ABSTRACT

Coexistence in Multi-Exploiter Systems

by

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The coexistence between members of a multi-exploiter system, whereby one victim (the host or prey) is attacked by two or more exploiters (predators, pathogens or parasites), was examined in the case of *Coccus viridis*; a sessile scale insect coffee pest that is attacked by a predatory Coccinellid, *Azya orbigera,* and an entomopathogenic fungus, *Lecanicillium lecanii*. Experimental inclusion of *A. orbigera* resulted in lower proportions of scales infected with *L. lecanii*; the trend further supported by field survey data from the previous year. Selective consumption by *A. orbigera* of late stage *C. viridis* instars that are more often infected by *L. lecanii* may act to remove the most susceptible individuals from the population thus lowering levels of infection in the presence of beetles. Lefkovitch stage-based matrix models provide evidence that *L. lecanii* is an important natural enemy of *C. viridis,* and that exploitation of *C. viridis* life stages 1-3 currently limits population growth. Thus, for systems where *C. viridis* is a major problem, promoting a variety of natural enemies that attack different instars can effectively control the pest if combined with infection from *L. lecanii*. In addition, theoretical analysis of a model describing a generalist pathogen and a specialist predator that share a common victim resource, hints to the importance of non-linear indirect ecological interactions such as intraguild predation in promoting coexistence between all components of the multi-exploiter system. Such self-limiting processes may be integral to how biological control through biocomplexity can maintain pests at below threshold levels, while also limiting the spread of the control agents themselves.

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Wei Ying Ong

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Dedicated to Jack, the purple mountains and the fleeting sunshine.

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ABSTRACTS

Chapter 1: **PREDATION-CONTROLLED INFECTION**

The coexistence between members of a multi-exploiter system, whereby one victim (the host or prey) is attacked by two or more exploiters (predators, pathogens or parasites), was examined in the case of *Coccus viridis*; a sessile scale insect coffee pest that is attacked by a predatory Coccinellid, *Azya orbigera*, and an entomopathogenic fungus, *Lecanicillium lecanii*. Experimental exclusion and inclusion of *A. orbigera* adults and larvae for 24 hours on leaves with healthy *C. viridis* populations resulted in lower proportions of scales infected for leaves that were exposed to *A. orbigera* a week earlier. Field survey data from the previous year support experimental findings; lower proportions of scales were infected in July 2009 where *A. orbigera* were found in June 2009 (when all tested bushes had equal scale population sizes and no signs of *L. lecanii* infection). We attribute this decrease in infection to consumption of *C. viridis* by *A. orbigera*; removing susceptibles from the population. Fewer scale insects were found on bushes with larvae in the field survey, and fewer found for treatments including adult beetles in the experiment. Leaves and bushes with lower scale numbers tended to have a lower proportion of fungal infection as well. Adult *A. orbigera* may consume *C. viridis* at a greater rate than larvae, but migrate more often to different patches since they are capable of flight. We also extend the hypothesis that selection of larger, older *C. viridis* prey by *A. orbigera* is responsible for decreasing *L. lecanii* infection if as suspected, larger, older *C. viridis* are more susceptible to infection. Intraguild predation of *L. lecanii* through consumption of already infected *C. viridis* by *A. orbigera* may also decrease infection by removing infected individuals from the population. In any case, the presence of the predator in this multi-exploiter system appears crucial for regulation of the second exploiter, and as a result, coexistence of the entire system.

Chapter 2: **USE OF STAGE-BASED MATRIX MODELS IN EVALUATING BIOLOGICAL CONTROL AGENTS AND NATURAL ENEMIES**

We evaluated the effectiveness of several natural enemies in keeping *Coccus viridis*, a major homopteran scale insect pest under control in an organic shade-tree coffee farm located in Chiapas, Mexico. We used Lefkovitch stage-based matrix models to calculate population growth rates (λ) and elasticities for survival, transition and fecundity rates of each life stage in the *C. viridis* life cycle. The effect of *Lecanicillium lecanii*, an entomopathogenic fungus that attacks the scale insect was evaluated by determining the probability of infection for each life stage of *C. viridis* and applying the effect on population projection models. Vital rates for the model were derived from time lapse photography of scale insects exhibiting no signs of *L. lecanii* infection. Including infection in the model stabilized population growth (λ=1.05), suggesting that *L. lecanii* is an important natural enemy of *C. viridis*. We also found evidence that exploitation of *C. viridis* life stages 1-3 is currently limiting growth of the population, and that further exploitation of life stage 4 could dramatically decrease population growth. By simulating data that was altered to remove all effects of predation, we discovered that the degree of predation required to cause population decline given the established gradient for *L. lecanii* infection, was least for 3rd stage instars followed by 1st then 2nd. However, when stochasticity was introduced, attacks on the 1st life stage decreased λ most. Previous studies of this system have considered *Azya orbigera*, a Coccinellid beetle predator that attacks mainly 3rd and 4th stage instars as an important natural enemy responsible for controlling *C. viridis*. Our results highlight the importance of other predators, in particular, another Coccinellid of the genus *Diomus* known to attack 1st and 2nd stage instars. For systems where *C. viridis* is a major problem, promoting a variety of natural enemies that attack different instars with particular attention to 1st stage instars is encouraged when combined with use of the naturally occurring biological control agent *L. lecanii*.

Chapter 1

PREDATION-CONTROLLED INFECTION

INTRODUCTION

The classic Lotka-Volterra predator-prey model excludes coexistence between two predators and one limited resource. However, in nature, examples of multiple predators or a predator and pathogen with one shared prey/host are common (Gilg 2003, Fenner 1959, Inouye 1981). In the Lotka-Volterra model, competition between two predators ultimately drives the weaker competitor to extinction, followed by stable cycling of the stronger competitor with the prey (Lotka 1925; Volterra 1927). However, the Lotka-Volterra equations do not account for effects of logistical growth, critical densities of hosts for the spread of infections, handling time, or seasonality on these multi-predator systems. Armstrong and McGehee (1976) demonstrated that slight alterations incorporating these kinds of effects can lead to stable coexistence in multi-predator systems.

Interactions between predators or pathogens as well as their associations with prey/hosts may also help explain the stability of these multi-exploiter systems. Consider for instance, the invasion of a pathogen in a previously stable predator-prey relationship. Previous theoretical work has shown that this invasion can destabilize the former relationship by driving prey densities below levels that can sustain predator densities; however, an alternate equilibrium point at densities lower than in the absence of the pathogen can also occur (Anderson et al. 1986).

These opposing outcomes are particularly important to understand in the case of biological pest control in economically valuable crops such as coffee. Interactions between control agents, their targets and associated predators or pathogens must be closely studied in order to insure the success and stability of biological control over time.

Vance-Chalcraft et al. (1995) suggest the particular importance of intraguild predation, whereby two species that share hosts or prey also engage in trophic interactions with one another through predation or parasitism. For instance, the introduction of a generalist control agent that preys on both a target pest species as well as a natural enemy of the target species has the potential to shift the equilibrium point of the pest species to higher densities by diminishing the negative effects of parasitism (Snyder 2001).

In another example of intraguild predation, a predator that prefers or unintentionally consumes prey infected with pathogens (thereby consuming the pathogen as well) can diminish reservoirs of the disease and lower total contact time between susceptible and infected individuals by removing infected individuals from the pool (Ostfeld and Holt 2004). Thus, predator removal would result in an increase of pathogens, since infected prey survive longer in the absence of predators. In one example, predator culling by gamekeepers resulted in increased parasite loads of red grouse (Hudson et al. 1992). Ostfeld and Holt (2004) also point out that even in the case of predators preferring to consume healthy prey (higher food quality), removal of predators still results in an increase of both the total abundance and fraction of infected individuals in the population by increasing the total prey population, thus allowing higher numbers of pathogen to persist (2004).

Despite the plethora of theoretical studies on these multi-predator and predator, pathogen, prey/host systems, few empirical studies have explored how the relationship between predators and pathogens structures their own communities. We wish to understand how interspecific interactions self-stabilize multi-exploiter systems in nature, and how this may influence the control of common pest problems in agroecosystems. In this study, we examine the interactions between a homopteran coffee pest, *Coccus viridis* (the green coffee scale), its Coccinellid predator, *Azya orbigera*, and the pathogen, *Lecanicillium lecanii*, an entomopathogenic fungus that attacks *C. viridis*. This system is particularly interesting to study because of the immobility of the host/prey, *C. viridis*. Its sessile nature makes the demographics and epidemiology of the population relatively easy to quantify and follow. Once attached to the leaf or stem of a coffee bush, adult *C. viridis* remain relatively immobile, only moving in cases of extreme dehydration (Perfecto pers. comm.). Adults produce an abundance of nymphs which undergo three

larval stages before reaching adulthood, becoming less mobile with each additional stage of growth (Fredrick 1943). Immobility makes *C. viridis* an easy target for predation by *A. orbigera*, a voracious consumer of *C. viridis* in both its adult and larval stages (Uno 2007, Liere and Perfecto 2008). *C. viridis* populations are also heavily regulated by regular epizootics of the fungal disease, *L. lecanii* (Easwaramoorthy and Jayaraj 1978, Jackson et al. 2009). Here, we tested to what extent *L. lecanii* was hindered or spread by *A. orbigera*. In theory, this predator could either act as a stabilizing force limiting the spread of *L. lecanii* by reducing the disease reservoir in the *C. viridis* population or alternatively, a destabilizing fore, by helping spread the disease as adults travel from large clusters of infected scales unwittingly carrying conidia to new, uninfected patches.

METHODS

Study Sites, Experiments, and Field Surveys

Our enclosure experiments were conducted in an organically managed coffee farm in the Soconusco region of Chiapas, Mexico, named Finca Irlanda (15º11' N, 92º20' W). Finca Irlanda is approximately 300 ha in size with elevations ranging between 900 and 1150 m. The farm receives about 4500 mm rain/yr and consists of approximately 1200-2500 coffee plants/ha. Experiments were set up in three sites within Finca Irlanda, which we will refer to as Heidi 1 (H), Porfidio Diaz (P), and Quebradita (Q). At each site, all plants having at least three leaves infected with ≥20 scale insects each and no visible signs of *L. lecanii* infection were included, thirteen plants (7- Q, 5-P, 1-H) in total. Starting in 28 May 2010, three leaves in each of three plants in Quebradita were covered with 3.5'x7'clear plastic sealable bags and randomly selected to include either 1) an adult *A. orbigera*, 2) a larval stage *A. orbigera* or 3) nothing as a control. The number of scales was censused at the beginning and end of the experiment. Bags were sealed at the base of the leaves and organisms left inside for approximately 24 hours and then removed. After seven days, bags were removed from leaves and all scales inspected for white halos of mycelia characteristic of *L. lecanii* infection. To increase sample size, 10 replicates were added from the 10^{th} -14^{th} of June 2010. Plastic bags were replaced with

3.5'x7' mesh bags in this later set of replicates to increase air flow and decrease microclimatic effects. Because of logistical constraints, five replicates from this later set were run for eight instead of seven days. Statistical tests were run (see below) to test for differences, and then lumped.

To corroborate experimental results from this study, data from field surveys of Quebradita conducted in June and July of 2009 were analyzed to look for patterns between *L. lecanii*, *A. orbigera* and *C. viridis* populations. Each coffee bush (428) in the entire 12 x 7 ha plot was surveyed for the number of scale insects, average percentage of *L. lecanii* infection, and the number of *A. orbigera* adults and larvae. A first census of *C. viridis* and *L. lecanii* densities was conducted from June 6-8, 2009. This was followed by a second census of the same plot from July 8-11, 2009. Each coffee bush was given a quick visual examination to determine whether there were less than twenty (0 category), twenty to fifty (50 category) or greater than fifty scales total. If the plant had greater than 50 scales, each individual branch was surveyed and placed into one of the following categories: low (0-6 scales), medium (7-30), high (31-70), or super (›70). For plants with greater than 50 scales, number of scales total was estimated by multiplying the number of branches in each of the above branch categories by 0, 15, 46, and 150, respectively, and summing. Otherwise, total number of scales was estimated at 0 or 50. Each plant was censused in sequential, numerical order. Logistical and geographical barriers prevented a random survey.

