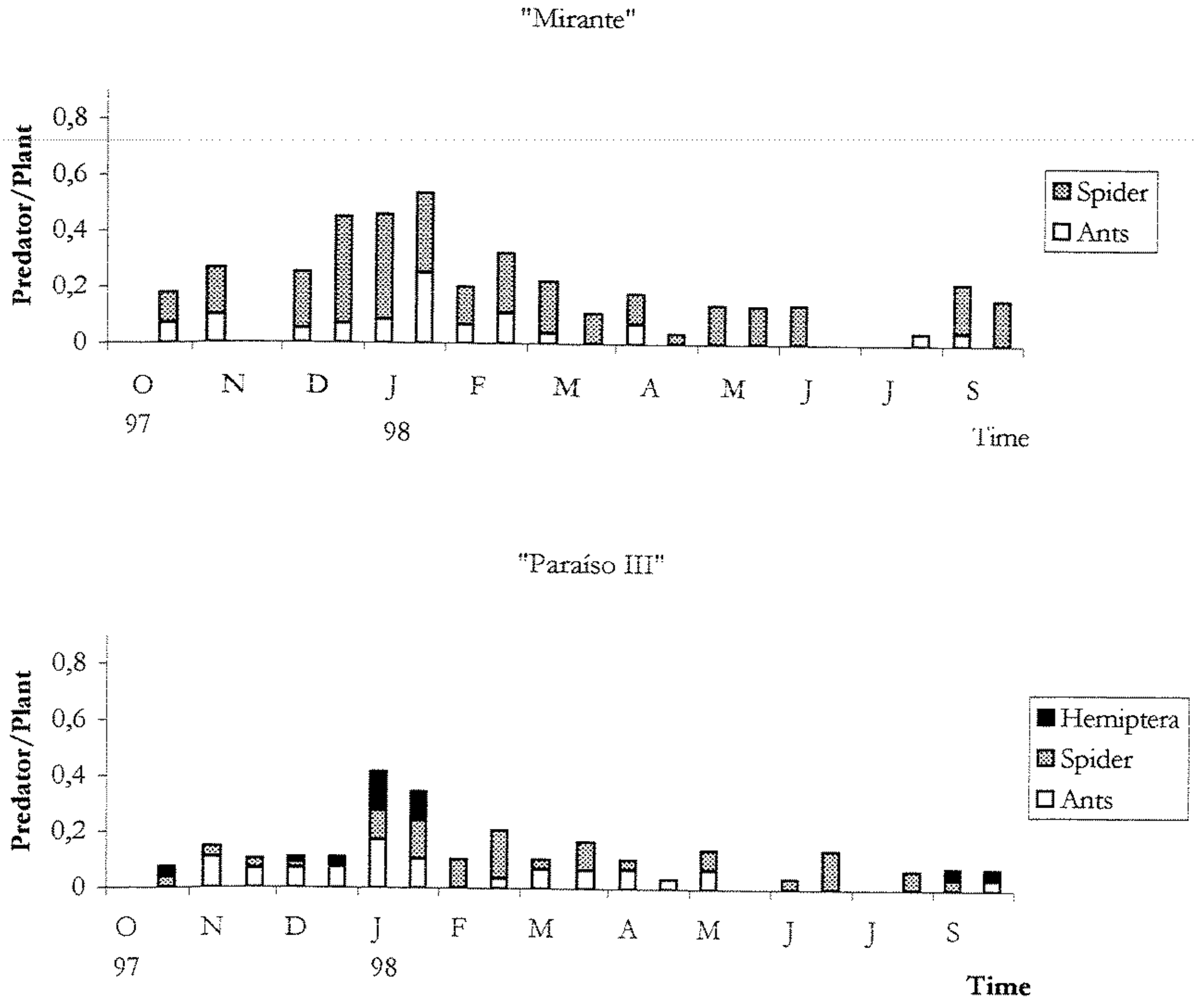
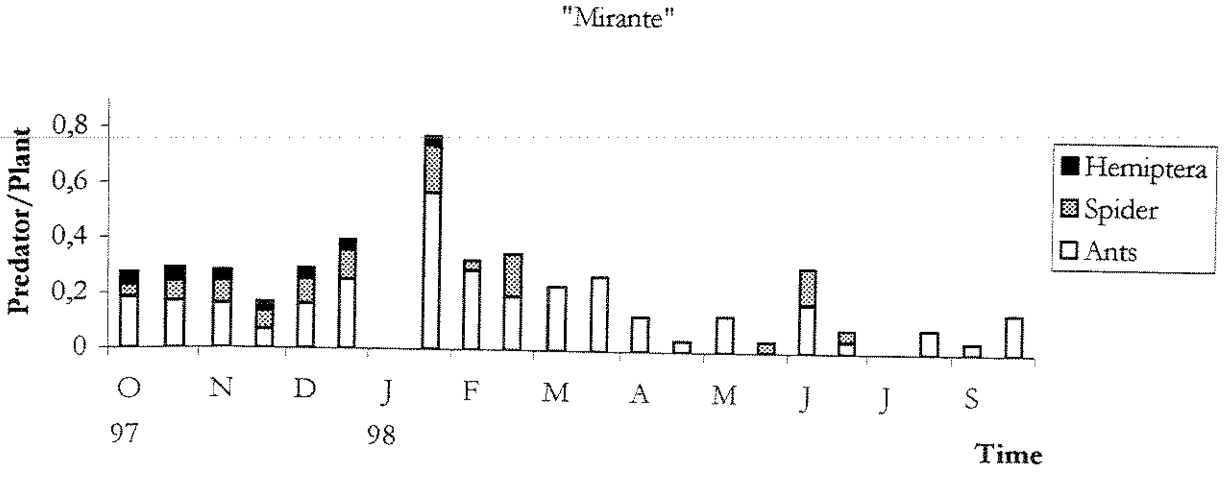


