

PARASITOID COEXISTENCE: LINKING SPATIAL FIELD PATTERNS WITH MECHANISM

ELIZABETH T. BORER,¹ WILLIAM W. MURDOCH, AND SUSAN L. SWARBRICK

Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, California 93106 USA

Abstract. Coexistence of resource specialists can be maintained through various spatial mechanisms, each potentially inducing different resource dynamics. We used observational data to suggest plausible spatial coexistence mechanisms for *Aphytis melinus* and *Encarsia perniciosi*, two parasitoids of California red scale. *Aphytis* consistently produced more progeny from scale insects on leaves, and *Encarsia* produced progeny equally from scale insects on leaves and stems. To investigate the mechanism inducing this pattern, we performed field experiments to test two possible explanations. In the absence of substrate choice and at a given abundance of scale, the number of progeny produced by each species was the same on stems as on leaves. When given a choice, *Encarsia* had no substrate preference, while *Aphytis* preferred scale insects on leaves, producing results qualitatively similar to the field observations. Ecological theory suggests that this form of habitat preference may facilitate coexistence. Although this mechanism of coexistence is clearly compatible with biological control by multiple natural enemies in the red scale system, theory dealing with this type of interaction has not thoroughly examined the predictions for resource dynamics.

Key words: *Aphytis melinus*; behavior; biological control; California red scale; *Encarsia perniciosi*; parasitoid coexistence; resource segregation.

INTRODUCTION

Ecological theory has shown that stable coexistence of two resource specialists on a limiting resource requires that intraspecific competition be stronger than interspecific competition. Stronger intraspecific competition can be induced spatially via various mechanisms such as resource partitioning (e.g., MacArthur 1958), aggregated resource use (e.g., Atkinson and Shorrocks 1981, Ives 1991), or by dispersal of the poorer competitor to uninhabited patches in the landscape (Levins and Culver 1971, Hastings 1980).

For several reasons, successful biological control communities provide model systems for developing a detailed understanding of both species interactions and mechanisms of coexistence. First, species introduced as biological control agents generally are resource specialists that specialize on the pest species. Second, biological control communities are often composed of only a few strongly interacting species (Takagi and Hirose 1994, Pedata et al. 1995). Third, when biological control is successful, natural enemies maintain the pest at low levels and therefore limit it through predation (or herbivory). Finally, in most successful biological control systems in which the resource is limited, multiple, albeit few, natural enemies coexist (Argov and Rossler 1993, Takagi and Hirose 1994, Pedata et al. 1995, Murdoch et al. 1998).

California red scale is an insect pest of citrus that has been maintained under successful biological control by multiple coexisting parasitoids for nearly half a century (DeBach and Sundby 1963). In many citrus groves, including our study groves, two parasitoids coexist. These parasitoids have reduced their shared red scale resource to levels nearly 200 times lower than the average density set by its resources (DeBach et al. 1971), suggesting potentially strong resource competition between parasitoid species.

Here, we evaluate the evidence for the most important spatial and temporal mechanisms by which the two parasitoids coexist on California red scale. This manuscript has the following structure. We begin with a brief introduction to the biology of the species (*Natural history*). The next section (*Field observations*) is an analysis of two long-term observational data sets consisting of field-collected juvenile parasitoids across multiple spatial scales through time in which we examine the broad spatial and temporal patterns of parasitism. We use these long-term data sets to suggest the most important scales for further, experimental, investigation. Our observational work shows that the only strong and consistent pattern is numerical dominance by one parasitoid species in scales on leaves but similar juvenile abundances of both species in scales on stems. The following sections of the manuscript (*Methods, Results*) present two field experiments in which we mechanistically examine the patterns found in the observational data. In these sections, we present experiments designed to determine whether the heterogeneous distribution of the parasitoid species across

Manuscript received 16 September 2002; revised 14 July 2003; accepted 28 July 2003. Corresponding Editor: A. R. Ives.

¹ E-mail: borer@nceas.ucsb.edu

stems and leaves can be attributed to differences in scale quality between substrates or behavioral preferences that differ between the two parasitoid species.

NATURAL HISTORY

Aonidiella aurantii (Mask.), California red scale, is a homopteran armored scale that was introduced accidentally into the United States in the late 1800s (Clausen 1978). This citrus pest can infest all aboveground parts of a tree, though the densities of red scale are often far higher in the scaffolding of a tree than on the leaves and fruit (Murdoch et al. 1989). Ebeling (1959) and Bodenheimer (1951) provide details of the red scale biology. Juvenile females pass through three instars and two molts before maturing. Males have two instars followed by a pupal stage, and they emerge as winged adults concurrent with the female third instar. Red scale has ~3 generations per year. Red scale individuals grow in diameter with increasing age, and their size is dependent on the quality of the substrate on which they settle (Walde et al. 1989, Hare et al. 1990).

Aphytis melinus (DeBach) is a hymenopteran ectoparasitoid introduced into California in 1956 to control red scale. Rosen and DeBach (1979) describe *Aphytis*' life history. In brief, *Aphytis* can produce progeny from second- or third-instar scale, but it rarely attacks scales during either molt or mature scale stages (Yu and Luck 1988). Among vulnerable scales, *Aphytis* prefers scales larger than ~0.39 mm² (small third-instar scales), particularly for producing female progeny (Luck and Podoler 1985). There is some evidence that *Aphytis* uses color-based searching and searches on leaves and fruit rather than on the structural parts of the tree (Gregory 1985). *Aphytis* begins its adult life with only a partial complement of eggs, and produces approximately four eggs each day after protein meals acquired by feeding on red scale (Collier 1995).

DeBach and Sundby (1963), Yu et al. (1990), McClain et al. (1990a), and Baroffio (1993, 1997) describe the biology and ecology of *Encarsia perniciosi* (Tower), a hymenopteran endoparasitoid on red scale, introduced for biological control in 1949. In contrast to *Aphytis*, *Encarsia* can produce progeny from all juvenile stages of red scale except the very smallest first instars, although it appears to prefer small second-instar scales for oviposition (Yu et al. 1990). There is some evidence from color traps that *Encarsia*, like *Aphytis*, may use a color-based search strategy but, unlike *Aphytis*, chooses to search on the structural parts of the tree (McClain et al. 1990b). *Encarsia* begins its adult life with a full egg complement of ~32 eggs (E. T. Borer, unpublished data), and does not feed on red scale as an adult. Although *E. perniciosi* can attack multiple scale species, California red scale is the only resource available in our study area.

When both *Aphytis* and *Encarsia* eggs are laid in the same scale, the juvenile *Aphytis* generally consumes

the juvenile *Encarsia*. In all cases, only one parasitoid species emerges from the contested scale. Thus, depending on the rate of multiparasitism, field data on developing juveniles may underestimate the actual parasitism rate of *Encarsia*; however these data provide a good estimate of adult parasitoid recruitment for both species.