In order to control for *C. viridis* density effects on infection rates, only data in the 50 category for scales in June was analyzed. For each of these bushes, *L. lecanii* infection was estimated for the entire bush from 0 - 100% in 5% intervals. To more accurately *census A. orbigera* populations, surveys were done about a week after *C. viridis* and *L. lecanii* surveys to minimize disturbance to the flying insect community. The first *A. orbigera* census was done from June 16-19, 2009, eight days after the first *C. viridis*, *L. lecanii* survey. The second *A. orbigera* census was conducted on July 20-27, 2009, nine days after the second *C. viridis*, *L. lecanii* survey. At every bush, all larvae were counted, and all adult beetles captured, sexed, and released at the end of the survey.

Statistical Analyses

To test for effects of *A. orbigera* on the levels of *L. lecanii* infection, general linear hypothesis tests were conducted using a model that specified infection as a binomial distribution with a logit function where success and failure of infection was tallied for each experimental treatment and control group then compared using tukey contrasts with a false-discovery rate adjustment. To test for effects of site, a similar model was written, replacing treatment for site as the predictive variable. Sites were again compared using tukey contrasts with a false discovery rate adjustment.

Effects of bag type and number of experimental days elapsed were tested using t-tests after transforming proportion scales infected with the arc sine square root to meet conditions of normality. Linear models were created to check for correlations between proportion infection and 1) number of scales at beginning of experiment 2) number of scales at end of experiment, and 3) number of scales consumed during course of the experiment. To test for differences in scale consumption between adult and larval stage beetles, a t-test was run on data after normalizing using a square root transformation.

To test for a relationship between *A. orbigera* and *L. lecanii* populations, the *A. orbigera* census data from June 2009 was compared to the *L. lecanii* census data from July 2009, one month after the beetle census. In this way, we hoped to test for links between locations of beetles in one month and degrees of fungal infection in the next month. These tests were independent of scale density since all coffee plants began with the same initial average of 50 scales, none of which showed any signs of *L. lecanii* infection in the first June survey. Each plant was individually tagged with an ID number allowing us to know how the *C. viridis*, *A. orbigera*, and *L. lecanii* populations within each bush (58 bushes total) changed over the course of one month. To test for effects of *A. orbigera* on infection, general linear hypothesis tests were conducted using a model that specified infection as a binomial distribution with a logit function where success and failure of infection was tallied for plants with 1) adult *A. orbigera* in June, 2) larval *A. orbigera* in June, and 3) both adult and larval *A. orbigera* in June or 4) no *A. orbigera* in June and compared using tukey contrasts with a false-discovery rate adjustment.

To insure that effects on *L. lecanii* were related to beetle presence in June, not July, seven general linear models were compared using AICs where number of scales infected

was predicted by presence of 1) adult *A. orbigera* in June, 2) larval *A. orbigera* in June, 3) both adult and larval *A. orbigera* in June , 4) adult *A. orbigera* in July, 5) larval *A. orbigera* in July, 6) both adult and larval *A. orbigera* in July and 7) a control null model.

RESULTS

Azya orbigera enclosure experiment

At the conclusion of the enclosure experiment, leaves exposed to adult *A. orbigera* had 50% less *L. lecanii* infection than control leaves. There was also a significant decrease of 41% less scales infected on leaves exposed to *A. orbigera* larvae (Figure 1.1, Table 1.1). When data from larval treatments was lumped with adult treatments, presence of *A. orbigera* significantly decreased the proportion *L. lecanii* infection on average 1.8 times (t=-2.51, p=0.02) from controls (Figure 1).

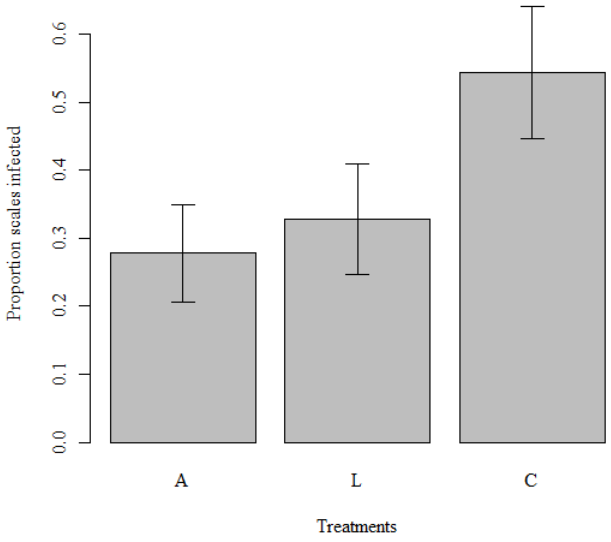

Figure 1.1. Effect of *A. orbigera* presence on infection in enclosure experiment. L= Larvae, A= Adult, C= Control treatments. 95% confidence intervals. Presence of *A. orbigera* significantly decreased proportion of scales infected with *L. lecanii*

Table 1.1. Enclosure Experiment Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts					
Fit: glm(formula = Infection ~ Treatment, family = binomial("logit"))					
Linear Hypotheses:					
	Estimate	Std. Error	z value	Pr(>|z|)	
L - A	0.53	0.11	4.99	8.95E-07	***
C - A	0.96	0.10	9.27	< 2.00E-16	***
C - L	0.43	0.11	4.10	4.18E-05	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
(Adjusted p values reported -- fdr method)					

L= Larvae, A= Adult, C= Control treatments. For every leaf, the total numbers of infected and healthy scales were tallied and modeled as a binomial distribution with a logit function using treatment as the predictive variable. Infection was compared across treatments using tukey contrasts. Infection was lowest for larvae treatments, followed by adults and controls. All differences were significant.

There were significant differences in the degree of fungal infection between sites, with the highest average of 60% infection in Porfidio Diaz compared to 30% in Quebradita and 10% in Heidi 1 (Figure 1.2, Table 1.2). However, each site showed a similar trend of lower infection rates in *A. orbigera* treatments as compared to controls (Figure 1.3).

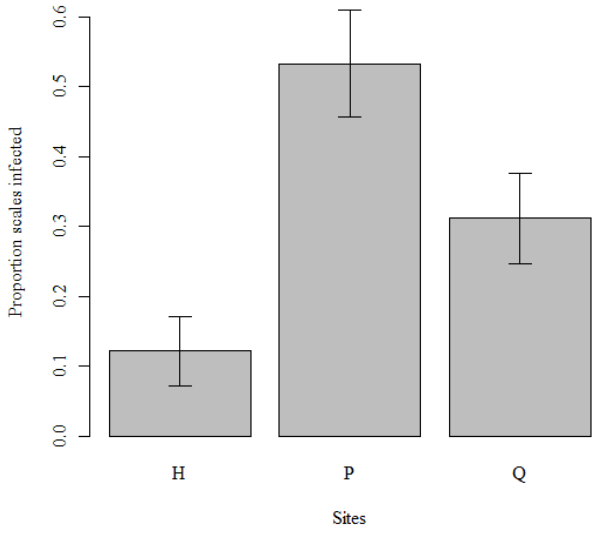

Figure 1.2. Effect of site on infection in enclosure experiment. H= Heidi 1, P= Porfidio Diaz, Q= Quebradita. 95% Confidence intervals. Porfidio Diaz had the highest levels of infection followed by Quebradita then Heidi 1. All differences were significant.

Table 1.2. Enclosure Experiment Simultaneous Tests for General Linear Hypotheses- Site

Multiple Comparisons of Means: Tukey Contrasts					
Fit: glm(formula = Infection ~ Site, family = binomial("logit"))					
Linear Hypotheses:					
	Estimate	Std. Error	z value	Pr(>|z|)	
P – H	2.10	0.28	7.46	1.30E-13	***
Q – H	1.26	0.28	4.50	6.75E-06	***
Q – P	-0.84	0.09	-9.50	< 2.00E-16	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
(Adjusted p values reported -- fdr method)					

H= Heidi 1, P= Porfidio Diaz, Q= Quebradita. For every leaf, the total numbers of infected and healthy scales were tallied and modeled as a binomial distribution with a logit function using site as the predictive variable. Infection was compared across sites using tukey contrasts. Infection was lowest for Heidi 1, followed by Quebradita and Porfidio Diaz. All differences were significant.

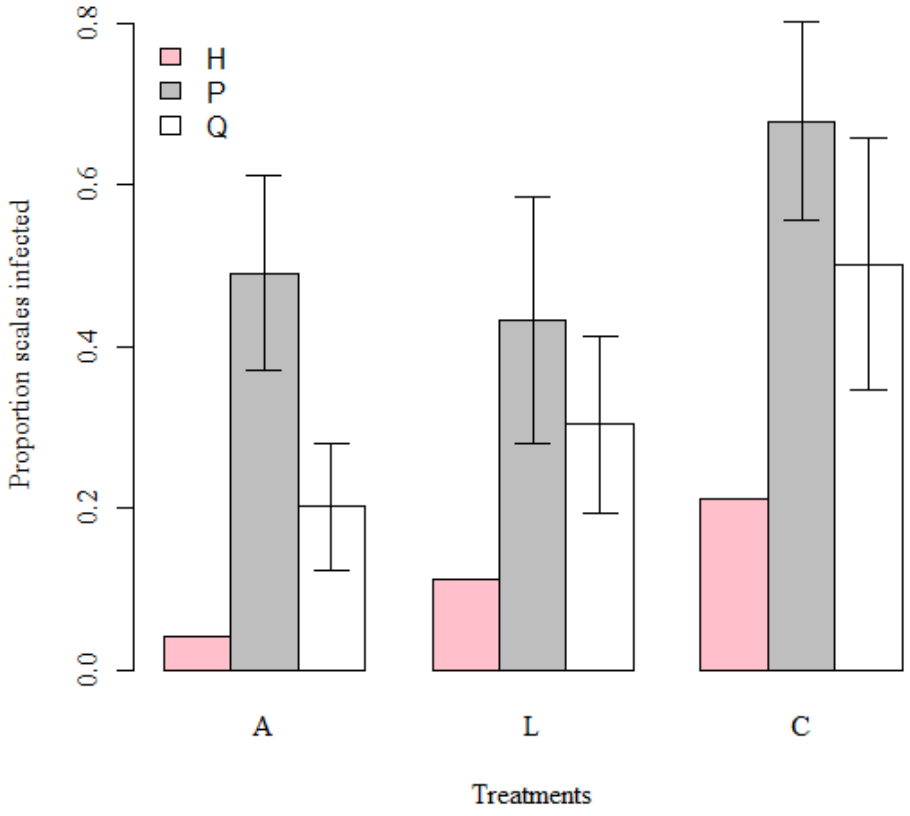

Figure 1.3. Effect of treatment by site on infection in enclosure experiment. H= Heidi 1, P= Porfidio Diaz, Q= Quebradita. A=Adults, L=Larvae, C=Controls. 95% Confidence intervals. All sites show similar trend: consistently lower infection in *A. orbigera* treatments compared to controls. Only 1 repetition was conducted in Heidi 1.