Figure 3.3 Frequency of S. chalybea potential predators found on Bidens segetum at "Mirante" and "Paraíso III" trails in 1997-1998 life cycle, Serra do Japi, SP.



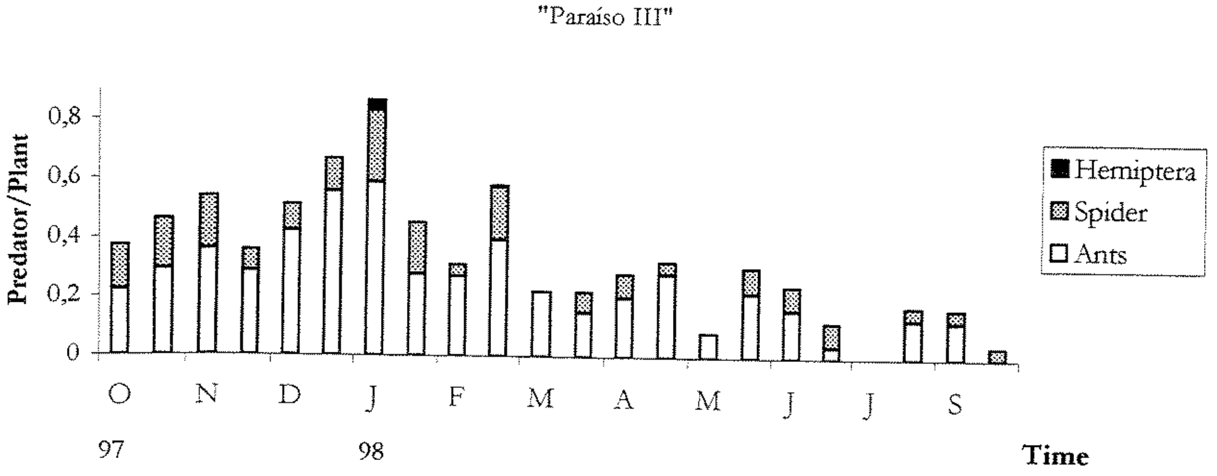


Figure 3.4 Frequency of S. areolata potential predators found on Mikania cordifolia at "Mirante" and "Paraíso III" trails in 1997-1998 life cycle, at Serra do Japi, SP.