FIELD OBSERVATIONS

Methods

We collected data on the distribution and abundance of *Encarsia*, *Aphytis*, and red scale on grapefruit trees near Fillmore, California in 1984–1985 and 1987–1989. Sampling methods for these studies are described in Murdoch et al. (1989, 1995). Although data were collected during both studies on a large number of manipulated and unmanipulated trees, here we analyze the field collections of *Aphytis* or *Encarsia* juveniles in red scale insects sampled from only unmanipulated trees used as experimental controls. These data include information on the total number of scales and presence of developing *Aphytis* and *Encarsia* juveniles in samples from leaf, stem (photosynthetic), and wood (non-photosynthetic) collected from trees at randomly designated locations throughout the grove. The samples from the 1984–1985 study comprised 16 trees sampled on 32 dates, while the 1987–1989 study samples comprised 10 trees sampled on 25 dates. Although these data have been previously analyzed to examine population regulation of the red scale (Murdoch et al. 1989, 1995), they have never been analyzed to examine parasitoid coexistence.

To examine patterns in the field observational data, we arcsine-square-root transformed the proportion of juveniles that were *Aphytis* ($\arcsin[\sqrt{Aphytis/(Aphytis + Encarsia)}]$) and used linear regression to determine whether there was evidence in either study for spatial (by sample tree or substrate) or temporal (by sample date) variability of juvenile parasitoids in unmanipulated citrus trees under field conditions. We also examined the substrate \times tree and substrate \times date interactions. (Some tree-date combinations dropped out of the analyses due to lack of parasitism.) We treated tree as a random effect in these models and nested tree within date to account for repeated measurements on multiple dates. All data manipulation and analyses were performed in SAS (SAS Institute 2001).

Results of field observations

The proportion of juvenile *Aphytis* differed among dates, among trees within dates, and was different between substrates (leaves as compared to stems) (Table 1). The mean squares values in Table 1 provide a quantitative partitioning of the variance in this proportion, suggesting that the proportion of juvenile *Aphytis* varied most strongly between leaves and stems, and was more similar among dates and trees in a grove.

TABLE 1. Results of ANOVA for the effect of date, tree within date, substrate, and their interactions on proportion of juvenile *Aphytis*.

Factor	1984–1985 study				1987–1989 study			
	df†	MS	F	P	df†	MS	F	P
Date	31, 513.18	0.5423	1.78	0.0074	21, 297.97	0.4605	1.71	0.0326
Tree(Date)	363, 172	0.3043	1.38	0.0078	174, 124	0.2689	1.49	0.0095
Substrate								
Leaf	1, 172	5.4296	24.71	0.0001	1, 124	5.1198	28.33	0.0001
Wood	1, 172	0.7230	3.29	0.0714	1, 124	2.7940	15.46	0.0001
Leaf × Date	29, 172	0.2055	0.94	0.5660	20, 124	0.1399	0.77	0.7395
Leaf × Tree	15, 172	0.1336	0.61	0.8659	9, 124	0.1647	0.91	0.5178
Wood × Date	22, 172	0.4541	2.07	0.0053	7, 124	0.7322	4.05	0.0005
Wood × Tree	15, 172	0.3967	1.81	0.0372	9, 124	0.4603	2.55	0.0102

Notes: Substrate within each tree is a categorical variable with three levels (leaf, stem, wood); therefore only two degrees of freedom are available. Leaf and wood are included explicitly in the model, while stem is included implicitly to avoid singularity. Tree is treated as a random effect and is also nested within date to account for repeated measures. Proportional data ($Aphytis/(Aphytis + Encarsia)$) were arcsine-square-root transformed prior to analysis.

† Denominator df for Date were adjusted for random effects using Satterthwaite's approximation.

Variability among dates.—Although the proportion of *Aphytis* to total parasitism varied among dates, on average both parasitoids responded similarly to resource variability, as depicted for the 1987–1989 field study (Fig. 1). For example, the mean proportion of scales containing *Aphytis* was positively correlated with the mean proportion of scales containing *Encarsia* on each date for these data (0.49 on leaves and 0.60 on stems).

Variability among trees.—The proportion of *Aphytis* to total parasitism was variable among trees on each date (Table 1), suggesting the possibility of spatial segregation at the scale of trees. Several lines of evidence suggest that spatial variability at the scale of trees is not a primary explanation for coexistence, however. First, the numbers of progeny of the two species were positively related among trees ($r = 0.5$). Because both species' juveniles tracked scale abundance closely (*Aphytis*, $r = 0.732$, $P < 0.0001$; *Encarsia*, $r = 0.502$, $P < 0.0001$; Borer 2002), we examined the proportion of scales parasitized by each species per tree, normalizing by date. After normalizing scale abundance by date, *Aphytis* density was independent of *Encarsia* density ($r = -0.07$), suggesting that individual trees are not consistently dominated by either species. Each of these results corroborates the significant nested tree parameter in the model shown in Table 1, and taken together these results indicate that, although at any point in time the proportion of *Aphytis* and *Encarsia* varied among trees, interspecific segregation was not common at the scale of trees. Although these data do not support spatial segregation in parasitism per se, variability in the proportion of parasitoids at the scale of trees may act, to some extent, to facilitate coexistence. We focus our attention on the stronger substrate-scale response, here, and return to consider the role of variability at the scale of trees in the discussion.

Variability between substrates.—The strongest and most consistent result from both studies was that ju-

venile *Aphytis* were more abundant than juvenile *Encarsia* in scales on leaves and stems (Tables 1 and 2, Fig. 1). The proportion of *Aphytis* was >0.8 on leaves in both studies, suggesting dominance by *Aphytis* on this substrate, but was closer to 0.5 on stems (Table 2). In addition, scale density was always higher on stem samples than on leaf samples in each study, yet the combined parasitism rate by *Aphytis* and *Encarsia* on stems was equal to or less than that on leaves (Table 2).

The relative abundance of juvenile *Aphytis* showed no consistent pattern on wood (Table 1). Juvenile production on wood was not significantly different from that on stems in the 1984–1985 study, while in the 1987–1989 study, it was significantly lower on wood than on stems (Table 2). In both studies, juvenile production also varied strongly among sample dates (Table 1, Fig. 1). These analyses suggest that the relative production of *Aphytis* and *Encarsia* from scales on wood is not consistent through time.

While the variability in juvenile production at several spatial and temporal scales may contribute to coexistence, the strongest and most consistent pattern arising from the analysis of these observational data was the numerical dominance of *Aphytis* on leaves and the more even distribution of parasitism by both species on stems. This pattern could have arisen from at least two mechanisms. First, *Aphytis* and *Encarsia* juveniles may survive differentially on leaves and stems. Many studies have shown strong effects of host quality on survival (e.g., Harvey et al. 1995, Jervis and Kidd 1996), and red scale insects are smaller on stems than on leaves (Walde et al. 1989). *Aphytis* juveniles might experience higher mortality than *Encarsia* juveniles in small scales, causing each species to, effectively, have a distinct resource (scales on stems and scales on leaves) mediated by substrate-specific host quality.

