

A Survey of Syrphid Predators of *Nasonovia ribisnigri* in Organic Lettuce on the Central Coast of California

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ABSTRACT Organic lettuce, *Lactuca sativa* L., producers on California's Central Coast rely on endemic syrphid flies (Diptera: Syrphidae) to suppress populations of *Nasonovia ribisnigri* Mosley (Homoptera: Aphididae) and other aphids affecting lettuce. Growers are using various forms of habitat manipulation to enhance biological control. We surveyed syrphids collected from organic romaine in and around the Salinas Valley from March through September 2005 to gain a better understanding of the species responsible for aphid suppression and to examine possible implications for biocontrol. The primary species of syrphid fly reared were *Toxomerus marginatus* (Say) (39%), *Platycheirus stegnus* (Say) (27%), *Sphaerophoria sulfuripes* (Thomson) (13%), and *Allograpta obliqua* (Say) (10%). *Syrphus opinator* Osten Sacken (2%), *Toxomerus occidentalis* (Curran) (1.3%), and *Eupeodes volucris* Osten Sacken (1%) were less common. *Sphaerophoria pyrrhina* Bigot, *Scaeva pyrastris* (L.), *Platycheirus obscurus* Say, *Allograpta exotica* Wiedemann, and *Eupeodes americanus* Wiedemann each made up <1% of the syrphids reared. *T. marginatus* and *Sphaerophoria sulfuripes* were commonly collected from romaine plants with few or no detectable aphids. *P. stegnus* was observed to deposit in clusters of eggs, and was only reared in significant numbers from highly infested fields. Approximately 5% of syrphid larvae overall were parasitized by either *Diplazon* sp. (Hymenoptera: Ichneumonidae) or *Pachyneuron* sp. (Hymenoptera: Pteromalidae).

KEY WORDS conservation biocontrol, organic agriculture, insectary crops, aphid predators, Syrphidae

California produces ≈70% of the lettuce, *Lactuca sativa* L., greens consumed in the United States (NASS 2006). Over three quarters of the state's lettuce is grown in the Central Coast county of Monterey, which contains the highly productive Salinas Valley (California Farm Bureau 2006). Several varieties of both leaf and head lettuce are grown in the Salinas Valley, and sold boxed, as hearts, and in preprepared salad mixes. Lettuce is planted in Monterey County from January to August, and it is harvested from April to December. Production of organic lettuce has grown dramatically on California's Central Coast during the past 10 yr (Klonsky and Richter 2005, ERS 2006). Several large-scale producers of conventionally grown vegetables have expanded to include organic production in response to increased national demand (Dimitri and Greene 2002, Carol 2004, ERS 2006).

Nasonovia ribisnigri Mosley is a European aphid species that has spread to Asia, the Middle East, and North and South America (Blackman and Eastop 2000). This aphid became established in California in 1998, and it has become the most economically important arthropod pest of lettuce in California's Central Coast region (Chaney 1999). *N. ribisnigri* colo-

nizes the innermost leaves of the lettuce plant, contaminating areas that cannot be treated easily with insecticides (MacKenzie and Vernon 1988, Liu 2004). This contamination renders lettuce unmarketable. In addition to *N. ribisnigri*, *Aulocorthum solani* (Kaltbach); green peach aphid, *Myzus persicae* (Sulzer); and potato aphid, *Macrosiphum euphorbiae* (Thomas) are pests of Central Coast lettuce. Conventional lettuce growers use frequent scouting and application of organophosphate, neonicotinoid, and other insecticides to suppress incipient populations of *N. ribisnigri*.

Organic lettuce producers on California's Central Coast rely almost exclusively on an endemic complex of syrphid flies to remove aphid infestations before harvest (Chaney 1998, Colfer 2004). Many large-scale organic lettuce producers intercrop lettuce with quick-flowering annuals or "insectary crops" to provide floral resources to syrphid adults with the intention of increasing egg laying by syrphids in adjacent lettuce. Syrphid adults feed on nectar as an energy source and require pollen as a protein source for gamete production (Chambers 1988). The most commonly planted insectary crop is sweet alyssum, *Lobularia maritima* L. (Desv.) (Chaney 1998). Coriander, *Coriandrum sativum* L., and plant mixtures marketed

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as "good bug" blends also are planted by growers with the intention of enhancing syrphid activity in lettuce fields.