Adult *A. orbigera* ate on average 11 more scales (t=2.06, p=0.05) than larvae (Figure 1.4). No significant effect of bag type (t=-0.62, p=0.55), or number of experimental days elapsed was found (t=1.19, p=0.27). There was also no relationship between the number of scales eaten (R^2=-0.03, p=0.65), number of scales at the beginning of the experiment (R^2=0.02, p=0.22), or number of scales at the end of the experiment (R^2=-0.03, p=0.99) with proportion scales infected.

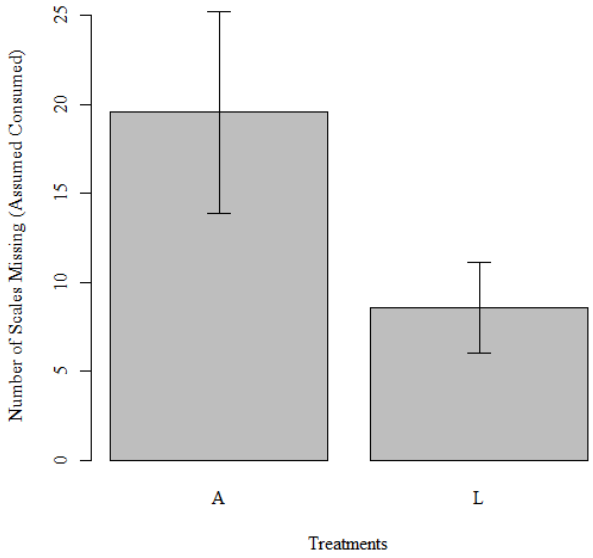

Figure 1.4. Difference in consumption of scales by *A. orbigera* adults and larvae in enclosure experiment. A= Adults, L=Larvae. Adult *A. orbigera* consumed on overage 20 *C. viridis* scales compared to 9 for larvae in the 24 hour window in which they were allowed to forage on leaves. No scales were missing on control leaves.

2009 Comparative Field Survey

Plants with larval *A. orbigera* in June 2009 had twelve times less infection in July 2009 than plants without larvae. Similarly, plants with adult *A. orbigera* in June had three times less infection in July than plants without adults. Collectively, plants with either or both adults and larvae had eight times less infection in July than plants without any *A. orbigera* in June (Figure 1.5, Table 1.3).

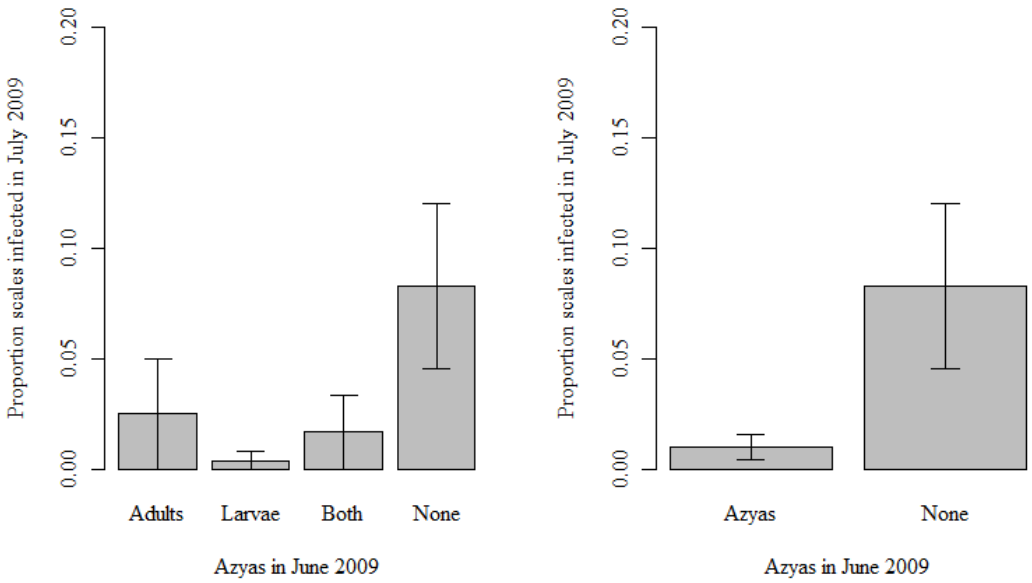

Figure 1.5. 2009 Field Survey, *L. lecanii* infection on plants with and without *A. orbigera.* Coffee plants (n=58) in the Quebradita site with an average of 50 scale insects with no signs *of L. lecanii* infection in June 2009 were monitored for presence of *A. orbigera* larvae and adults. Subsequent census of *L. lecanii* in July 2009 showed drastically lower levels of infection for plants that had *A. orbigera* (n=20) the month before, especially those with larvae (n=13). The '*Azyas*' category in the graph on the right includes all plants where *A. orbigera* adults (n=4), larvae, or both (n=3) were found in June 2009. 95% Confidence intervals.

Table 1.3. 2009 Field Survey, Simultaneous Tests for General Linear Hypotheses: effect of *A. orbigera* on *L. lecanii* infection levels

Multiple Comparisons of Means: Tukey Contrasts					
Fit: glm(formula = Infection ~ Presence of *A. orbigera* types, family = binomial("logit"))					
Linear Hypotheses:					
	Estimate	Std. Error	z value	Pr(>|z|)	
Both – Adults	0.80	1.23	0.65	6.20E-01	
Larvae - Adults	0.10	1.23	0.08	9.38E-01	
None - Adults	3.47	1.01	3.44	1.15E-04	**
Larvae - Both	-0.70	1.01	-0.70	6.20E-01	
None – Both	2.67	0.72	3.72	6.08E-04	***
None - Larvae	3.37	0.71	4.72	1.42E-05	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
(Adjusted p values reported -- fdr method)					

Tukey contrasts used to compare the mean infection for several groups, adjusting p values using the fdr method to account for multiple comparisons. Infection is a binomially distributed matrix of the number infected and healthy scales per bush. Presence of larval and adult *A. orbigera* in June 2009 significantly decreased infection of scale insects by *L. lecanii* in July 2009. Plants with both adults and larvae did not have significant differences in infection from plants with no signs of beetles. This difference is likely due to low sample size; plants with larvae (n=13), adults (n=4), both (n=3), none (n=38).

When comparing GLMs predicting infection based on presence of *A. orbgiera* types in June versus July, presence of both adult and larvae in June was selected as the best predictor of infection with the lowest AIC value (Table 1.4). Models predicting infection based on *A. orbigera* in June had consistently lower AICs than models predicting infection based on *A. orbigera* in July (Table 1.4), although all July models had lower AICs than the null.

Table 1.4. AIC table for models predicting *L. lecanii* infection in July 2009Field Survey

Model predictor	df	AIC	AIC difference from Null	
Null	1	1013.78	0	
Larvae in July	2	981.42	32.36	
Adults in July	2	990.64	23.14	
Combined *A. orbigera* in July	2	970.73	43.05	
Larvae in June	2	919.54	94.24	
Adults in June	2	952.16	61.62	
Combined *A. orbigera* in June*	2	874.42	139.36	*Best Model

Seven general Linear Models predicting *L. lecanii* infection in July 2009 survey were compared using AIC values. General Formula= Infection (matrix of # infected and healthy scales per plant) ~ Model predictor, family= binomial ("logit"). All models have lower AIC values than Null suggesting that beetle presence in both June and July are related to infection levels in July. However, June models have consistently lower AICs than July models, with the combined June *A. orbigera* as the best model with an AIC of 874.42. Since all plants began with no infection, we believe that June beetle presence decreased infection for July. These plants with low infection levels likely retained their June beetle population into July, possibly attracting more.

Although all plants started out with around 50 scale insects, plants found with larval *A. orbigera* in June 2009 had on average 14 fewer scales in July 2009 than plants without larvae (Figure 1.6, Table 1.5). Plants with adult *A. orbigera* in June also had fewer scales than plants with no sign of beetles, but the average difference of approximately 6 scales was only marginally significant (p=0.069). Overall, plants with any sign of *A. orbigera* in July had on average 13 fewer scales than those with no signs (Figure 1.6, Table 1.5). The same trend was found when looking at the presence of *A. orbigera* in July, with fewer scales when *A. orbigera* were present (Figure 1.7, Table 1.6). Only one plant had both an adult and larval *A. orbigera* in July.

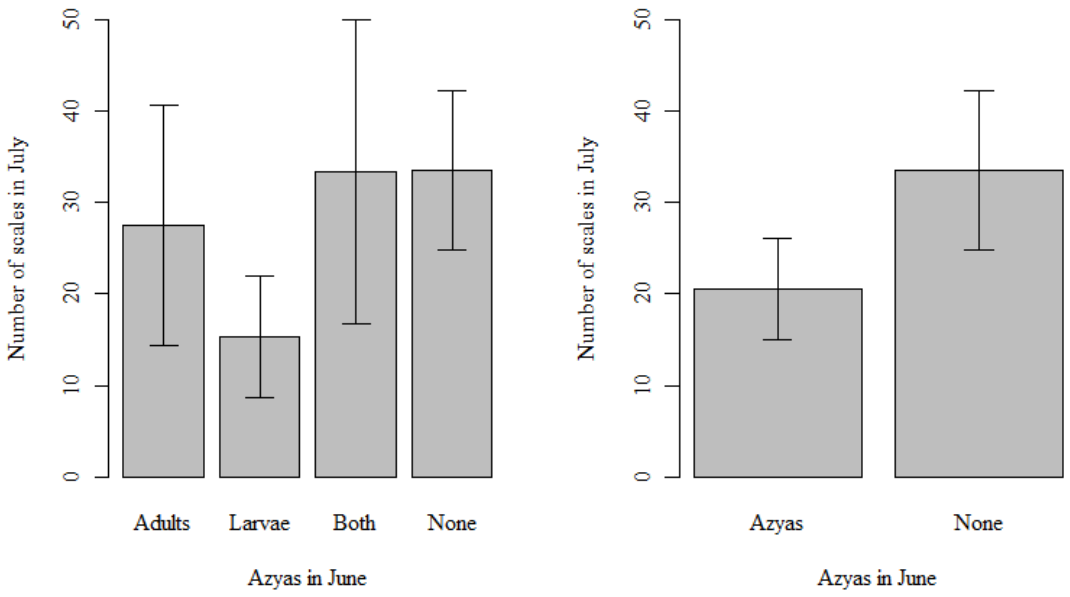

Figure 1.6. 2009 Field Survey, Number of scales in July for plants with and without *A. orbiger*a in June. All plants began with approximately 50 scales in June 2009. Plants where *A. orbigera* larvae were found in June had significantly fewer scales ($p<0.001$) than those that had none. Plants with adult beetles also had fewer scales, though this difference is only marginally significant ($p=0.69$). '*Azyas*' category in b) includes 'Adults' (n=4), 'Larvae' (n=13), and 'Both' (n=3) categories from a). 58 coffee plants total. 95% Confidence intervals.

Table 1.5. 2009 Field Survey, Simultaneous Tests for General Linear Hypotheses: effect of June *A. orbigera* on July *C. viridis* population

Multiple Comparisons of Means: Tukey Contrasts					
Fit: glm(formula = Scales in July ~ Presence of *A. orbigera* types in June, family =poisson)					
Linear Hypotheses:					
	Estimate	Std. Error	z value	Pr(>|z|)	
Both – Adults	0.19	0.14	1.39	1.97E-01	
Larvae - Adults	-0.58	0.12	-4.89	1.99E-06	***
None - Adults	0.20	0.10	1.99	6.93E-02	.
Larvae - Both	0.77	0.12	6.31	8.21E-10	***
None – Both	0.01	0.10	0.06	9.56E-01	
None - Larvae	0.78	0.08	10.24	< 2.00E-16	***
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1					
(Adjusted p values reported -- fdr method)					

Tukey contrasts used to compare the mean number of scale insects for several groups, adjusting p values using the fdr method to account for multiple comparisons. Poisson distribution specified for count data. Larvae significantly reduced scale population. There was a marginal significant decrease for adults. Category 'Both' includes bushes where both adults and larvae were found. There was significantly greater

numbers of *C. viridis* in these bushes than those with only larvae, but this is likely due to low sample size; plants with larvae (n=13), adults (n=4), both (n=3), none (n=38).