The second spatial mechanism that could have produced this pattern is that *Aphytis* may prefer to attack

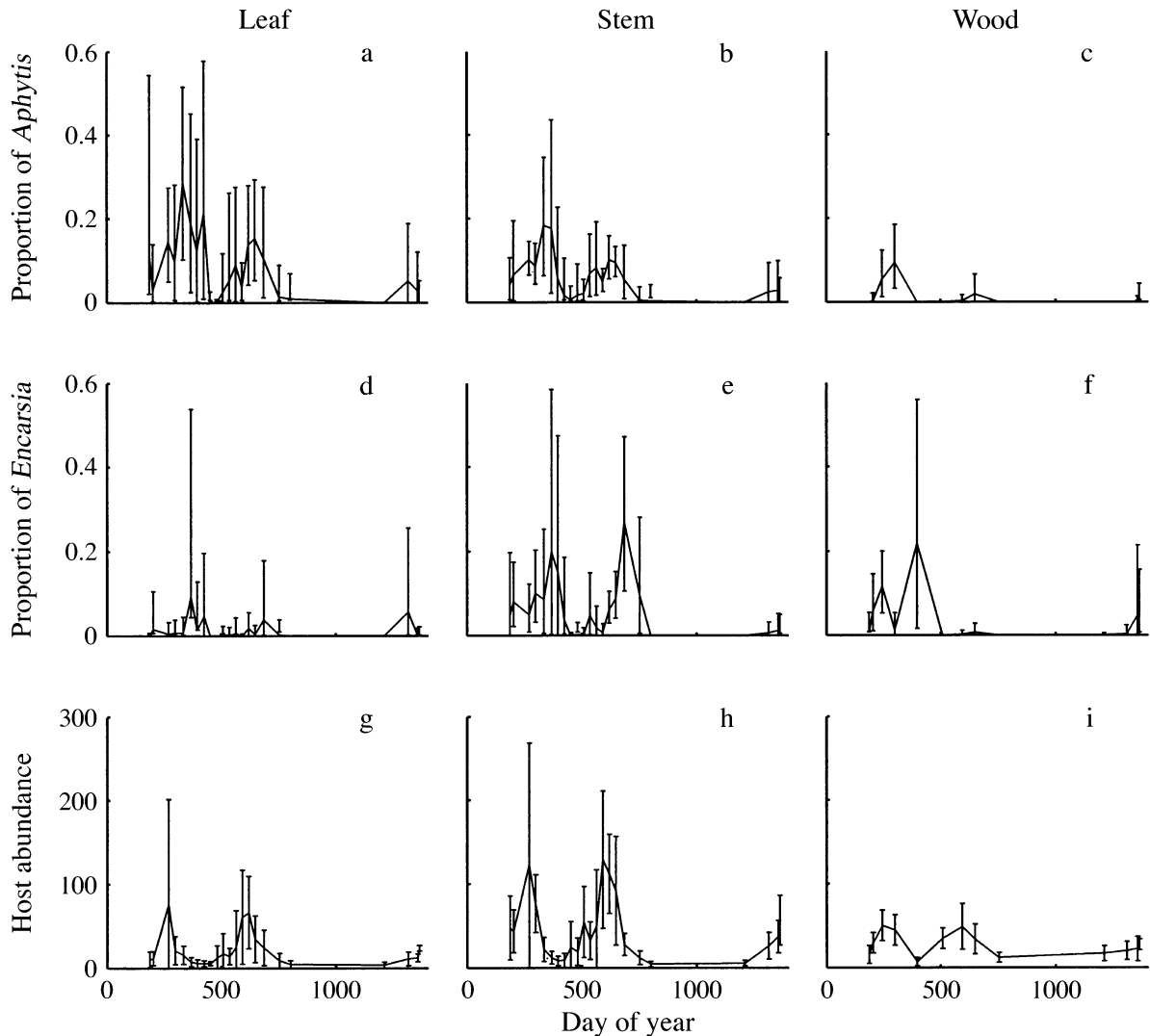


FIG. 1. Juvenile parasitoid and scale abundances are more variable among dates than they are among trees within a date, and proportion parasitism by both parasitoids appears to track scale abundance. Plots show patterns of parasitoid juveniles and scale abundance on the unmanipulated control trees from a field study conducted in 1987–1989. Error bars (± 2 SD) represent variability among trees within each date. Error bars for the proportional data (plots a–f) were calculated from arcsine-square-root transformed data, then back transformed for plotting, to bound values between 0 and 1.

scales on leaves, *Encarsia* may prefer scales on stems, or both. Theory suggests that coexistence can be maintained via resource preferences such as segregation or, more generally, different parasitism rates by each species on different patches (e.g., MacArthur 1958, Kakhashi et al. 1984, Klopfer and Ives 1997). In particular, Klopfer and Ives (1997) show that density-independent aggregation that produces a spatial cross-correlation between species' distributions that is less than perfect (i.e., $r < 1$), such as different inherent substrate preferences, can facilitate coexistence.

In the following sections, we examine potential mechanisms causing this consistent, strong substrate-scale pattern by addressing the question: Is the observed difference in parasitoid production between

leaves and stems due to (1) a differential ability of the two species to produce progeny on leaves and stems or (2) behavioral partitioning of scale resources through substrate preferences?

METHODS

Field experiments

We performed both field experiments in a lemon grove in Fillmore, California, between May and September 2000. We randomly chose 10 trees within the grove and used each tree in both experiments. In both experiments, each tree received all treatments; thus each experiment was a randomized complete block design, with trees as blocks. For logistical reasons, the

TABLE 2. Mean proportion of scales parasitized by *Aphytis* and *Encarsia* per sample, mean proportion *Aphytis* juveniles in each sample, mean number of scale per sample, and mean scale density per sample for each substrate type in two field observational data sets.

Substrate	<i>Aphytis</i> / total scale	<i>Encarsia</i> / total scale	<i>Aphytis</i> / total parasitism	Total scale/ sample	Scale density (no./cm ²)
June 1984–December 1985					
Leaf	0.176	0.015	0.906	4.840	0.057
Stem	0.087	0.065	0.634	13.689	1.296
Wood	0.013	0.012	0.572	7.370	7.370
July 1987–March 1989					
Leaf	0.126	0.029	0.830	3.809	0.052
Stem	0.084	0.081	0.563	3.534	0.289
Wood	0.028	0.055	0.423	5.183	5.183

trees were divided into two temporally staggered sets of five trees each. On each tree, we cleaned all existing scale and parasitoids from the leaves and branches of 14 twigs, and we secured each twig inside a mesh bag. Bags were made of 118- μ m polyester mesh (Sefar America, Inc., Kansas City, Missouri, USA), and each was 30 cm (diameter) \times 60 cm (length). Parasitoids are capable of searching the entire caged twig daily. In both field experiments, twigs had similar surface areas, and each twig was a replicate.

We started the experiment by adding California red scale crawlers (the juvenile dispersive stage) to the leaves and/or stems of each bagged twig, depending on the treatment (see *Methods: Experiment 1: Substrate-mediated juvenile survival* and *Methods: Experiment 2: Behavioral preference*). We allowed the scale insects to settle and grow to mid-second instars (\sim 28 d). Crawlers that settled on leaves in stem-only treatments (and vice versa) were removed manually prior to parasitoid introductions.