Although conservation biocontrol of *N. ribisnigri* is largely successful, each year experienced growers are unable to harvest $\approx 5\%$ or more of their lettuce due to aphid contamination (Phil Foster and Ramy Colfer, personal communication). The syrphids either do not arrive in sufficient quantities in the field, or they only suppress aphid infestations after the narrow harvest window (Chaney 1998).

Preliminary surveys indicated that several syrphid species are involved in suppressing *N. ribisnigri* and other aphids in organically grown lettuce on California's Central Coast (Chaney 1998). However information on the relative importance of these species is lacking. Our goal was to survey the syrphid species suppressing aphids in romaine in and around the Salinas Valley to clarify the role of these different species. We hope that improved understanding of the species complex can be used to enhance conservation biological control of aphids in lettuce and help us understand why it sometimes fails. In addition, we hope that this information will be useful for integrating biological control of *N. ribisnigri* into conventional production, if the materials conventional growers currently rely on become less available.

We hypothesized that different growing environments and distinct aphid densities might favor distinct syrphid species and that therefore we would not find the same species predominating at each sample site. To assess the influence of site and aphid density on the syrphid species present, we collected romaine plants three to five times during one or more crop cycles at five farms located in different areas of the survey region. We sampled an additional nine sites less intensively during the course of the survey.

Preliminary surveys indicated a limited role for other natural enemies in suppressing *N. ribisnigri*. We recorded the presence of other natural enemies in the collected romaine, but we did not attempt to quantify the densities of other natural enemies.

Materials and Methods

To determine whether the same syrphid species predominate throughout the growing area, we collected syrphid eggs, larvae, and pupae from organically grown romaine lettuce from 14 farms in and around the Salinas Valley over a 6-mo period. We collected romaine from ranches in Monterey, San Benito, and Santa Cruz counties from 16 March through 20 September 2005. To have a better understanding of the role of different syrphid species at distinct aphid infestation levels, we collected romaine plants three to five times during the crop cycle at five of these farms. An interval of 1–2 wk passed between collection dates for a given crop. The final sample was collected at or near harvest.

Twenty romaine plants were collected randomly from the field on a given collection date and placed in plastic bags for transport to the University of Califor-

nia Cooperative Extension laboratory in Salinas, CA. In the laboratory, we examined plants leaf by leaf beneath a jeweler's magnifying glass (Electrix Illumination model E507, Electrix Lighting, Buffalo Grove, IL) for the presence of aphids and natural enemies, including syrphid eggs, larvae, and pupae. We recorded the number and species of aphid in each plant. The presence of other predators, parasitized aphids and fungus-killed aphids was recorded.

We recorded the number of syrphid eggs, larvae, and pupae collected from each plant. Eggs, larvae, and pupae were placed individually in a petri dish (100 by 25 cm, Fisher, Pittsburgh, PA) and kept in a growth chamber (model MB60-B, Percival Scientific, Perry, IA) with a photoperiod of 16:8 (L:D) h at 20–25°C and 60–75% RH. Eggs and larvae were provided with *N. ribisnigri*; pea aphid, *Acyrtosiphon pisum* Harris; and/or foxglove aphid on pieces of lettuce leaf or faba bean, *Vicia faba* L., leaf every 3 d until pupation. Upon reaching the adult stage, syrphids were allowed 1 d to expel abdominal fluid, then frozen, and later pinned and placed in a reference collection. The species and gender of each individual was recorded. Data were kept on rearing mortality for the five intensively sampled sites.

Romaine was only grown at most farms in the survey area for a portion of the survey period. Therefore, it was not possible to sample each site at the same time or during the entire time span. The southernmost site, the Wilson Ranch near San Ardo, was sampled from late March through early July. The Nevins Ranch near Greenfield was sampled from July through September. The Storm Ranch in Salinas was sampled from June through September. The Cassidy Ranch in Aromas was sampled in July and August. The Home Ranch in Gilroy was sampled in August and September.