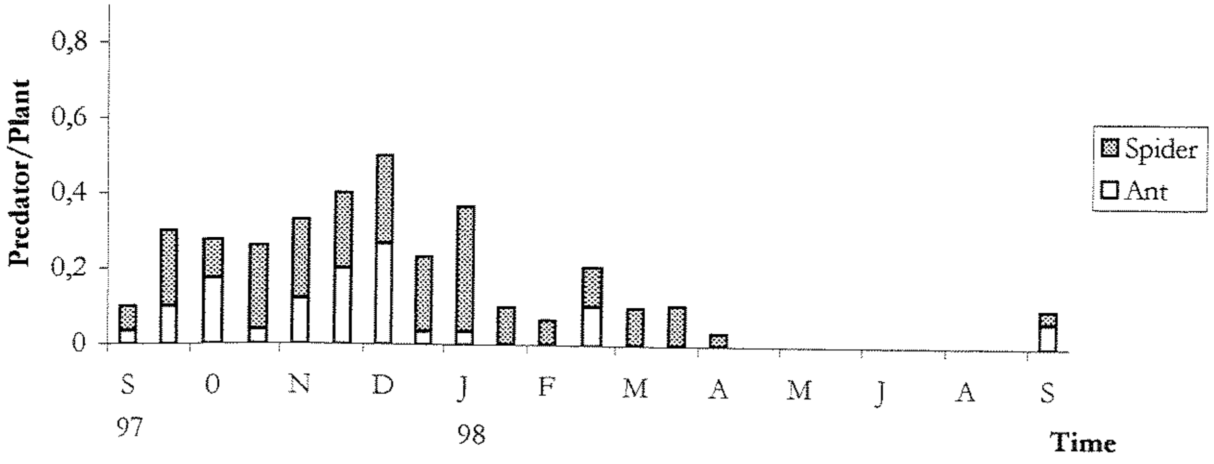


Figure 3.5 Frequency of A. phaeopoda potential predators found on Baccharis trimera at "Paraíso III" trail in 1997-1998 life cycle, at Serra do Japi, SP.