We then added five 2-d-old laboratory-reared *Aphytis* and/or *Encarsia* females into the bags, depending on the treatment, and left them in each bag for 6 d. We chose this duration of exposure to keep the expected production of parasitoid eggs/female the same for both species. *Encarsia* begins life with a full complement of \sim 32 eggs. *Aphytis* females produce \sim 4 eggs/d throughout their lifetime, and we introduced them into the treatments as mated two-day-old females (i.e., females with eight mature eggs). After 8 d as an adult, females of each species should have produced \sim 32 mature eggs apiece.

After removing the parasitoids from the bags, we allowed their progeny to develop into pupae, which required \sim 20 d. We then cut down each replicate twig, returned it to the laboratory, and recorded the incidence of live and dead scale on each substrate, as well as the abundance of parasitoid pupae of each species.

Experiment 1: Substrate-mediated juvenile survival

This experiment assessed the differential ability of each species to produce progeny from scale on either leaves or stems. We added red scale to either the leaves

or stems of each bagged twig, and subsequently introduced *Aphytis*, *Encarsia*, or no parasitoids. Parasitoids were given no choice of substrate on which they laid their progeny, which allowed us to quantify parasitoid performance on scale on each substrate in the absence of interspecific competition, and in the absence of oviposition choice.

This experiment had a two-factor design: parasitoid (five *Aphytis*, five *Encarsia*, or neither present) crossed with substrate (leaves, stems). All six parasitoid \times substrate treatments were randomly assigned to twigs on each of the 10 trees for a total of 60 observations.

The treatments were applied across a gradient of scale abundance. The number of scale insects per twig ranged from eight to 584 on leaves and from 23 to 365 on stems. Scales were somewhat more difficult to apply to stems, and across twigs in this experiment, 65% of the scales were on leaf-only twigs (mean = 323, median = 348) and 35% were on stem-only twigs (mean = 179, median = 174).

Experiment 2: Behavioral preference

This experiment allowed us to determine whether patterns of parasitism were affected by the substrate on which the red scale insect had settled or by the presence of a competitor. We added scale insects to both substrates (leaves and stems) on each bagged twig, and thus in every treatment, parasitoids chose whether to oviposit on stem or leaf scales.

We applied a factorial combination of *Aphytis* and *Encarsia* at two levels across a gradient of scale abundance: five *Aphytis* or five *Encarsia* females/twig, five of each species together on a twig, or no parasitoids. Each of the four *Aphytis* \times *Encarsia* treatments was applied randomly to one twig with high and one with low scale insect abundance on each of the 10 trees for a total of 80 observations. The gradient of scale insect abundance spanned a range from resource-limitation to resource super-abundance, determined by the ratio of scale insects to potential parasitoid egg production/twig (from <1 scale/egg to >25 scales/egg). By keeping the number of parasitoid females/species constant, we could directly compare the juvenile parasitoids pro-

duced by five individuals alone to five individuals in the presence of a competitor species.

Statistical analyses

We analyzed the data from Experiment 1 to quantify the importance of substrate-specific oviposition and survival in the production of the observed field pattern. Based on the species' biology, we expected a decrease in the relative number of juvenile parasitoids with increasing scale abundance (e.g., a Type II functional response), suggesting that the most appropriate model for these data should be nonlinear. We used the Gauss-Newton algorithm for nonlinear least squares to fit the model $\mu = \beta_0 A^{(\beta_1 + \beta_2 S)}$. In this model, μ is the number of progeny produced, the β 's are unknown coefficients, S represents substrate (leaf = 0, stem = 1) on which the scales occur, and A represents abundance of available scales on each twig. We found that other nonlinear model forms produced similar results.

Next, we determined whether either species shifted its production of progeny from leaf to stem (or vice versa) within a twig depending on scale insect abundance or interspecific competition. For this analysis, we used logistic regression to analyze data from Experiment 2, and each bag represented a single experimental unit. In this model $\log(p/(1-p)) = \beta_0 + \beta_1 A + \beta_2 C + \beta_3 AC$. Here, p is the conditional probability that a parasitized scale occurs on a leaf, C represents the presence ($C = 1$) or absence ($C = 0$) of interspecific competitors, and A represents scale abundance on a twig. In this experiment, scales on both stems and leaves were available to each searching parasitoid, so substrate was not independent within a bag. To control for covariance of parasitism within a bag, we used a quasi-likelihood approach to adjust for over- or underdispersion in these data (McCullagh and Nelder 1989). In addition, because of the wide range in the number of scale in each bag, we weighted all bags equally such that each provided a single data point in the analysis.

Finally, we assessed data from Experiment 2 to determine whether the presence of interspecific competitors or the availability of scales affected the number of progeny each parasitoid species produced. As in Experiment 1, we expected a nonlinear relationship between scale availability and number of juvenile parasitoids (e.g., a Type II functional response), so we employed the Gauss-Newton algorithm to fit a nonlinear least squares model: $\mu = \beta_0 A^{(\beta_1 + \beta_2 C)}$. In this model, each parameter retained the definitions from above. Other biologically based nonlinear models produced similar results.

For all statistical analyses, we used SAS (SAS Institute 2001).

RESULTS

Inherent substrate mediated survival

In Experiment 1, we found no difference in the suitability of scales on stems and leaves for either para-

sitoid. When presented with scales on either stems or leaves (but not both) in Experiment 1, *Aphytis* produced similar numbers of progeny from scales on each substrate, given similar numbers of scale insects (Fig. 2a). The same was true of *Encarsia* (Fig. 2b). Both species produced more progeny with increasing scale abundance, but this increase was not related to substrate-specific differences in scale availability or quality (Table 3).

Field experiments vs. field observations

The pattern of domination by *Aphytis* on leaves but more equal abundances on stems observed in our field data (Table 2) was reproduced in Experiment 2. In this experiment, in treatments in which both species were together, *Aphytis* made up $78 \pm 4.5\%$ (mean ± 1 SE) of the juvenile parasitoids on leaves, but $58 \pm 6.6\%$ on stems. *Aphytis* produced $\sim 63\%$ of the progeny on both substrates combined. Regardless of scale abundance, the proportion of *Aphytis* in the total parasitism was always higher on leaves than stems.

Resource segregation

In the absence of interspecific competition in Experiment 2, when scales were available on both leaves and stems, each species' preferences mirrored the field-observed patterns of their juvenile abundances (Fig. 3, open squares). *Aphytis* strongly preferred scales on leaves over those on stems ($67 \pm 3\%$ of *Aphytis* juveniles were on leaves), whereas *Encarsia* showed no preference ($52 \pm 4\%$ of the juveniles were on leaves). These patterns were maintained in the presence of interspecific competitors (Fig. 3, Table 4), although the relative abundance of *Encarsia*'s juveniles on leaves when *Aphytis* was present dropped slightly to $45 \pm 3\%$. In spite of the huge range in scale insect availability among twigs, neither species' relative preference shifted with increasing scale abundance (Fig. 3, Table 4).

Due to experimental logistics, it was somewhat easier to apply red scale to leaves, so on each twig of Experiment 2 leaves contained a higher proportion of the scales than stems (0.58 on leaves). This discrepancy had low variability among experimental twigs (variance = 0.014), however, and does not contradict the important result that under identical conditions *Aphytis* and *Encarsia* showed different behavioral preferences for scales on leaves and stems.