To remove any potential variability due to lettuce type, we only sampled romaine lettuce. There was however considerable variability in the field environment from which romaine was sampled. Romaine was collected from fields of varied size (2–20 ha) and management practices. Romaine was grown either from seed or transplants, either in 101-cm (40-in.) beds or 203-cm (80-in.) beds (measured center to center). Growers intercropped the romaine with alyssum as an insectary plant at the Aromas, Gilroy, and Salinas sites. An intercrop mixture of flowering species referred to as "good bug blend" was used at the San Ardo site. No insectary crop was planted at the Greenfield site.

The landscape surrounding each field was likewise variable. The Aromas ranch is primarily surrounded by wooded habitat. The Gilroy, Greenfield, and Salinas ranches are adjacent to conventional fields where pesticides are applied. The Gilroy and Greenfield sites also border natural habitat, whereas the Salinas site is near commercial and residential centers. The San Ardo fields are in the center of a 340-ha organic farm that borders wooded hills.

The effects of aphid density on egg-laying probability were assessed using generalized linear mixed

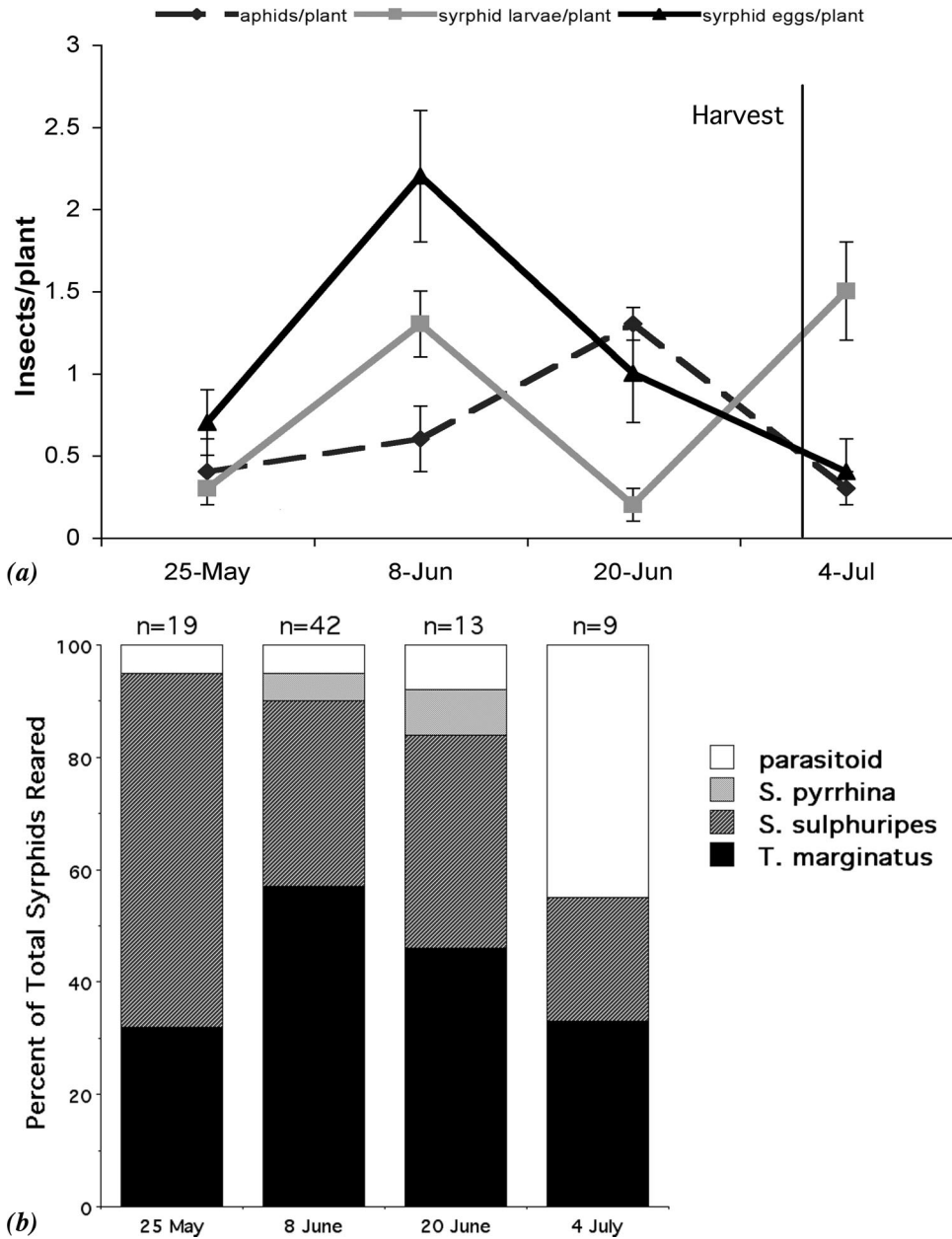


Fig. 1. Aphid and syrphid populations at a low aphid density site (San Ardo). (a) Average \pm SEM per plant densities of aphids, syrphid eggs, and syrphid larvae. Transplanted, 15 May; harvested, 4 July; $n = 20$ romaine plants per sample date. (b) Syrphid species composition and percentage of parasitism of reared syrphids from each sample date.

models. A logistic regression was performed with fixed effects for species, aphid class (0–2 or 3+), and species by aphid class interaction. To account for time and location effects, a random effect for site by date was added to the model. The interaction of species by aphid class was studied using pairwise comparisons. Statistical significance was declared at the 0.05 level. The analysis was carried out using a data set comprised of one crop from the Aromas site, one crop from the Gilroy site, two crops from the Greenfield site, two

crops from the Salinas site, and one crop from the San Ardo site.

Results

Survey. In total, 1,087 individual syrphids were reared to adulthood from eggs, larvae, and pupae collected from organically grown romaine lettuce at 14 sites between 16 March and 20 September 2005. These syrphids were reared from romaine collected on 51