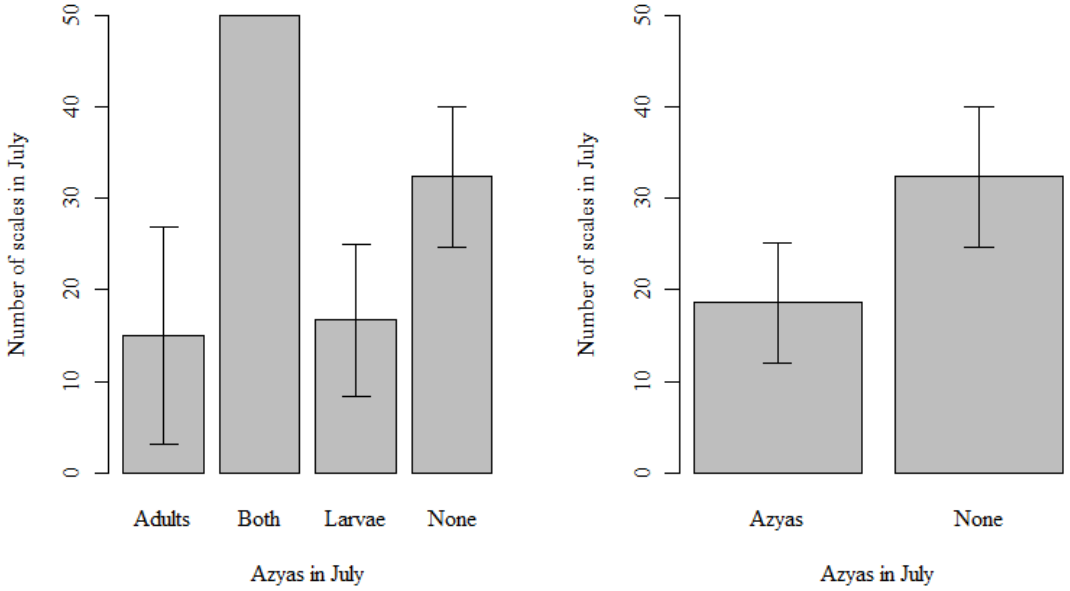

Figure 1.7. 2009 Field Survey, Number of scales in July for plants with and without *A. orbigera* in July. Test for correlation between current July beetle population and number of scales in July. All plants originally had a count of 50 scales. Scales on plants with *A. orbigera* adults (n=4), larvae (n=9), both (n=1) or none (n=44) in July 2009 were counted. Same trend as Figure 6; fewer scales where adults and larvae are present.

Table 1.6. 2009 Field Survey, Simultaneous Tests for General Linear Hypotheses: effect of July *A. orbigera* on July *C. viridis* population

Multiple Comparisons of Means: Tukey Contrasts					
Fit: glm(formula = Scales in July ~ Presence of A. orbigera types in July, family = poisson)					
Linear Hypotheses:					
	Estimate	Std. Error	z value	Pr(>|z|)	
Both – Adults	1.20	0.19	6.29	6.45E-10	***
Larvae - Adults	0.11	0.15	0.69	4.90E-01	
None - Adults	0.77	0.13	5.84	8.07E-09	***
Larvae - Both	-1.10	0.16	-6.73	5.17E-11	***
None – Both	-0.43	0.14	-3.02	3.00E-03	**
None - Larvae	0.66	0.09	7.73	6.39E-14	***
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1					
(Adjusted p values reported -- fdr method)					

Tukey contrasts used to compare the mean number of scale insects for several groups, adjusting p values using the fdr method to account for multiple comparisons. Poisson distribution specified for count data. Plants with larvae and adults in July had significantly reduced scale populations in July. Category ‘Both’ includes bushes where both adults and larvae were found. There was significantly greater numbers of *C.*

viridis in these bushes than all other categories, but this is due to low sample size; plants with larvae (n=9), adults (n=4), both (n=1), none (n=44).

There was also a positive relationship between the number of scales in July 2009 and the proportion of scales infected in July 2009 (R^2=0.15, p=0.002, Figure 1.8).

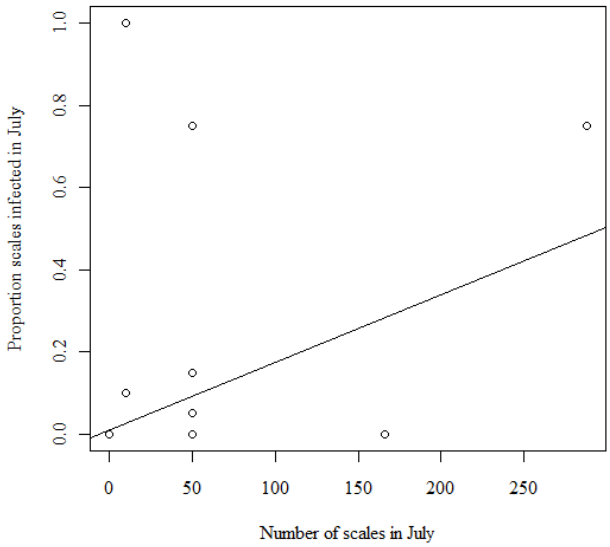

Figure 1.8. Positive relationship between proportion infection and scale number in July 2009. R^2=0.15, p=0.002, m=0.01

DISCUSSION

Enclosure experiment

Experimental manipulation showed that the addition of larval and adult *A. orbigera* to leaves with healthy *C. viridis* populations lowered the proportion of scales that are subsequently infected with *L. lecanii*. The first and simplest explanation is that the beetles lowered the number of susceptible individuals by consuming healthy scales, thus lowering the overall proportion infected. This is corroborated by the fact that less infection occurred in bags with adult *A. orbigera*, which ate on average twice as many scales than larval *A. orbigera*. Consequently, larval treatments had higher proportions of infected scales than adult. Regardless, larvae did consume some scales, which may explain why larval treatments had lower levels of infection than the controls (no

predation). However, there was no direct relationship between either the number of scales eaten or the total number of scales at the end of the experiment and proportion infected. This suggests that the fungus diminishing effect from beetle predation is more complicated than simply reducing the number of overall scales, a question we will address later on.

Site had a significant effect on infection, with Porfidio Diaz having the most infection followed by Quebradita and finally Heidi 1. However, within each site, *A. orbigera* treatments had consistently lower levels of infection than controls; supporting the hypothesis that *A. orbigera* is limiting spread of infection despite variability in degree between sites. Interestingly, Porfidio Diaz experienced significantly less predation than Quebradita, which again supports the hypothesis that increased predation leads to lower infection. However, Porfidio Diaz was the site of a fungal inoculation study the year before, which may have easily left residual levels of fungus (Jackson et al. in review).

2009 Field Survey

Field survey results were relatively consistent with experimental results, showing an overall decrease in infection for plants that had *A. orbigera* present when compared to plants without a month prior to the final census. However, contrary to experimental results, field surveys showed higher infection rates for adult than for larval *A. orbigera*. In addition, there were fewer scales on plants with larvae than with adults suggesting that larvae consumed more scales and had a consequently larger effect on fungus levels than adults. Past work studying the functional response rates of adult and larval *A. orbigera* has shown that adults consume a slightly higher number of scales than larvae within a 24 hour period, although the difference is not significant. Adults consumed a maximum of 40 scales whereas larvae consumed a maximum of 37 (Liere et al. in review). However, both the functional response study and the enclosure experiment constrained beetle foraging to 24 hours. Comparing experimental and field survey results suggest that adult beetles consume scales at a faster rate (within 24 hours) than larvae, but since the field survey did not constrain adult beetles to any one bush, we suspect that high rates of migration in flight-capable adult beetles and constant attacks from *Azteca instabilis*, the

aggressive ant mutualist of the scale insect (Perfecto and Vandermeer 2008) decreased adult consumption per plant. In its larval stage, *A. orbigera* experiences little to no harm from *A. instabilis* due to a protective coating of sticky filaments, which dissuade attack by ants (Liere and Perfecto 2007). Considering that the field survey was conducted over the span of an entire month, the effect of larvae is likely stronger than of adults because larvae are incapable of flight, and may remain on specific plants for much greater lengths of times since they are not threatened by *A. instabilis*. In contrast, experimental enclosure of adult beetles for 24 hours is an unrealistic length of time to expect an adult beetle to remain foraging on one patch while under attack by *A. instabilis*. Ants were also excluded in the enclosure experiment, which artificially lowered predation risk and likely increased scale consumption by adults.

Possible mechanism for decreased infection

A preliminary study where leaves ranging in size of healthy *C. viridis* populations were sealed in plastic trays and observed for subsequent *L. lecanii* infection over one week showed evidence of a critical population size (>50 scales) necessary to have infections higher than 20% (Ong et al. unpublished). There is also some evidence to suggest that the proportion of infected scales in the 2009 field survey increased with the total number of scales in the population (Figure 1.10). This is expected since we predict higher contact rates between infected and susceptible individuals as population size increases (Mollison 1995). However, the positive trend found between population size and proportion infected in the field survey is largely driven by one plant with 300 scales and high levels of infection. Even when including this point, the slope is at a very low value of 0.01 with an R^2 value of 0.15, suggesting that variables other than total population size are also responsible. As previously stated, no direct relationship between population size or number of scales eaten and proportion infected was found in the enclosure experiment. Field observations of leaves with high infection levels despite low numbers of scales were also common.

We propose the preferential consumption of larger scales by *A. orbigera* as a possible mechanism to explain this conundrum. If we assume that most scales are under constant

exposure to fungal spores that persist in the environment (Easwaramoorthy and Jayaraj 1978), we may predict that the older a scale is (and subsequently larger), the longer its exposure to the fungal spores and the greater its chance of infection. Arnold and Herre found that endophyte infection was influenced more by duration of exposure than by age of *Theobroma cacao* leaves (2003), but if we suspect that exposure is constant in our system, age should correlate with length of exposure. Indeed, many infections are age dependent; increases in age result in the weakening of immune responses and changes in organ systems making the hosts more susceptible to certain infections (Gardner 1980). Field observations support this hypothesis since infected scales were mostly adults, while younger nymphs and crawlers were usually found healthy unless the population experienced a complete epizootic. Another possibility is that *L. lecanii* requires a significant amount of incubation time before infection in *C. viridis* becomes apparent. The older a scale, the longer an infection has to incubate. If older scales are more likely infected than younger, selective consumption of these scales by *A. orbigera* could drastically reduce the number of susceptible individuals in the population, keeping the average age of the population relatively young and infection levels subsequently low. Previous food choice experiments where *A. orbigera* was offered a range of scale sizes to consume provide evidence of a preference for larger, older scales (Iverson et al. unpublished).

Intraguild Predation

Latent infection in a majority of *C. viridis* individuals is likely considering field observations of spontaneous infections occurring in scales that are neither adjacent nor nearby already sporulating cadavers. *A. orbigera* appear to prefer consuming healthy scales, but we do not know whether the beetles can distinguish between healthy scales and those that are infected but not yet sporulating. In at least one case, a female adult *A. orbigera* was recorded on tape consuming part of a scale visibly infected with white mycelia from *L. lecanii*. However, the beetle was starved and had no other food options. The scale in question appeared freshly infected; retaining a significant amount of liquid volume despite the presence of a growing ring of white mycelia. Coccinellids are known

to consume fungus-infected prey when starved or under great stress (Pell and Vandenberg 2001). Given this, when infection levels are high and food availability short, consumption of infected (sporulating or non-sporulating) scales is quite plausible. Such intraguild predation may help explain how infection levels drop in the presence of beetles.