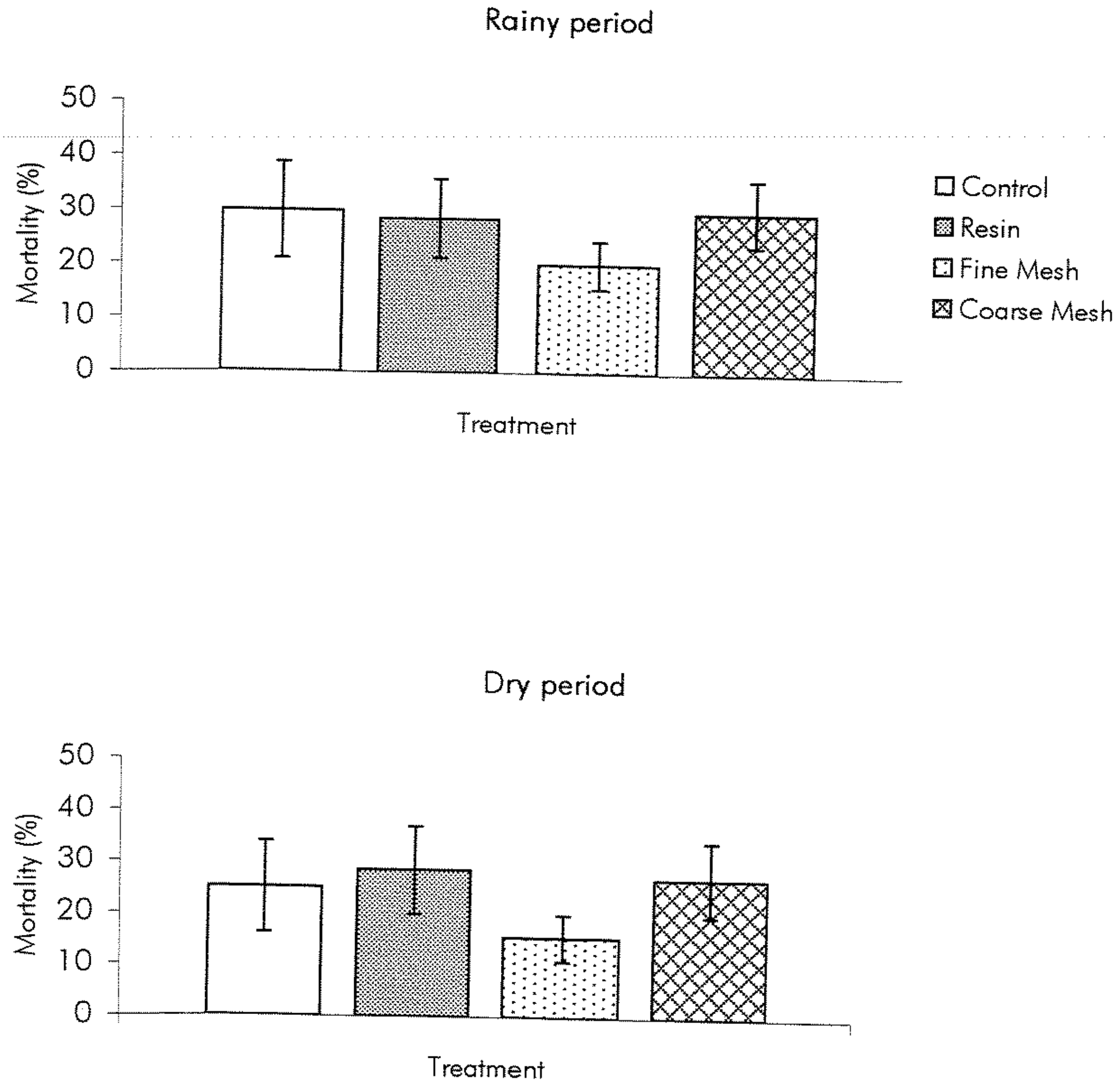


Figure 3.6 Mean and standard error of general mortality rates of 2nd or 3rd instar larvae of Stolas chalybea on Bidens segetum submitted to four different experimental treatments to test for predator influence during a rainy (in 1998) and a dry period (in 1999), at Serra do Japi, SP.

Síntese Geral

Entre as 15 espécies de plantas das tribos Heliantheae, Astereae e Eupatorieae registradas em ambas as trilhas estudadas, os cassidíneos Stolas chalybea, Stolas areolata e Anacassis phaeopoda só consumiram uma espécie de planta hospedeira, cada um, Bidens segetum, Mikania cordifolia e Baccharis trimera, respectivamente. Isso está de acordo com os dados de Jolivet (1988) e Buzzi (1994) classificando a maioria das espécies de Cassidinae já conhecidas como monófaga ou bastante especializada. As folhas foram as partes da planta hospedeira consumidas por S. chalybea e S. areolata; enquanto que A. phaeopoda consumiu as alas dos caules de sua planta hospedeira (folhas são raramente observadas em B. trimera).

A presença de inimigos naturais nas espécies de plantas taxonomicamente próximas (muitas vezes do mesmo gênero) das hospedeiras de cada Cassidinae, pode ser uma explicação para que estas não tenham sido utilizadas pelos besouros.

As desovas das espécies de Stolas foram significativamente maiores do que a de A. phaeopoda. O padrão de oviposição de desovas na superfície abaxial de folhas se repetiu para as duas espécies de Stolas, seguindo também um padrão já encontrado para outras espécies de Chrysomelidae (e.g.: Flowers, 1991,; Charlet, 1992; Frieiro-Costa, 1995). Desovas de S. areolata também foram frequentemente encontradas em plantas vizinhas às suas hospedeiras. Esta estratégia pode ser eficiente para reduzir o encontro com inimigos naturais visualmente ou quimicamente orientados.

Larvas se mantiveram agregadas durante o início de seu desenvolvimento e também foram mais frequentemente encontradas na superfície abaxial das folhas da planta hospedeira. Porém o número médio de ovos na desova e de larvas por agrupamentos reduziu ao longo de seu desenvolvimento. A diferença na redução do número médio de indivíduos em cada estágio de desenvolvimento investigado pode estar refletindo as taxas de mortalidade distintas de ovos e larvas de S. chalybea, S. areolata and A. phaeopoda. A ocorrência das larvas na superfície abaxial das folhas também foi reduzida com seu desenvolvimento e adultos de ambas as espécies de Stolas foram mais frequentemente encontrados na superfície adaxial das folhas. Este padrão de localização de ovos e larvas em suas plantas hospedeiras também pode estar relacionado com defesa contra inimigos naturais. Cox (1996) mencionou que ovos e larvas de Chrysomelidae representam alvos expostos para predadores e parasitóides, porque devido ao hábito sedentário estes se tornam mais previsíveis nas suas plantas hospedeiras. Paleari (1997) e Sá e Macêdo (1999) citam a localização na superfície inferior de folhas como estratégias para reduzir tal vulnerabilidade. O mesmo padrão não se repete para larvas mais maduras e adultos porque, provavelmente, estes desenvolveram outros tipos de defesa, como o sangramento reflexo, por exemplo, excretando substâncias, muitas vezes tóxicas, pelas articulações e boca.