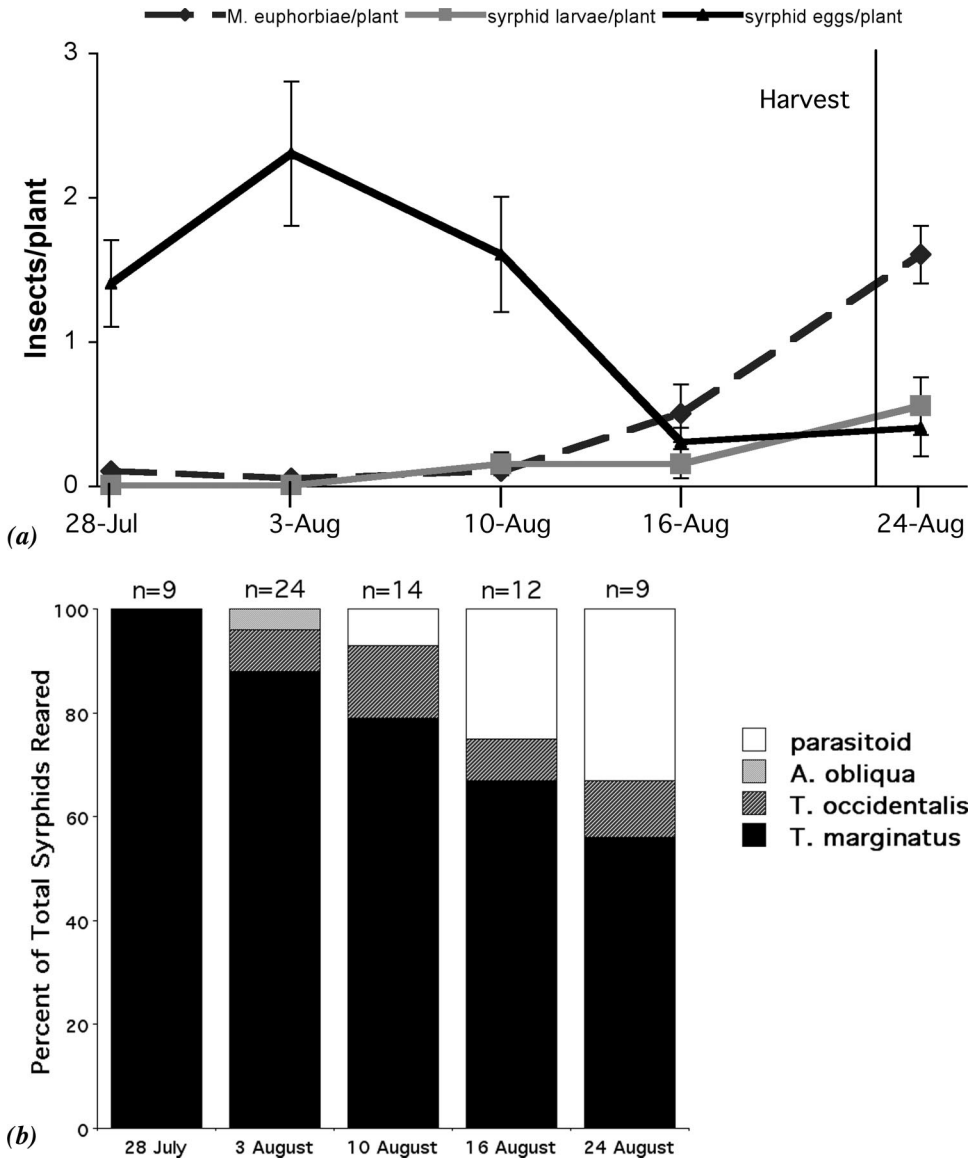


Fig. 2. Aphid and syrphid populations at a low aphid density site (Greenfield), *Nasonovia*-resistant romaine. (a) Average \pm SEM per plant densities of aphids, syrphid eggs, and syrphid larvae. Planted, 28 June; harvested, 24 August; $n = 20$ romaine plants per sample date. (b) Syrphid species composition and percentage of parasitism of reared syrphids from each sample date.

dates during that period, during which >1,000 heads of romaine were examined.

The primary species reared were *Toxomerus marginatus* (Say) (39%) and *Platycheirus stegnus* (Say) (27%). *Sphaerophoria sulfuripes* (Thomson) (13%) and *Allograpta obliqua* (Say) (10%) were also common. *Syrphus opinator* Osten Sacken (2%), *Toxomerus occidentalis* (Curran) (1.3%), and *Eupeodes volucris* Osten Sacken (1%) were less common. *Sphaerophoria pyrrhina* Bigot, *Scaeva pyrastris* (L.), *Platycheirus obscurus* Say, *Allograpta exotica* Wiedemann, and *Eupeodes americanus* Wiedemann each made up <1% of the syrphids reared. Five percent of larvae overall were parasitized, primarily by the larval-pupal parasitoid *Diplazon* sp. (Hymenoptera:

Ichneumonidae). *Pachyneuron* sp. (Hymenoptera: Pteromalidae), a gregarious parasitoid, also was reared from syrphid pupae at low levels.

T. marginatus and *S. sulfuripes* were reared from each of the five major collection sites (Aromas, Gilroy, Greenfield, Salinas, and San Ardo). *P. stegnus* and *A. obliqua* were not reared from material collected in the southernmost site, San Ardo. Only 19 syrphid pupae were collected from all sites. *S. sulfuripes* emerged from five pupae, two produced *A. obliqua*, five were parasitized, and seven pupae died.

Mortality. Mortality in the rearing process was high, especially among eggs. Only 60% of the 1,568 syrphid eggs and larvae collected from the five prin-