CONCLUSION

In conclusion, predation of *C. viridis* and intraguild predation of *L. lecanii* by *A. orbigera* may reduce overall levels of infection in *C. viridis* populations. Experimental removal of all predators and other organisms increased the proportion of scales that were infected suggesting that the presence of *A. orbigera* significantly decreases fungal infection. The balance between enemies in this multi-exploiter system appears to shift populations from either coexistence to extinction depending on the strength of *A. orbigera* predation. Selective predation of the beetle on scales that are larger but coincidentally more likely to become infected may help contain levels of infection below some threshold limit for epizootics. This in turn keeps the population of *C. viridis* healthy enough to sustain the *A. orbigera* population.

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Chapter 2

USE OF STAGE-BASED MATRIX MODELS IN EVALUATING BIOLOGICAL CONTROL AGENTS AND NATURAL ENEMIES

INTRODUCTION

Population projection models using Leslie (age-based) and Lefkovitch (stage-based) matrices are very useful for understanding how population structure, for instance, life stage, age, and fecundity, influences population growth over time (Leslie 1945, Lefkovitch 1965). These methods are traditionally employed in the conservation of endangered and threatened species. In these systems, stage-based population models are used to determine which life stages (eggs, pre-juveniles, juveniles, or adults) have the greatest impacts on population growth, which can help to better inform management decisions. For instance, in the case of Loggerhead sea turtles, a Lefkovitch matrix determined that survival of adult turtles had the strongest influence on total population growth, informing management decisions to concentrate conservation efforts on adult turtles rather than nesting sites (Crouse et al 1987).

The utility of these techniques for conservation is obvious, but somewhat less obvious is their applicability to biological control. Population projection models usually require large data sets that follow individual specimens throughout their entire life cycles, thus limiting their use in conservation. However, this kind of data is incredibly accessible in studies of agricultural insect pests, the subjects of which are infamous for having large population sizes and short life cycles. Many insect pests undergo a series of larval stages before reaching maturity (Bommarco 2001) making the use of stage-based matrix models appropriate and feasible, yet few studies take advantage of this opportunity. Age and stage-based models can determine the "weak points" or "weak stages" in pest life cycles by manipulating vital rates (survival, transition, fecundity) of each stage class, projecting total population size over time, and determining which stage and vital rate is most

sensitive or elastic—causing population growth to fluctuate most. Yet the implications for pest management remain relatively unnoticed; with only a handful of studies applying the techniques to arthropods under laboratory conditions and invasive plant species (Habtewold 1995, Parker, 2000, Shea et al. 2005). This paper seeks to expand the scope of influence for these models to field studies of agricultural pests and their enemies. These simple models can help predict how effective potential biological control agents are prior to release, based solely on the demographics of the pest and knowledge of which life cycle stages the control agent most commonly attacks. For systems with a variety of biological control agents and natural enemies that target different, specific instars, these models can help us choose which agents to deploy and which natural enemies to protect.

The focus of this study is *Coccus viridis*, a parthenogenetic and oviparous homopteran scale insect (Frederick 1943) that is considered a pest in many coffee plantations (Waterhouse 1997). *C. viridis* undergoes three instar molts before reaching maturity, becoming larger and more convex with each additional molt (Frederick 1943). There are several natural enemies of *C. viridis* including *Azya orbigera*, a Coccinellid beetle predator, and *Lecanicillium lecanii*, an entomopathogenic fungus that is available commercially as a biological control agent and occurs naturally in many organic, shade-grown coffee agroecosystems (Singh 1995). *A. orbigera* prefers to consume 3rd and 4th stage instars (Iverson et al. unpublished), and field observations suggest that levels of *L. lecanii* infection are also higher in these instars. But how much does the *C. viridis* population as a whole depend on the survival of these late stage instars, and how does the combined effect of the fungal pathogen and beetle predator on the same stage instars influence total population growth?

METHODS

The parthenogenicity and sessile nature of *C. viridis* make the application of a Lefkovitch matrix model particularly simple and appropriate. All *C. viridis* individuals are female and reproduce asexually to produce female progeny; therefore a standard Lefkovitch model effectively describes the entire population, rather than just half of the

population that is usually described when modeling sexually reproducing organisms. The transition matrix **A:**

$$A=\begin{bmatrix} s_{11} & 0 & 0 & f_4 \\ s_{12} & s_{22} & 0 & 0 \\ 0 & s_{23} & s_{33} & 0 \\ 0 & 0 & s_{34} & s_{44} \end{bmatrix} A \leftarrow \begin{bmatrix} s_{11(1-p_1)} & 0 & 0 & f_4 \\ s_{12(1-p_1)} & s_{22(1-p_2)} & 0 & 0 \\ 0 & s_{23(1-p_2)} & s_{33(1-p_3)} & 0 \\ 0 & 0 & s_{34(1-p_3)} & s_{44(1-p_4)} \end{bmatrix} \quad (2.1)$$

corresponds to the same stage survival (s_{ii}), transition (s_{ii+1}), and fecundity rates (f_i) of the *C. viridis* life cycle (Figure 2.1). To include the effect of *L. lecanii* on the growth of the population, each survival and transition rate was multiplied by the probability of surviving infection $(1 - p_i)$ for each life stage, referred to in this paper as **A'** (2.1).

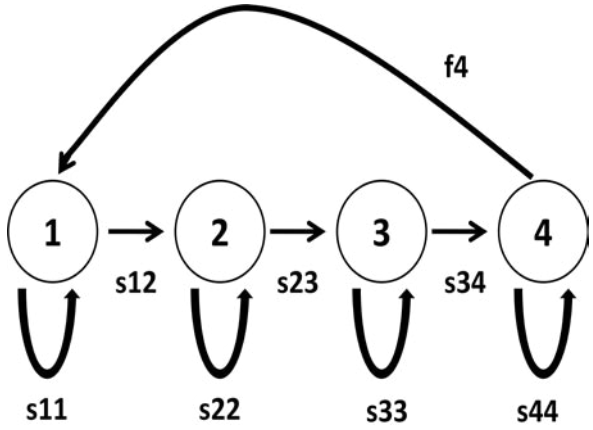


Figure 2.9. Life cycle of *C. viridis*. 1st instar larvae have some rate s_{11} of surviving and staying in the same stage and some rate s_{12} of transitioning to the next stage. This is true for all instars. Stage 4 represents the adult stage with some fecundity rate, f_4 of producing 1st stage larvae.

Transition matrix **A** was multiplied by a vector of initial *C. viridis* numbers for each life stage $\mathbf{N_0}$, to give a new vector of population sizes, $\mathbf{N_1}$. Populations were projected for 100 time steps, with each time step representing 18 days in the life of *C. viridis*. The model is summarized by the following equation:

$$[\boldsymbol{A}] \times \boldsymbol{N_t} = \boldsymbol{N_{t+1}} \quad (2.2)$$

Parameter estimation

The parameters for **A** were estimated using a stock of photographs taken on an organic, shade grown coffee farm named *Finca Irlanda* located in the southern state of Chiapas, Mexico. Photographs were taken of several different *C. viridis* populations (on individual coffee leaves) from bushes of *Coffea arbabica* growing in the same rustic coffee farm plot on two dates: June 9th and June 27th of 2008 (See Moguel and Toledo 1999 for details on coffee management types). In order to estimate rates of survival and transition to the next stage, we analyzed five leaves from two coffee bushes that showed no signs that any scales were infected by *L. lecanii* in the 18 day time period of the photographs (an obvious white halo of mycelia is characteristic of infection). Infection is included later as a separate parameter so that it can vary independently of natural survival and transition rates (2.1). Owing to their sessile nature, individual *C. viridis* specimens from each of the four stage classes were easily tracked from one time period to the next. The average same stage survival and transition rates for each stage class were calculated and incorporated into transition matrix **A**. Probability of infection for each life stage (p_1, p_2, p_3, p_4) was estimated by taking the average number of scales infected with *L. lecanii* from 17 leaves and included in **A'**. Average population sizes for 1st-4th stage *C. viridis* were calculated from the same dataset and used to parameterize the initial population vector $\mathbf{N_0}$ (Table 2.1). Fecundity was estimated at 85 eggs for each adult 4th stage *C. viridis* based on available literature sources (Frederick 1943).

Table 2.1. *C. viridis* population demographics

Instar	N	P_i
1	315	0.02
2	56	0.22
3	29	0.39
4	29	0.34

N= average population sizes for each instar (i), P_i= average proportion of scales infected with *L. lecanii* from 17 populations of *C. viridis.*

Sensitivity analysis

The asymptotic population growth of *C. viridis* was calculated from the dominant eigenvalue of transition matrices **A** and **A'**, termed λ (Caswell 1989). To determine "weak stages" in the *C. viridis* life cycle we examined matrix elasticities, which measure how sensitive λ is to proportional changes in matrix coefficients (vital rates) (See Caswell 1989 for detailed explanation and mathematical notation). To separate the effects on λ from infection-induced mortality and predation-induced mortality, vital rates calculated from infection free leaves were adjusted to remove mortality such that all scale insects in a given stage class either survive and remain in their stage class or survive and transition to the next stage class i.e.) $s_{11}+s_{12}=1$ (Table 2.2). This effectively removes predation from the system. By calculating the matrix elasticities in the absence of predation we can examine how infection alone influences λ. In addition, we can manually reduce vital rates as a proxy for predation on each stage class, thus allowing us to visualize how instar-specific predators would affect λ in the presence of *L. lecanii*.

Stochasticity

In order to incorporate stochasticity into the model, each of the transition rates were parameterized as random numbers pulled from a Beta distribution with values a and b derived from the averages and variances of the parameters used in the static transition matrix **A** post adjustment to remove predation (a', b', Table 2.2). Same stage survival rates were made equal to 1-transition rates. The resulting vital rates were multiplied by the probability of surviving infection (2.1) and plotted against lambda for 100 random runs. Adult fecundity was also randomized (negative binomial distribution with size=85, and μ=85). Effect of predation was tested on each instar by multiplying the same stage survival and transition rate of that instar by values ranging from 0 to 1 in 0.01 increments; the probability of surviving predation. Adult fecundity was also tested by varying the parameter from 0 to 1000 in increments of 10.

Table 2.2. Vital rate parameter estimation							
	s_{11}	s_{12}	s_{22}	s_{23}	s_{33}	s_{34}	s_{44}
μ **- Original**	5.73E-01	1.14E-01	6.03E-01	5.97E-02	2.54E-01	4.42E-01	8.64E-01
o^2	6.74E-02	3.80E-02	2.71E-03	7.42E-03	6.62E-02	7.79E-03	1.26E-02
a	3.90E-01	9.47E-02	6.76E-02	2.60E-02	2.01E-01	8.59E-02	3.33E-01
b	2.90E-01	7.33E-01	4.45E-02	4.09E-01	5.91E-01	1.08E-01	5.25E-02
μ' **- No Mortality**	7.29E-01	2.71E-01	7.72E-01	2.28E-01	4.06E-01	5.94E-01	1.00E+00
$o^{2\prime}$	4.30E-02	4.30E-02	1.13E-03	1.13E-03	2.76E-02	2.76E-02	0.00E+00
a'	4.29E-01	1.59E-01	6.45E-02	1.91E-02	1.62E-01	2.38E-01	--
b'	1.59E-01	4.29E-01	1.91E-02	6.45E-02	2.38E-01	1.62E-01	--

Parameters for μ are derived from mean survival and transition rates of *C. viridis* on *L. lecanii* free leaves. Mortality in this dataset is likely due to predation. μ' values were adjusted to remove this source of mortality, so that the sum of survival and transition rates of each instar always equals 1. Beta distribution values a and b were calculated from μ and σ*:* $\boldsymbol{\mu = E(X) = \frac{a}{a+b}}$; $\boldsymbol{Var(X) = E(X-\mu)^2 = \frac{ab}{(a+b)^2(a+b+1)}}$. Note that S_{44} has no complementary transition rate, thus is always equal to 1 in the absence of predation.