Effect of competition on parasitism rates

Overall, both species were negatively affected by the presence of the other (Fig. 4, Table 5), demonstrating the negative effect of interspecific competition. *Encarsia* produced no progeny on three twigs, all of which were shared with *Aphytis*, and both species produced their maximum number of progeny in the absence of interspecific competitors.

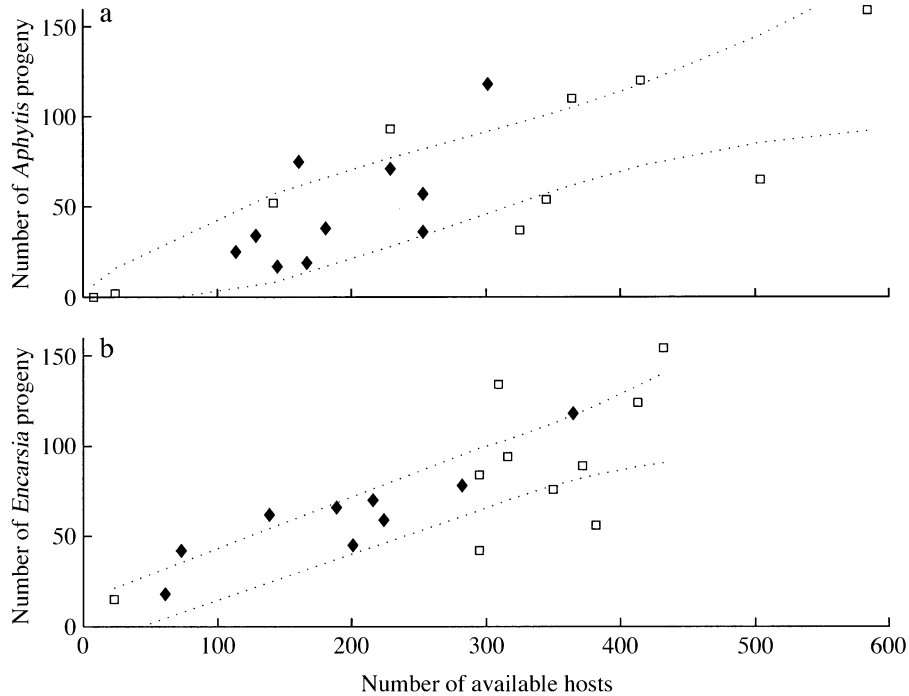


FIG. 2. When provided red scale hosts on only stems (solid diamonds) or leaves (open squares) (but not on both) in Experiment 1, (a) *Aphytis* and (b) *Encarsia* both produce increasing numbers of progeny solely as a function of the abundance of available hosts. The number of juvenile parasitoids produced (a combination of egg allocation and juvenile mortality) is not impacted for either species by the substrate on which the scales settled. Dotted lines represent the 95% confidence intervals. The full model is presented in Table 3.

Effect of scale abundance on parasitism rates

Fig. 4 shows that the number of progeny produced from Experiment 2 twigs increased with scale abundance for both species (also see Table 5). Each parasitoid responded somewhat differently to scale abundance when alone, however (Fig. 4, open squares). Without interspecific competition, the number of *Aphytis* progeny increased linearly with scale abundance over the range provided in this experiment (Fig. 4a, solid line). However, under the same conditions, the abundance of *Encarsia* juveniles was a saturating function of scale abundance (Fig. 4b, solid line). At high scale abundances, in particular, *Encarsia* produced far fewer progeny per available red scale than did *Aphytis*.

The difference between the species probably reflects the fact that *Aphytis* females host feed and develop new eggs throughout their lives, whereas *Encarsia* females emerge with their lifetime complement of ~32 eggs. The maximum number of progeny produced by the five *Encarsia*/twig (131 progeny) occurred on a twig with 321 scales (Fig. 4b) and is close to the expected maximum egg production (160, see *Methods*). Thus, under these experimental conditions, *Encarsia* was egg-limited on twigs with moderate to high scale abundance. *Aphytis*' steeper linear increase with scale abundance suggests that it was less egg limited at high scale abundance than was *Encarsia*. On two twigs, *Aphytis* was able to produce more eggs than the expected maximum over the 6 d of the experiment.

TABLE 3. Production of *Aphytis* and *Encarsia* juveniles with increasing scale abundance on leaf vs. stem substrates from Experiment 1 (scales on leaves or stems, but not both).

Factor	df	Estimate	95% CI
Model for <i>Aphytis</i> (n = 20)			
Intercept (β_0)	1	0.2338	-0.685-1.152
Scale abundance (β_1)	1	0.9178	0.441-1.395
Substrate (β_2)	1	0.0088	-0.028-0.046
Model for <i>Encarsia</i> (n = 19)			
Intercept (β_0)	1	0.4588	-1.158-2.076
Scale abundance (β_1)	1	0.8534	0.337-1.370
Substrate (β_2)	1	0.0057	-0.017-0.028

Note: Models for both species are significant at $P < 0.0001$.

DISCUSSION

Field observations of the distribution of juvenile *Aphytis* and *Encarsia* showed that *Aphytis* was always numerically dominant on leaves while *Aphytis* and *Encarsia* were always found in similar abundances on stems. Our experiments suggest that these differences arise from *Aphytis*' preference for leaves and *Encarsia*'s lack of preference. We showed that differential juvenile survival or other substrate-induced differences in the suitability of hosts do not cause the field-observed difference in species distributions between leaves and stems. Our experiments show that both