Regressões múltiplas entre Cassidinae e plantas hospedeiras indicaram que ovos e larvas de S. chalybea são mais frequentes em plantas com maior quantidade de ramos novos e folhas. Isto pode indicar que folhas jovens sejam preferidas como recurso para larvas e que estas são mais frequentes em plantas onde seu recurso é mais abundante. Por outro lado, devido a relação negativa entre o número de folhas ou ramos na planta hospedeira e a abundância de S. areolata e A. phaeopoda, é possível que estes besouros não sejam tão frequentes onde seu recurso é mais abundante, mas provavelmente onde este apresenta melhor qualidade. Plantas menores também podem atrair menos inimigos naturais visualmente orientados, o que pode explicar também a ocorrência de S. areolata e A. phaeopoda em plantas como menores números de ramos ou folhas.

A altura da planta hospedeira foi o parâmetro em que todas as espécies estudadas apresentaram relações positivas. A relação entre Chrysomelidae e plantas hospedeiras maiores tjá havia sido detectada por outros autores como Windig (1993) e Macêdo et al. (1994).

A. phaeopoda foi encontrado mais frequentemente em indivíduos de plantas expostos ao sol, padrão este mais frequente para B. trimera. Porém, para as duas espécies de Stolas não houve diferença significativa entre sua frequência em plantas no sol ou sombra, indicando que estes crisomelídeos podem ser resistentes à exposição ao sol, ou estarem submetidos às menores temperaturas de plantas localizadas em sítios sombreados.

O padrão geral de ocorrência das espécies de Cassidinae estudadas foi de atividade durante o período de setembro à junho (aproximadamente). Após o período de ausência de atividade, a diapausa, indivíduos adultos foram os primeiros a serem encontrados no campo. Estes iniciaram suas atividades reprodutivas ainda na primavera, mas o pico de reprodução foi atingido no verão. Ovos e larvas foram mais frequentemente observados neste mesmo período, indicando a importância de temperaturas mais altas na reprodução dos Cassidinae estudados. Durante o outono, as atividades reprodutivas foram reduzidas e foram completamente interrompidas em meados de maio. Adultos entraram em diapausa à partir de julho, permanecendo neste estado durante toda a estação seca/inverno. Algumas relações positivas significativas entre abundância de Cassidinae e dados de temperatura, inclusive com o intervalo de tempo(lag), podem indicar a influência deste fator na reprodução e flutuação populacional dos Cassidinae conforme sugerido por Vasconcellos-Neto (1980), Delinger

(1986), entre outros. O padrão temporal de ocorrência das espécies de crisomelídeo foi coincidente com o padrão estacional da região, quando se verifica uma estação quente e úmida favorável aos insetos e outra fria e seca, desfavorável à reprodução.

Além da temperatura, a fenologia das plantas hospedeiras de *S. chalybea*, *S. areolata* e *A. phaeopoda* influenciaram a abundância e reprodução de adultos. Estes apresentaram uma sincronia entre sua ocorrência e a presença de certas feno-fases de suas plantas hospedeiras. Este mesmo fato já foi detectado por diversos autores (e.g.: Campbell e McCaffrey, 1991; Clark e Clark, 1991; Vasconcellos-Neto, 1991; Medeiros, 1991; Crowe, 1995).

Nós observamos que a população de *S. chalybea* pareceu ser mais abundante na trilha do “Paraíso III”, onde sua planta hospedeira apresentou maior abundância relativa. A população de *S. areolata* pareceu ser mais abundante na trilha do “Mirante”. Entretanto, essa diferença não seguiu o padrão de abundância da sua planta hospedeira nas trilhas estudado. Nós sugerimos que a variação na abundância de *S. areolata* possa ser explicado por influência de fatores abióticos, que podem atuar de maneiras distintas em ambas as trilhas devido à diferença de quase 100 metros de altitude entre as mesmas ou por pressão de inimigos naturais.