RESULTS

Static transition matrix **A** using μ values (no effect of *L. lecanii*, effect from natural predation rates) produced a lambda of 1.32. Elasticity was highest for adult same stage survival s_{44} (2.3)

$$\begin{array}{c} \\ [1,] \\ [2,] \\ [3,] \\ [4,] \end{array} \begin{array}{c} \begin{array}{cccc} [,1] & [,2] & [,3] & [,4] \end{array} \\ \begin{bmatrix} 0.099 & 0.000 & 0.000 & 0.129 \\ 0.129 & 0.109 & 0.000 & 0.000 \\ 0.000 & 0.129 & 0.031 & 0.000 \\ 0.000 & 0.000 & 0.129 & \mathbf{0.246} \end{bmatrix} \end{array} \quad (2.3)$$

Elasticity matrix for transition matrix **A** based on parameters derived from infection free leaves (μ). Top row indicates fecundity rates for the different instars (s_{11}, f_2, f_3, and f_4). Matrix element [2,1] = transition rate from instar 1 to 2 (s_{12}), [2,2] = same stage survival of instar 2 (s_{22}), etc. Bold print indicates highest elasticity value (s_{44}).

Including infection from *L. lecanii* using static transition matrix **A'** with μ values (effect of *L. lecanii*, effect from natural predation rates) caused lambda to decrease to 1.05. Elasticity remained highest for s_{44}, although elasticity for s_{11} increased dramatically from 0.099 to 0.157. Instars with high probabilities of infection had decreased elasticity

values, while those with low probabilities of infection (Table 2.1) had increased values (2.4).

$$\begin{array}{c} \\ [1,] \\ [2,] \\ [3,] \\ [4,] \end{array} \begin{array}{c} \begin{array}{cccc} [,1] & [,2] & [,3] & [,4] \end{array} \\ \begin{bmatrix} 0.157 & 0.000 & 0.000 & 0.136 \\ 0.136 & 0.111 & 0.000 & 0.000 \\ 0.000 & 0.136 & 0.024 & 0.000 \\ 0.000 & 0.000 & 0.136 & \mathbf{0.163} \end{bmatrix} \end{array} \quad (2.4)$$

Elasticity matrix for transition matrix **A'** based on parameters derived from infection free leaves (μ). Top row indicates fecundity rates for the different instars (s_{11}, f_2, f_3, and f_4). Matrix element [2,1] = transition rate from instar 1 to 2 (s_{12}), [2,2] = same stage survival of instar 2 (s_{22}), etc. Bold print indicates highest elasticity value (s_{44}).

When μ' values were used in **A** (no effect from *L. lecanii* or predation), lambda increased to 2.07. Elasticity of s_{44} decreased, and reached a high of 0.158 in all transition rates and adult fecundity (2.5).

$$\begin{array}{c} \\ [1,] \\ [2,] \\ [3,] \\ [4,] \end{array} \begin{array}{c} \begin{array}{cccc} [,1] & [,2] & [,3] & [,4] \end{array} \\ \begin{bmatrix} 0.086 & 0.000 & 0.000 & \mathbf{0.158} \\ \mathbf{0.158} & 0.094 & 0.000 & 0.000 \\ 0.000 & \mathbf{0.158} & 0.039 & 0.000 \\ 0.000 & 0.000 & \mathbf{0.158} & 0.148 \end{bmatrix} \end{array} \quad (2.5)$$

Elasticity matrix for transition matrix **A'** based on parameters derived from infection free leaves (μ). Top row indicates fecundity rates for the different instars (s_{11}, f_2, f_3, and f_4). Matrix element [2,1] = transition rate from instar 1 to 2 (s_{12}), [2,2] = same stage survival of instar 2 (s_{22}), etc. Bold print indicates highest elasticity values (f_4, s_{12}, s_{23}, s_{34}).

Applying μ' values to **A'** (effect from *L. lecanii*, no effect from predation) gave a lambda of 1.67. The elasticity of all transition rates and adult fecundity increased to 0.163 (2.6).

$$\begin{array}{c} \\ [1,] \\ [2,] \\ [3,] \\ [4,] \end{array} \begin{array}{c} \begin{array}{cccc} [,1] & [,2] & [,3] & [,4] \end{array} \\ \begin{bmatrix} 0.121 & 0.000 & 0.000 & \mathbf{0.163} \\ \mathbf{0.163} & 0.092 & 0.000 & 0.000 \\ 0.000 & \mathbf{0.163} & 0.028 & 0.000 \\ 0.000 & 0.000 & \mathbf{0.163} & 0.107 \end{bmatrix} \end{array} \quad (2.6)$$

Elasticity matrix for transition matrix **A'** based on adjusted parameters (μ'), removing predation-induced mortality. Top row indicates fecundity rates for the different instars (s_{11}, f_2, f_3, and f_4). Matrix element [2,1] = transition rate from instar 1 to 2 (s_{12}), [2,2] = same stage survival of instar 2 (s_{22}), etc. Bold print indicates highest elasticity values (f_4, s_{12}, s_{23}, s_{34}).

By varying each vital rate in **A'**, μ' from 0 to 1 (0-1000 for f_4) while holding all other rates constant, we discovered that only decreases in transition rates and fecundity cause λ

to drop below 1 (Figure 2.2). This corresponds with the high elasticity values for all transition rates and adult fecundity. Given a standard change across all vital rates, s_{23} appears to effect λ most severely; however, λ will drop below 1 at a higher value for fecundity and vital rate s_{34} than any other vital rates.

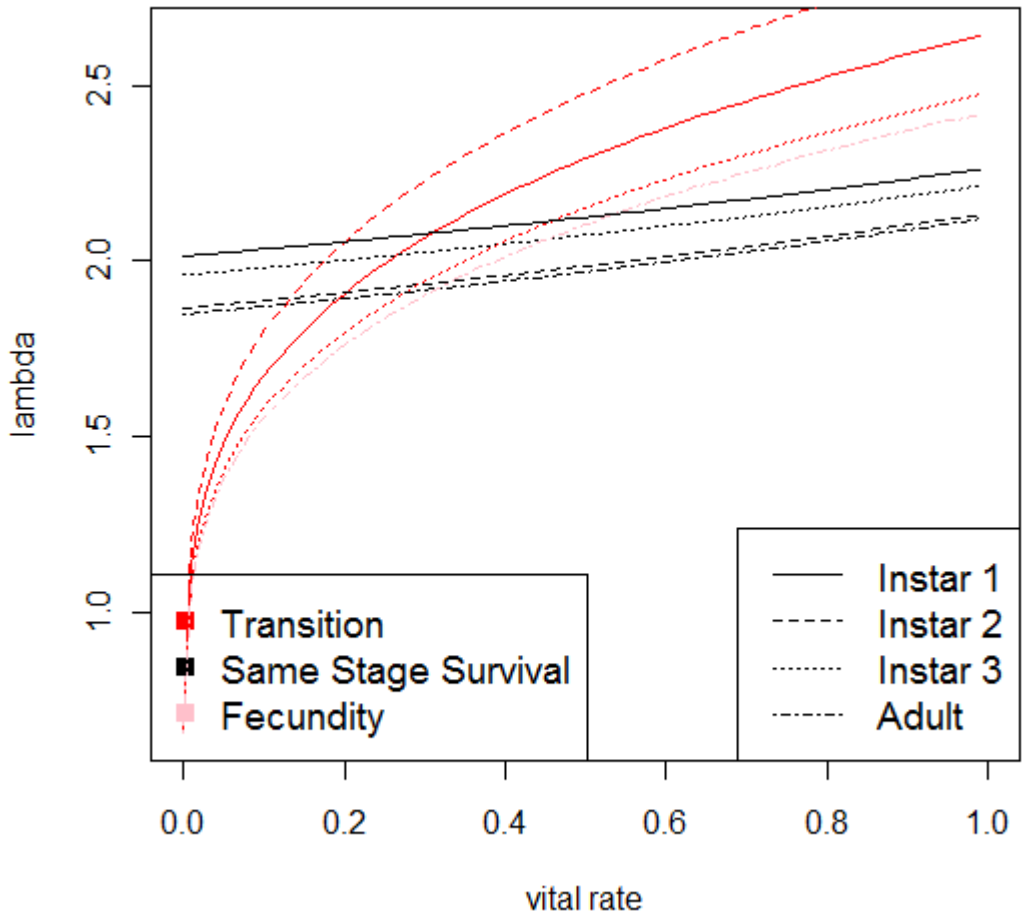


Figure 2.10. Varying vital rates from transition matrix A' with adjusted μ' values to simulate predation. Low transition and fecundity rates drive lambda below 1. X axis for adult fecundity varies from 0 to 1000.

Stochastic runs (n=100) of **A** based on μ' values produced an average lambda of 1.18. Including *L. lecanii* with **A'** based on μ' values decreased the average lambda to 1.03. There is a strong, generally positive relationship between same stage survival of instar 1 and lambda (Figure 2.3).

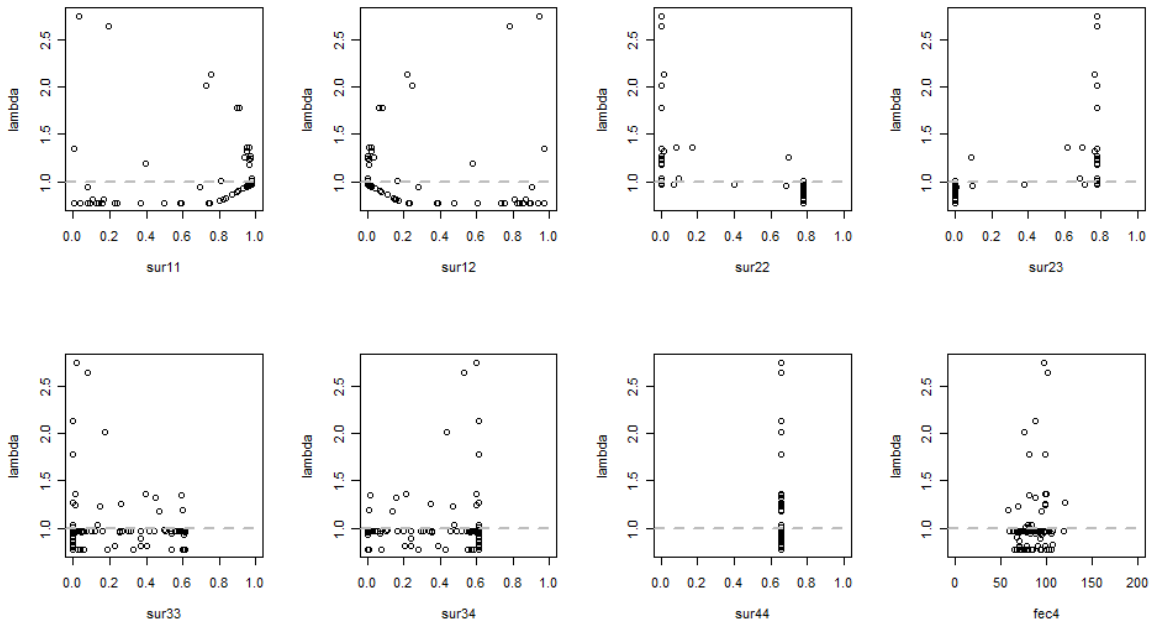
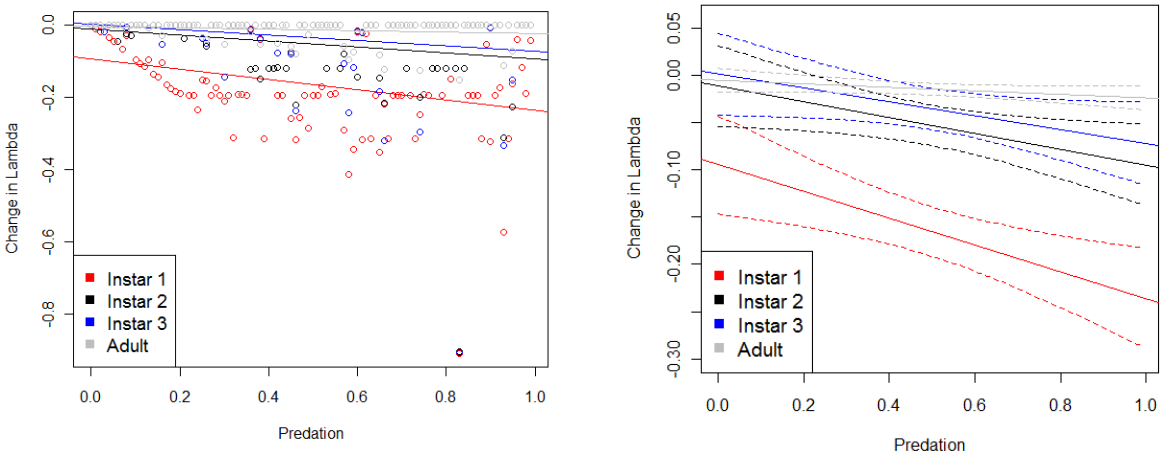