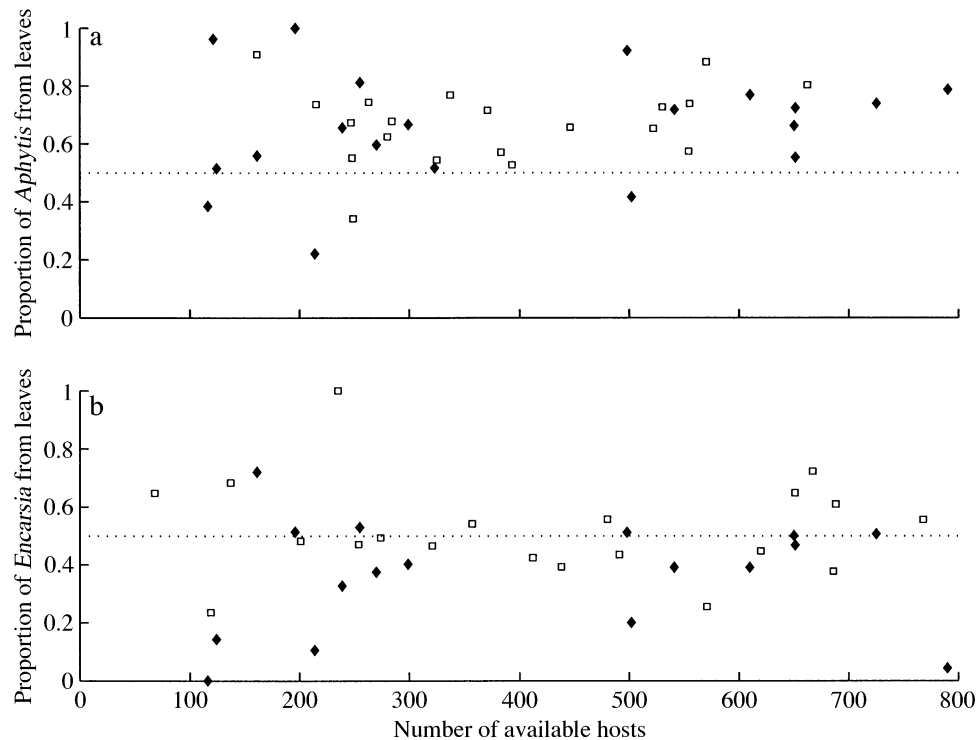


FIG. 3. In Experiment 2, the relative number of juvenile parasitoids from leaves and stems remained constant across a broad range of scale abundance with (solid diamonds) and without (open squares) interspecific competitors for both (a) *Aphytis* and (b) *Encarsia*. The y-axis represents the proportion of progeny produced on leaves (no. progeny on leaves/no. progeny on a twig). *Aphytis* produced twice as many progeny from scales on leaves (mean = 0.67 from leaves) while *Encarsia* produced progeny at similar rates on each substrate (mean = 0.45 from leaves). Dotted lines represent equal number of juveniles from leaves and stems. The full model is presented in Table 4.

Aphytis and *Encarsia* can reduce the efficiency of the other species via interspecific competition, but habitat preference by *Aphytis* may reduce the impact of interspecific competition in field populations.

The experimental data suggest that *Aphytis*' preference for red scale on leaves controls the pattern of juvenile abundance observed in the field. Across a nearly 10-fold range of scale abundance, *Encarsia* produced the same number of progeny from each substrate, while *Aphytis* consistently produced twice as many progeny

from leaves as from stems. This *Aphytis* pattern is caused by a preference for scale on leaves because, when offered only scales on stems or leaves, *Aphytis* produced progeny from each at the same rate. Gregory (1985) found that *Aphytis* is attracted to the colors of leaves and fruit, and Walde et al. (1989) identified a possible reason for this attraction: on average, scale insects on leaves are somewhat larger than scale insects on stems. Although we did not measure the sex of *Aphytis* juveniles in this study, *Aphytis* lays female eggs

TABLE 4. Logistic regression for both parasitoid species, assessing the probability that a parasitized scale occurs on a leaf in Experiment 2 (scales on both leaves and stems) as a function of scale insect abundance and presence/absence of a competing parasitoid species.

Factor	df	Estimate	SE	F	P
Model for <i>Aphytis</i> (n = 40)					
Intercept (β_0)	1, 36	0.4116	0.5190		
Scale abundance (β_1)	1, 36	0.0008	0.0013	0.38	0.5407
Competition (β_2)	1, 36	-0.0487	0.6321	0.01	0.9390
Abund \times Comp (β_3)	1, 36	-0.0000	0.0015	<0.01	0.9779
Model for <i>Encarsia</i> (n = 37)					
Intercept (β_0)	1, 33	0.1871	0.4237		
Scale abundance (β_1)	1, 33	-0.0002	0.0009	0.07	0.7956
Competition (β_2)	1, 33	-0.8918	0.6155	2.13	0.1542
Abund \times Comp (β_3)	1, 33	0.0006	0.0013	0.18	0.6747

Note: *Encarsia* produced no progeny on three twigs, and these dropped out of this analysis.

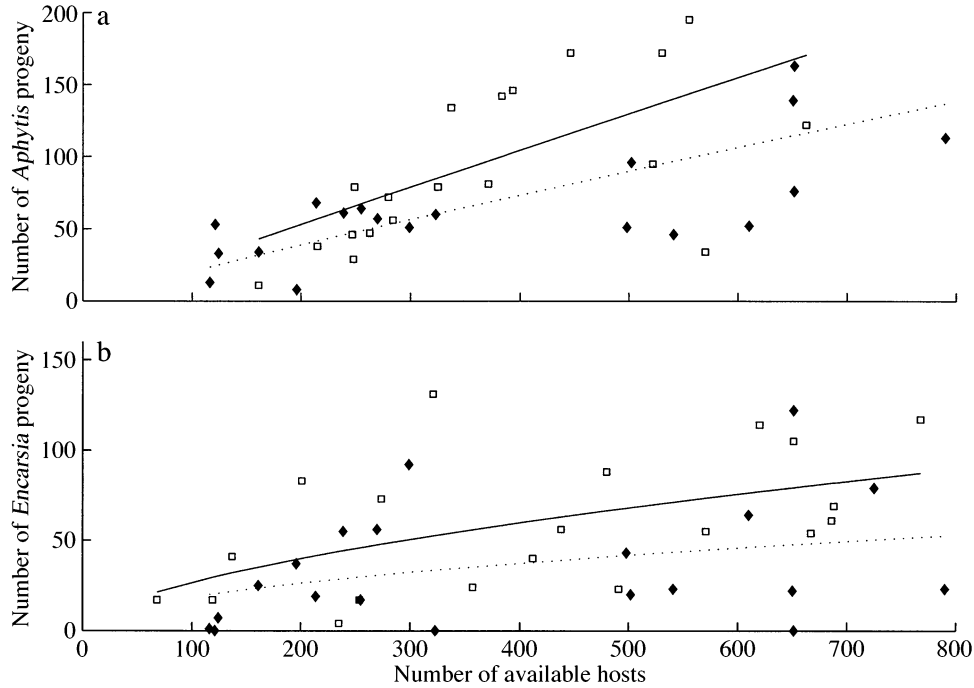


FIG. 4. Juvenile parasitoids increased with scale abundance and were depressed on twigs with interspecific competitors (solid diamonds) relative to those without competitors (open squares) for both species in Experiment 2. The solid lines represent the model prediction for each species without interspecific competitors, and the dotted lines represent the model prediction with competitors. The full model is presented in Table 5.

preferentially in larger scales (Luck and Podoler 1985). But *Aphytis*'s preference was not absolute; at all scale abundances *Aphytis* produced about a third of its progeny from scales on stems.

Many examples exist demonstrating that the realized niche of an inferior competitor is smaller than its fundamental niche. Coexistence in those species pairs is maintained by the existence of a refuge for the poorer competitor. For example, Connell's (1961) barnacles, Bovbjerg's (1970) crayfish, Pimm et al.'s (1985) hummingbirds, and Martin and Martin's (2001) warblers coexist because there is a balance between habitat specialization by the more aggressive competitor and broader environmental tolerance by the poorer com-

petitor. The region lacking the better competitor serves as a refuge for the poorer competitor.

The patterns of resource use in the competing barnacles, crayfish, and birds are similar to those we observed in our system. *Encarsia*, the poorer within-host competitor, produced similar numbers of progeny from scale resources on both stems and leaves. *Aphytis*, the dominant within-host competitor on all substrate types, showed a preference for a subset of *Encarsia*'s fundamental niche: red scale insects on leaves. But our results showed that the interaction between these two parasitoids differed in one very important aspect from those observed in the systems cited above: *Encarsia* does not have an absolute refuge from competition with

TABLE 5. The number of progeny of each species produced in Experiment 2 (scale insects on both leaves and stem) as a function of scale insect abundance and presence/absence of a competing parasitoid species.