Nós acreditamos que a Segunda hipótese esteja mais fortemente relacionada às diferenças da abundância de *S. chalybea* e *S. areolata* nas duas trilhas porque potenciais predadores observados em suas plantas hospedeiras foram menos abundantes nas trilhas onde a abundância de cada uma das espécies de Cassidinae foi maior em relação ao número de plantas hospedeiras encontradas na trilha. Entretanto, experimentos de exclusão de predadores de larvas de *S. chalybea* não indicaram diferenças de pressão de predação entre as duas trilhas por diferentes grupos de predadores. Por outro lado, nós não sabemos se parasitóides atuam diferentemente em diferentes locais e se o mesmo padrão se repete para predadores de ovos e adultos.

Parasitóides foram importantes fatores de mortalidade de ovos e larvas de *S. chalybea* e *S. areolata*, principalmente. Em *S. chalybea* nós estimamos que somente 40% dos ovos observados no campo eclodiram. A mortalidade de ovos foi a maior entre todos os estágios imaturos (exceto pupa), o que também se repetiu em *S. areolata* e *A. phaeopoda*. Além de parasitismo, a mortalidade de ovos de *S. chalybea* é dividida quase que igualmente também com infecção por fungo e predação. Foram encontradas oito espécies de micro-himenópteros parasitóides em ovos de *S. chalybea*, enquanto que somente duas espécies foram encontradas em ovos de *S. areolata* e nenhuma foi encontrada em *A. phaeopoda*. A menor taxa de parasitismo e o menor número de espécies de parasitóides em S. areolata estão de acordo com as observações de Vinson (1976); Rowell-Rahier e Pasteels (1992); Köpf et al. (1997); Meiners e Hilker, (1997) sobre a redução de parasitismo quando os parasitóides usam pistas químicas da planta para encontrarem seus hospedeiros, já que nas trilhas estudadas suas plantas hospedeiras são menos abundantes do que B. segetum.

Uma mesma espécie de Tachinidae foi coletada parasitando larvas de S. chalybea e A. phaeopoda com a mesma frequência, aproximadamente. A ausência de especificidade deste parasitóide pode estar relacionada com a baixa população de Cassidinae no campo, conforme também foi observado por Keese (1997) em larvas do crisomelídeo Ophraella slobodkini. Larvas de S. areolata também foram parasitadas por um tachinídeo não identificado, porém mais frequente do que o parasitóide das demais espécies.

Conforme mencionado por Gomes-Filho (1997), eventos de predação são bastante efêmeros, e por esta razão provavelmente, poucos eventos deste tipo foram observados ao longo do trabalho. Alguns Asopíneos (Pentatomidae) nas fases de ninfa e adulta foram observados sugando larvas de S. chalybea.

Experimentos de exclusão de predadores indicaram que a mortalidade de larvas jovens de S. chalybea causada por diferentes grupos de predadores não diferiu estatisticamente em períodos de maior ou menor pluviosidade e que grupos de invertebrados que chegam às plantas por via aérea, tendem a ser os maiores responsáveis pela mortalidade de larvas jovens de S. chalybea. Este resultado não foi estatisticamente significativo, apesar de concordar com dados de literatura (veja revisão de Olmstead, 1996 por exemplo).

Os únicos inimigos naturais encontrados em adultos de Cassidinae, um nematódeo e a aranha Nephila clavipes, foram coletados junto a S. chalybea.

Os padrões de ocorrência observados para as espécies de Cassidinae estudadas, poderiam indicar, em uma análise inicial sem considerar a abundância dos mesmos, que os tamanhos das populações poderiam ser controlados por competição interespecífica, uma vez que plantas hospedeiras relativamente próximas taxonomicamente foram utilizadas e baixos números populacionais foram observados. Entretanto, observações em campo indicaram a grande abundância de plantas não utilizadas, levando a hipótese inicial a ser desconsiderada, já que o recurso não seria um fator limitante. Nós acreditamos que fatores climáticos e padrões de utilização de plantas hospedeiras podem influenciar os padrões temporais de ocorrência das populações de Cassidinae estudadas através das importantes relações detectadas neste trabalho.

Entretanto, nós destacamos a importância de inimigos através dos levantamentos e observações frequentes dos mesmos no campo atuando sobre as espécies de Cassidinae estudadas Isto pode indicar que seus papéis realmente parecem ser mais relevantes como causa de mortalidade de herbívoros, conforme tem sido sugerido por diversos autores (como Hairston et al., 1960; Cappaert et al., 1991; Keese, 1997; Cornell et al., 1998 entre outros) e atuando mais intensamente na determinação dos tamanhos populacionais dos mesmos.

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