Figure 2.11. Lambda results for stochastic vital rates based on μ' reduced by probability of infection. Note s_{44} does not vary since there is no transition state for adults and predation was removed from the system. Grey dotted line represents cutoff between population growth and decline (λ=1). High s_{11} values may increase λ.

Predation on instar 1 has the greatest negative effect on lambda (m= -0.095, R^2=0.08, p= 0.003), followed by instar 2, 3, and adult *C. viridis* (Figure 2.4, 2.5).



Figures 2.4, 2.5. Change in lambda due to reduction in vital rates from predation vector. Dotted lines represent 95% confidence intervals. At low predation rates, lambda values do not differ significantly across instars. However, as predation on each instar increases, the effect on instar 1 decreases lambda to a significantly greater extent than predation on any other instar.

These results correspond with high elasticity values for s_{11} compared to all other vital rates when averaging results from 100 random runs (Figure 2.6).

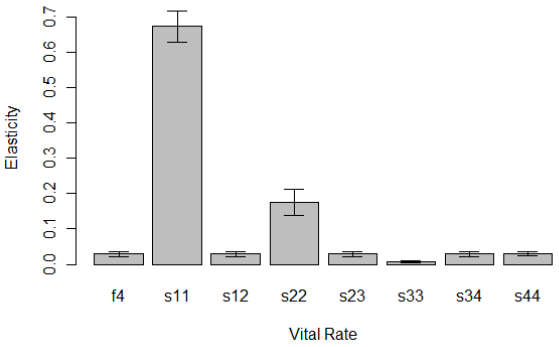


Figure 2.6. Elasticity results for stochastic vital rates based on μ' reduced by probability of infection. Elasticity measures fluctuation in λ given proportional changes in vital rates.

DISCUSSION

Conditions in the field

Our results suggest that in the absence of *L. lecanii*, natural predation rates do not effectively limit *C. viridis* population growth (λ=1.32). When the effect of *L. lecanii* is subsequently included, the *C. viridis* population ceases to grow geometrically, stabilizing at a λ of 1.05. *L. lecanii* is likely an important check on the population growth of *C. viridis*. Any additional attacks on adult *C. viridis* may cause λ to drop below 1 since all simulations with natural predation rates result in high elasticity values for adult survival (s_{44}). Current levels of control from predators and *L. lecanii* appear to keep populations of *C. viridis* in check; however any further predation on *C. viridis*, especially adult *C. viridis*, may cause the entire system to collapse. Decreasing probability of *L. lecanii* infection (Table 2.1) generally increases elasticity of same stage survival rates. Thus, attacks on instars that are generally unaffected by *L. lecanii* may decrease population growth most severely.

Understanding the roles of predation and infection

Removing *L. lecanii* and natural predation rates causes rapid population growth (λ=2.07), and redistributes elasticities so that f_4, s_{12}, s_{23}, and s_{34} are equally important in population control. Including *L. lecanii* with predation removed decreases population growth to 1.67 with elasticities remaining evenly distributed across f_4, s_{12}, s_{23}, and s_{34}. Thus, *L. lecanii* alone cannot limit *C. viridis* population growth. Since including natural predation rates causes the elasticity of s_{44} to increase above all other vital rates, predation on 1^{st}-3^{rd} stage larvae must be strongest in the field.

Decreasing any single transition or adult fecundity rate to a low enough level can cause the *C. viridis* population to decline (Figure 2.2), however, this near zero level is an unrealistic target for any single biological control agent. Combined predation on several instars as in the natural predation parameters derived from μ make for a much more effective and feasible control strategy. However, removing all predation helps us to understand which instars to focus attacks given established probabilities of *L. lecanii* infection for each instar. Our results show that less pressure on instar 3 is required than instar 1, followed by instar 2 to cause the same degree of decline in λ (Figure 2.2). Thus, if resources are limited, smaller attacks on instar 3 could be as effective as larger attacks on instar 1 and 2. Adult fecundity requires the smallest reduction to control population growth. However, this depends largely on the arbitrary range of values used for fecundity since fecundity need not vary from 0 to 1 as all transition and survival rates do. If the range is shortened, the degree to which fecundity must be reduced to attain control may appear greater.

Effects of stochasticity

Stochastic models excluding predation and infection result in overall population growth with an average λ of 1.18. Surprisingly, when *L. lecanii* is included in these stochastic models, the system re-stabilizes to a λ of 1.03. Thus, given natural fluctuations in vital rates, *L. lecanii* has the potential to control *C. viridis* even in the absence of all predators. Predation on instar 1, which has the highest average elasticity value (Figure

2.6) causes the greatest decline in λ (Figures 2.4,2.5), suggesting that targeting of instar 1 in a variable environment would cause the *C. viridis* population to decline dramatically. This is particularly interesting considering the fact that 1st stage instars are particularly resistant to infection from *L. lecanii*. Our results suggest that the control provided by *L. lecanii* on 3rd and 4th stage instars may heighten the importance of unaffected instars for overall population growth, supporting the notion that species complementarity provides effective biological control (Straub 2006).

Real world implications

Current levels of predation and infection appear effective in controlling the *C. viridis* population. Predation on the 1st-3rd instars of *C. viridis* limits population growth; achieved through a diversity of predators and other exploiters known to exist in the system (Vandermeer et al. 2010). Shade trees provide a diversity of habitats that encourage diversity in the insect community, which in turn provides effective biological control (Perfecto et al. 2011). *Azya orbigera* is known to attack mostly 3rd and 4th stage instars, which suggests that other predators are also, if not more important in limiting population growth. Another Coccinellid beetle in the genus *Diomus* was recently discovered (Iverson et al., unpublished) to attack mainly 1st and 2nd stage *C. viridis*. Stochastic simulations support the notion that *C. viridis* population growth depends greatly on the survival of its 1st stage instars. Thus, predation by *Diomus* may play an important, if not the main role in natural population control for this system. The natural history of *Diomus* is still largely unknown, but deserves exploration given the results of our study. Efforts should be made to maintain healthy populations of *Diomus* along with the suite of predators, parasitoids, and fungi that attack *C. viridis*.

Considering the stability of the system as it stands, introducing new biological control agents is unnecessary. In fact, introduction of new control agents that attack 4th stage instars could completely destroy the *C. viridis* population and cause large trophic cascades (Finke and Denno 2004). Although the parameters for this model were derived from a very specific plot of land in Chiapas, Mexico, general principals are easily extrapolated. In systems where *C. viridis* is a problem, our study suggests that a diversity

of natural enemies can provide effective control, especially those that attack earlier instars. Although life cycles could feasibly differ between populations of *C. viridis*, these differences are likely minimal considering basic growth restrictions. Including stochasticity in our model helps to account for some of this variation. With this in mind, other systems may experience dramatic declines in *C. viridis* if a focused attack on 1st stage instars is combined with stochastic events and infection from *L. lecanii*.

Stage-based matrix models in context

Stage-based matrix models are very useful but underused tools for understanding the population dynamics of important agricultural pests. By applying stage-based matrix models to a field study of *C. viridis*, we were able to determine that the population growth of the potential pest was under control, and thus not a threat. We were also able to elucidate which instars contributed most to population growth and make hypotheses as to which natural enemies were most responsible for keeping the population under control. Results indicate that attacks on several different life stages of the pest are currently controlling the growth of the pest, thus a variety of exploiters are probably responsible for control. Such results supports the claim that biodiversity provides effective natural control, and can help inform management decisions to maintain diversity on their farms, which in this case may take the form of a variety of shade trees. This information is valuable for implementing effective biological control programs in agricultural systems where *C. viridis* is considered a major economic threat. For these systems, our results on which natural enemies contribute most to control can help start effective biological control programs that encourage the growth and maintenance of these particular beneficials.

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Chapter 3

SYNTHESIS FOR BIOCOMPLEXITY: THEORY AND PRACTICE

INTRODUCTION

For political reasons, at the advent of the green revolution, public perceptions of agriculture were swayed to view the farm in terms of a battlefield where farmers waged war against their metaphorical enemies; agricultural pests (Russell 2001). Chemical pesticides, developed originally for biological warfare, were employed as weapons against these enemies (Russell 2001). To win the war, farmers needed to apply large sums of pesticides, which had unfortunate side effects on the environment, ecological networks, and human health (Pimentel 1992, Hayes 1982). In order to avoid these problems, biological control agents in the form of predators, parasites, and diseases of the pest were released in the hopes of controlling pests naturally. However, in several cases, introduction of a control agent caused severe and often times irreversible changes to an ecosystem through the unintended establishment and spread of the agent as an invasive species (eg. cane toads, Howarth 1983).

Both the deployment of specialist agents and use of synthetic pesticides were developed as "magic bullet" solutions to destroy enemy populations. However, by taking a more holistic approach to agriculture by viewing the farm as a delicate system of checks and balances between pests and their natural enemies, biocomplexity through biodiversity presents itself as an alternative resolution to the problem of pest control. The goal of control using biocomplexity is not to completely eradicate a pest, but instead to maintain the population below threshold levels through constant but constrained sources of exploitation from several natural enemies. Competition and indirect non-linear ecological interactions among natural enemies act to contain the spread of the control

agents themselves, thus lessening the risk of invasion, while also forcing coexistence of all members in the multi-exploiter system.

Results from previous chapters provide evidence that biocomplexity is helping to maintain populations of *C. viridis* below threshold levels, with observations hinting to the importance of intraguild predation in providing the necessary checks between competing natural enemies. In order to explore how such non-linear interactions help maintain coexistence, we developed a specific theoretical argument showing how intraguild predation of a generalist pathogen by a specialist predator can rescue the predator from extinction when pathogen virulence is high.