Factor	df	Estimate	95% CI
Model for <i>Aphytis</i> (n = 40)			
Intercept (β_0)	1	0.3037	-0.452 to 1.059
Scale abundance (β_1)	1	0.9748	0.568 to 1.382
Competition (β_2)	1	-0.0588	-0.107 to -0.011
Model for <i>Encarsia</i> (n = 40)			
Intercept (β_0)	1	1.8229	-3.144 to 6.790
Scale abundance (β_1)	1	0.5824	0.145 to 1.020
Competition (β_2)	1	-0.0785	-0.153 to -0.005

Note: Models for both species are significant at $P < 0.0001$.

Aphytis (e.g., multiparasitism). *Aphytis* attacks scale insects on all substrates, and both the observational and the experimental data show that *Encarsia* produced equal or fewer progeny than *Aphytis* on all substrates.

Absolute refuges are not the only possible spatial mechanism of coexistence, however. Scales on leaves and stems provide equal resources for both parasitoids: juvenile survival is invariant and species will attack scales at the same rate in both habitats if given no choice. When given a choice, however, *Aphytis* prefers to parasitize scales on leaves. Habitat preference is analogous to density-independent aggregation because parasitoids prefer resources in some patches more than in others, regardless of resource density. Parasitoid coexistence via density-independent aggregation is explored in models by May and Hassell (1981) and Kakehashi et al. (1984), but is generalized in a model by Klopfer and Ives (1997) to describe the entire range of relative species distributions among patches.

In Klopfer and Ives' model (1997), two parasitoids compete for resources in multiple patches, and one parasitoid always wins within-host competition. When the species aggregate to the same locations (i.e., have a spatial correlation of 1), intraspecific competition equals interspecific competition. All correlation values less than 1 (<1 to -1) reduce interspecific competition relative to intraspecific competition, thereby facilitating coexistence. Coexistence also is facilitated when habitat preference is explicitly incorporated and parasitism by each species is not inherently aggregated. In particular, the better within-host competitor must have a strong habitat preference whereas the inferior competitor needs only a weak preference for the other habitat to promote coexistence.

In light of this theory of coexistence via habitat preference, our experimental results suggest that an important mechanism of coexistence for *Aphytis* and *Encarsia* may operate at the between-substrate spatial scale. Although *Aphytis*' preference for scales on leaves would provide only a partial refuge for *Encarsia* from direct competition with *Aphytis*, this partial refuge may be sufficient to cause greater intraspecific than interspecific competition for each species.

Variability at other spatial scales may ease coexistence for *Aphytis* and *Encarsia*. In particular, our observational results have shown that large-scale (among-tree) variability in the relative abundance of each species occurs, suggesting a less-than-complete covariance that may reduce interspecific competition. Although we did not assess the evidence for extremely small-scale (within substrate) aggregation of egg-laying by either *Encarsia* or *Aphytis*, Reeve and Murdoch (1985) found no evidence for aggregation of parasitism by *Aphytis* at any spatial scale. In this study we have determined that *Encarsia* does not aggregate parasitism by tree or substrate within a tree, although to date, no study has assessed small-scale aggregation of parasitism by *Encarsia*.

Different spatial coexistence mechanisms can induce different dynamics in the shared resource, an issue particularly important in biological control where the resource species is an agricultural pest. Entomologists have argued for nearly a century, without resolution, over whether it is better to release only one, or multiple, natural enemies to control pests (Smith 1929, Hochberg 1996, Murdoch et al. 1998). Although most successful biological control systems contain multiple natural enemies (Argov and Rossler 1993, Takagi and Hirose 1994, Pedata et al. 1995, Murdoch et al. 1998), in most cases we do not know if natural enemy coexistence is beneficial to control or if control would be more effective with only a single species present. Recent reviews of competition models of biological control systems show that the mechanism of coexistence can, in fact, affect the efficiency of pest suppression (Mills and Getz 1996, Murdoch and Briggs 1996, Briggs et al. 1999, Murdoch et al. 2003).

Aphytis' preference for leaves may provide the mechanism by which *Aphytis* and *Encarsia* coexist, and in turn may affect resource dynamics and biological control in the red scale system. While various forms of aggregated resource use can promote coexistence, the resulting resource density may be higher in a system with multiple species than it would be with just the single most efficient resource exploiter (Kakehashi et al. 1984). The parasitoids in the California red scale system have maintained their shared resource for decades at extremely constant, low densities, however, suggesting that if pest density is elevated by the coexistence mechanism, the mechanism is, nonetheless, compatible with successful pest control.

ACKNOWLEDGMENTS

N. Barbee, S. Hoobler, J. Lee, J. McCarthy, H. Ross, E. Seabloom, and Ruth helped E. Borer in the field experiments. R. Nisbet, E. Seabloom, and A. Stewart-Oaten provided extremely helpful discussions about this paper, and anonymous reviewers provided useful comments on earlier versions of this manuscript. We particularly thank A. Ives for helpful discussions and suggestions. Funding for E. Borer was provided by NSF Research and Training grants BIR94-13141 and GER93-54870. The research was supported by NRI Competitive Grants Program/USDA grant #2001-35316-10989 to W. W. Murdoch and E. T. Borer.

LITERATURE CITED

- Argov, Y., and Y. Rossler. 1993. Biological control of the Mediterranean black scale, *Saissetia oleae* (Hom.: Coccidae) in Israel. *Entomophaga* **38**:89–100.
- Atkinson, W. D., and B. Shorrocks. 1981. Competition on a divided and ephemeral resource: a simulation model. *Journal of Animal Ecology* **50**:461–471.
- Baroffio, C. 1993. Description of the developmental stages of *Encarsia perniciosi* (Tower) using different techniques. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **66**:371–378.
- Baroffio, C. 1997. Some aspects of the biology of *Encarsia perniciosi* (Tower) in its host, *Quadraspidiotus perniciosus* (Comstock), and application of the results in a biological control program in central Switzerland (Canton Zug). *Mit-*