METHODS

The system is modeled as a series of three ordinary differential equations based on modified Lotka-Volterra predator-prey (or exploiter-victim) equations. The specialist predator A, modeled after *A. orbigera,* grows as a logistic function of the victim population size S (for scale insects) with saturation constant x_1, modified by its attack rate a_1, and mortality rate m_1 (3.1). The second exploiter L, a generalist pathogen modeled after *L. lecanii,* grows logistically at some natural rate of increase r_2 to its carrying capacity k_2 on a source other than the victim species S. The pathogen also increases according to a Holling type III functional response with saturation constant x_2 when p=2; the sigmoid curve that results representing a critical density of host species required for infection to spread (3.2). The pathogen attacks at a rate a_2, but is limited by a parameter representing the strength of intraguild predation *I*, such that when infection levels are high in the victim species, the specialist predator is forced to consume infected prey, thus reducing the attack rate of the pathogen (3.2). The victim grows logistically at some rate r to its carrying capacity k, limited also by the effect of the specialist predator and generalist pathogen on its population (3.3).

$$\frac{dA}{dt} = \frac{a_1 AS}{x_1+S} - m_1 A \qquad (3.1)$$

$$\frac{dL}{dt} = r_2 L \times \left(1 - \frac{L+S}{k_2+S}\right) + \frac{\left(\frac{a_2 LS^p}{1+I\frac{L}{A}}\right)}{x_2+S^p} - m_2 L \qquad (3.2)$$

$$\frac{dS}{dt} = rS \times \left(1 - \frac{S}{k}\right) - \frac{a_1 AS}{x_1 + S} - \frac{\left(\frac{a_2 LS^p}{1 + I\frac{L}{A}}\right)}{x_2 + S^p} \qquad (3.3)$$

Using these equations, we experimentally manipulated the system to create a situation where in the absence of intraguild predation (*I*=0), high virulence results in the extinction of the specialist predator. At this point, intraguild predation was added to see if we could "rescue" the lost predator.

RESULTS

As we increased the effect of intraguild predation on the attack rate of the generalist predator, the specialist predator was rescued leading to coexistence of all three components of the system. Bifurcation plots of parameter *I* demonstrate how increasing the effect of intraguild predation on the attack rate of the generalist pathogen affects the population dynamics of the specialist predator (Figure 3.1).

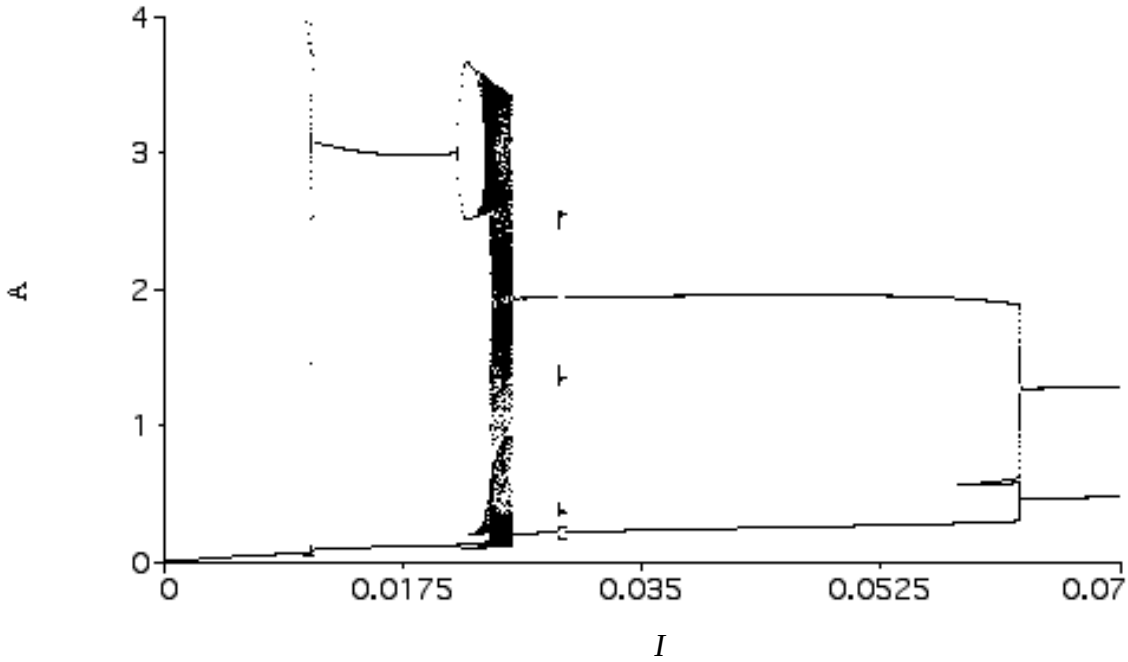

Figure 3.12. Bifurcation plot for the effect of intraguild predation *I* on population size of the specialist predator A. Parameter values: r = 0.129, r_2 = 0.001, k = 15, k_2 = 1, a_1 = 0.2, a_2 = 0.02, x_1 = 2, x_2 = 0.01, m_1 = 0.01, m_2 = 0.009, p = 2. All transients excluded by discarding first 300,000 iterations.

When the effect of intraguild predation is strong, a stable two point limit cycle immerges, but as this effect is reduced, the size of the cycle increases (Figure 3.2) followed by

chaotic window (Figure 3.3), a brief two point cycle interlude (Figure 3.4) and finally extinction of the specialist predator (Figure 3.1).

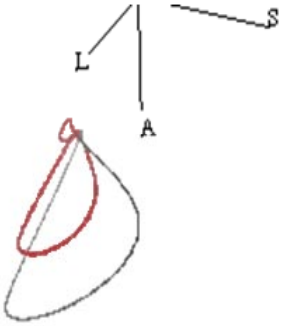

Figure 3.13. 3-D phase portrait showing an increase in the size of the limit cycle as the effect of intraguild predation is reduced. L = generalist pathogen, S = victim species, A = specialist predator. Smaller red limit cycle at high levels of intraguild predation: $I = 0.07$. As intraguild predation is reduced to $I = 0.035$, limit cycle increases in size (black lines). Parameter values are the same as those used to derive Figure 3.1.

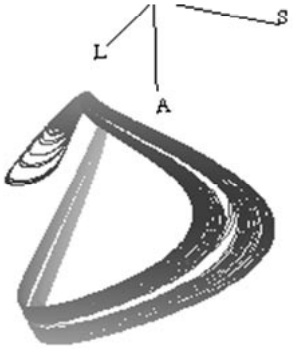

Figure 3.14. Chaotic attractor shifting between two stable manifolds at low levels of intraguild predation. L = generalist pathogen, S = victim species, A = specialist predator, I = 0.0235.

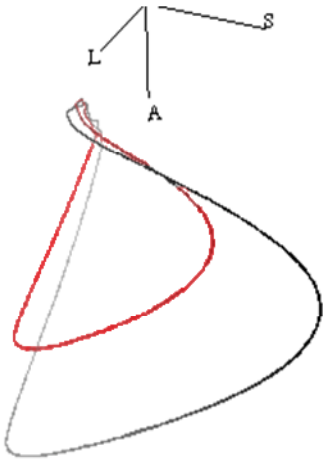

Figure 3.15. Phase portrait showing limit cycles growing in size towards extinction as intraguild predation decreases to 0. L = generalist pathogen, S = victim species, A = specialist predator. Red lines represent I = 0.16, black lines for I = 0.01. Parameter values are the same as those used to derive Figure 3.1.

The multi-exploiter system functions as a coupled oscillator producing at least five distinct manifolds, which alternate in stability as the effect of intraguild predation is reduced. The largest of the manifolds, although stable, grows so large as to cause extinction of the predator (Figure 3.1). As intraguild predation is reduced, a switch between manifolds occurs; producing a chaotic attractor (Figure 3.2) typical of most coupled oscillators. As the effect of intraguild predation is increased, oscillation size decreases leading ultimately to a robust 2 point limit cycle at values of *I* greater or equal to 0.07.

By including late transients (discarding values after 3000 iterations), complex behaviors immerge as trajectories switch between different manifolds (Figure 3.3).

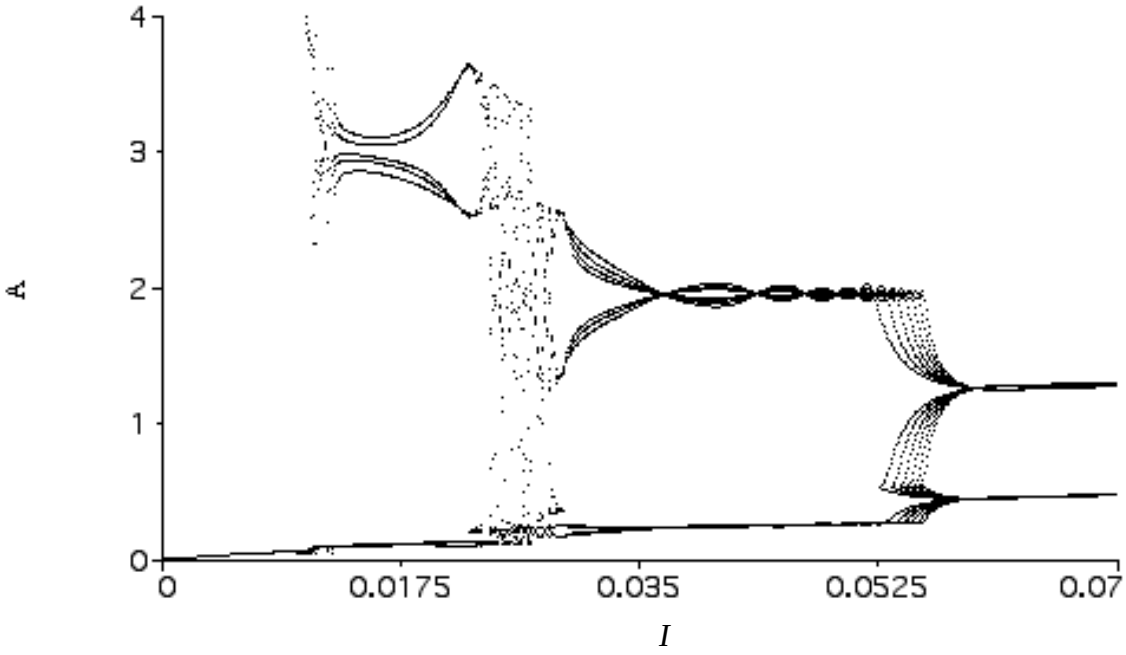

Figure 3.16 Complex behavior arising from late transients. Bifurcation of parameter *I* by the population of specialist predator A. Parameter values: r = 0.129, r_2 = 0.001, k = 15, k_2 = 1, a_1 = 0.2, a_2 = 0.02, x_1 = 2, x_2 = 0.01, m_1 = 0.01, m_2 = 0.009, p = 2. Latent transients included by discarding only the first 3,000 iterations.

DISCUSSION

Theoretical analysis of our model shows that increasing the effect of intraguild predation produces characteristic increases in the lengths of limit cycles followed by

chaos and ultimately extinction of the predator. Given the commonality of these dynamic behaviors and the prevalence of coupled oscillators in nature (Strogatz 1993), findings of chaotic and highly periodic population cycles may actually represent harbingers of an impending extinction event. The ability to forecast such an event is invaluable for studies ranging from conservation, invasive species, and for our purposes, biological control.

Our results indicate that intraguild predation can lead to coexistence in multi-exploiter systems mediated through dynamic, indirect effects between natural enemies. Increasing intraguild predation by one exploiter limits the attack rate of the second exploiter, acting as a natural check and balance system, which keeps the victim species at levels high enough to sustain both exploiters yet low enough to avoid pest status. These results support the use of biocomplexity through biodiversity as an effective pest control strategy. Intraguild predation, and potentially, many other non-linear indirect ecological effects, help to maintain the coexistence of multiple exploiters in the system. Coexistence of multiple exploiters is necessary for providing effective and continuous control of pests at low levels, but may also help in preventing the escape and invasion of the control agents themselves.

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