- teilungen der Schweizerischen Entomologischen Gesellschaft **70**:323–333.
- Bodenheimer, F. S. 1951. Citrus entomology in the Middle East with special reference to Egypt, Iran, Iraq, Palestine, Syria, Turkey. W. Junk, S-Gravenhage, The Netherlands.
- Borer, E. T. 2002. How do resource specialists coexist? Evidence from a biological control community. Dissertation. University of California, Santa Barbara, California, USA.
- Bovbjerg, R. V. 1970. Ecological isolation and competitive exclusion in two crayfish (*Orconectes virilis* and *Orconectes immunis*). *Ecology* **51**:225–236.
- Briggs, C. J., R. M. Nisbet, and W. W. Murdoch. 1999. Delayed feedback and multiple attractors in a host–parasitoid system. *Journal of Mathematical Biology* **38**:317–345.
- Clausen, C. P., editor. 1978. Introduced parasites and predators of arthropod pests and weeds: a world review. Agricultural Handbook No. 480. Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C., USA.
- Collier, T. R. 1995. Host feeding, egg maturation, resorption, and longevity in the parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). *Annals of the Entomological Society of America* **88**:206–214.
- Connell, J. H. 1961. The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. *Ecology* **42**:710–723.
- DeBach, P., D. Rosen, and C. E. Kennett. 1971. Biological control of coccids by introduced natural enemies. Pages 165–194 in C. B. Huffaker, editor. *Biological control*. Plenum, New York, New York, USA.
- DeBach, P., and R. A. Sundby. 1963. Competitive displacement between ecological homologues. *Hilgardia* **34**:105–166.
- Ebeling, W. 1959. Subtropical fruit pests. University of California, Division of Agricultural Sciences, Berkeley, California, USA.
- Gregory, W. A. 1985. In-flight response of citrus-inhabiting *Aphelinidae* (Hymenoptera) to trap colors and plant structures. Dissertation. University of California, Riverside, California, USA.
- Hastings, A. 1980. Disturbance, coexistence, history, and competition for space. *Theoretical Population Biology* **18**:363–373.
- Hare, J. D., D. S. Yu, and R. F. Luck. 1990. Variation in life history parameters of California red scale on different citrus cultivars. *Ecology* **71**:1451–1460.
- Harvey, J. A., I. F. Harvey, and D. J. Thompson. 1995. The effect of host nutrition on growth and development of the parasitoid wasp *Venturia canescens*. *Entomologia Experimentalis et Applicata* **75**:213–220.
- Hochberg, M. E. 1996. An integrative paradigm for the dynamics of monophagous parasitoid–host interactions. *Oikos* **77**:556–560.
- Ives, A. R. 1991. Aggregation and coexistence in a carrion fly community. *Ecological Monographs* **61**:75–94.
- Jervis, M., and N. Kidd. 1996. *Insect natural enemies: practical approaches to their study and evaluation*. First edition. Chapman and Hall, London, UK.
- Kakehashi, N., Y. Suzuki, and Y. Iwasa. 1984. Niche overlap of parasitoid in host–parasitoid systems: its consequence to single vs. multiple introduction controversy in biological control. *Journal of Applied Ecology* **21**:115–131.
- Klopfer, E. D., and A. R. Ives. 1997. Aggregation and the coexistence of competing parasitoid species. *Theoretical Population Biology* **52**:167–178.
- Levins, R., and D. Culver. 1971. Regional coexistence of species and competition between rare species. *Proceedings of the National Academy of Sciences (USA)* **68**:1246–1248.
- Luck, R. F., and H. Podoler. 1985. Competitive exclusion of *Aphytis lingnanensis* by a *Aphytis melinus*: potential role of host size. *Ecology* **66**:904–913.
- MacArthur, R. H. 1958. Population ecology of some warblers of northeastern coniferous forests. *Ecology* **39**:686–706.
- Martin, P. R., and T. E. Martin. 2001. Ecological and fitness consequences of species coexistence: a removal experiment with wood warblers. *Ecology* **82**:189–206.
- May, R. M., and M. P. Hassell. 1981. The dynamics of multiparasitoid–host interactions. *American Naturalist* **117**:234–261.
- McClain, D. C., G. C. Rock, and R. E. Stinner. 1990a. Thermal requirements for development and simulation of the seasonal phenology of *Encarsia perniciosi* (Hymenoptera: Aphelinidae), a parasitoid of the San Jose scale (Homoptera: Diaspididae) in North Carolina [USA] orchards. *Environmental Entomology* **19**:1396–1402.
- McClain, D. C., G. C. Rock, and J. B. Woolley. 1990b. Influence of trap color and San Jose scale (Homoptera: Diaspididae) pheromone on sticky trap catches of 10 aphelinid parasitoids (Hymenoptera). *Environmental Entomology* **19**:926–931.
- McCullagh, P., and J. A. Nelder. 1989. *Generalized linear models*. Second edition. Chapman and Hall, New York, New York, USA.
- Mills, N. J., and W. M. Getz. 1996. Modelling the biological control of insect pests: a review of host–parasitoid models. *Ecological Modelling* **92**:121–143.
- Murdoch, W. W., and C. J. Briggs. 1996. Theory for biological control: recent developments. *Ecology* **77**:2001–2013.
- Murdoch, W. W., C. J. Briggs, and T. R. Collier. 1998. Biological control of insects: implications for theory in population ecology. Pages 167–186 in J. P. Dempster and I. F. G. McLean, editors. *Insect populations in theory and practice*. Kluwer Academic, Norwell, Massachusetts, USA.
- Murdoch, W. W., C. J. Briggs, and R. M. Nisbet. 2003. *Consumer–resource dynamics*. Princeton University Press, Princeton, New Jersey, USA.
- Murdoch, W. W., R. F. Luck, S. L. Swarbrick, S. Walde, D. S. Yu, and J. D. Reeve. 1995. Regulation of an insect population under biological control. *Ecology* **76**:206–217.
- Murdoch, W. W., R. F. Luck, S. J. Walde, J. D. Reeve, and D. S. Yu. 1989. A refuge for red scale under control by *Aphytis*: structural aspects. *Ecology* **70**:1707–1714.
- Pedata, P. A., M. S. Hunter, H. C. J. Godfray, and G. Viggiani. 1995. The population dynamics of the white peach scale and its parasitoids in a mulberry orchard in Campania, Italy. *Bulletin of Entomological Research* **85**:531–539.
- Pimm, S. L., M. L. Rosenzweig, and W. Mitchell. 1985. Competition and food selection: field tests of a theory. *Ecology* **66**:798–807.
- Reeve, J. D., and W. W. Murdoch. 1985. Aggregation by parasitoids in the successful control of the California [USA] red scale: a test of theory. *Journal of Animal Ecology* **54**:797–816.
- Rosen, D., and P. DeBach. 1979. *Species of Aphytis of the world* (Hymenoptera: Aphelinidae). Series entomologica. Volume 17. W. Junk, Boston, Massachusetts, USA.
- SAS Institute. 2001. SAS version 8. 2. SAS Institute, Cary, North Carolina, USA.
- Smith, H. S. 1929. Multiple parasitism: its relation to the biological control of insect pests. *Bulletin of Entomological Research* **20**:141–149.
- Takagi, M., and Y. Hirose. 1994. Building parasitoid communities: the complementary role of two introduced parasitoid species in a case of successful biological control. Pages 437–448 in B. A. Hawkins and W. Sheehan, editors. *Parasitoid community ecology*. Oxford University Press, Oxford, UK.
- Walde, S. J., R. F. Luck, D. S. Yu, and W. W. Murdoch. 1989. A refuge for red scale: the role of size-selectivity by a parasitoid wasp. *Ecology* **70**:1700–1706.

- Yu, D. S., and R. F. Luck. 1988. Temperature-dependent size and development of California red scale (Homoptera: Diaspididae) and its effect on host availability for the ectoparasitoid, *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae). *Environmental Entomology* **17**:154–161.
- Yu, D. S., R. F. Luck, and W. W. Murdoch. 1990. Competition, resource partitioning and coexistence of an endoparasitoid *Encarsia perniciosi* and an ectoparasitoid *Aphytis melinus* of the California red scale. *Ecological Entomology* **15**:469–480.