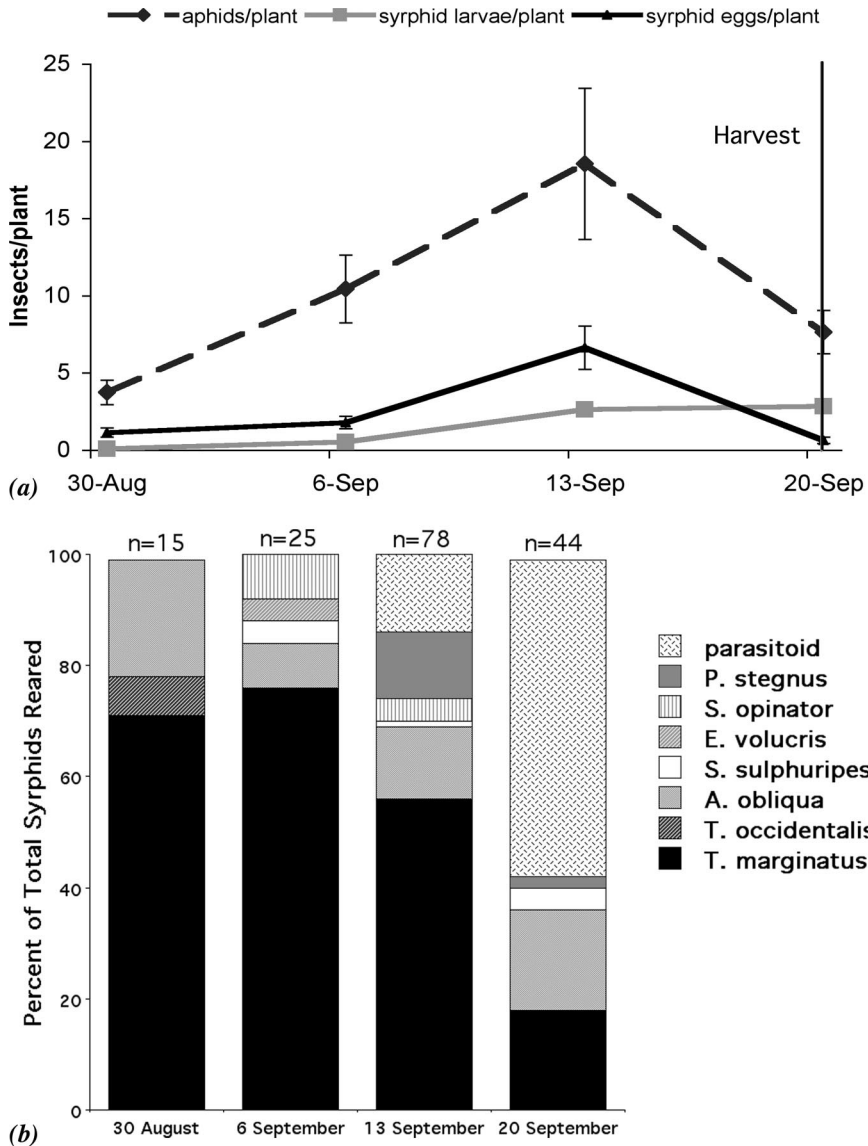


Fig. 3. Aphid and syrphid populations at a moderate aphid density site (Gilroy). (a) Average \pm SEM per plant densities of aphids, syrphid eggs, and syrphid larvae. Planted, 26 July; harvested, 20 September; $n = 20$ romaine plants per sample date. (b) Syrphid species composition and percentage of parasitism of reared syrphids from each sample date.

ciple sites were successfully reared to the adult stage. Twenty-three percent of the 1,039 eggs apparently did not produce a larva. We assume most of these eggs were not viable, although some may have produced larvae that died as neonates. Fourteen percent (146) of syrphids collected as eggs died as larvae, and 7% (76) died in the pupal stage. In total, 580 syrphids were reared from eggs collected from the five principle sites.

Of 529 syrphid larvae collected from the five principle sites, 21% (114) died in the larval stage and 12% (62) died as pupae. In total, 353 syrphid adults were reared from larvae collected at the five principle sites.

With the exception of two sample sites, in which mortality was unusually high because of a pathogen and insecticide use, mortality was relatively consistent across collection sites and dates. We assume that the individuals we were able to rear to adulthood are representative of the species complex present, but there is no way of knowing whether rearing mortality was higher among some species than others.

Species Composition from Repeated Sampling of Five Romaine Stands. The five repeatedly sampled sites presented a range of aphid infestation levels that included negligible, moderate, and high aphid densities. At the San Ardo and Greenfield sites, aphid levels did not reach economically significant levels, averag-

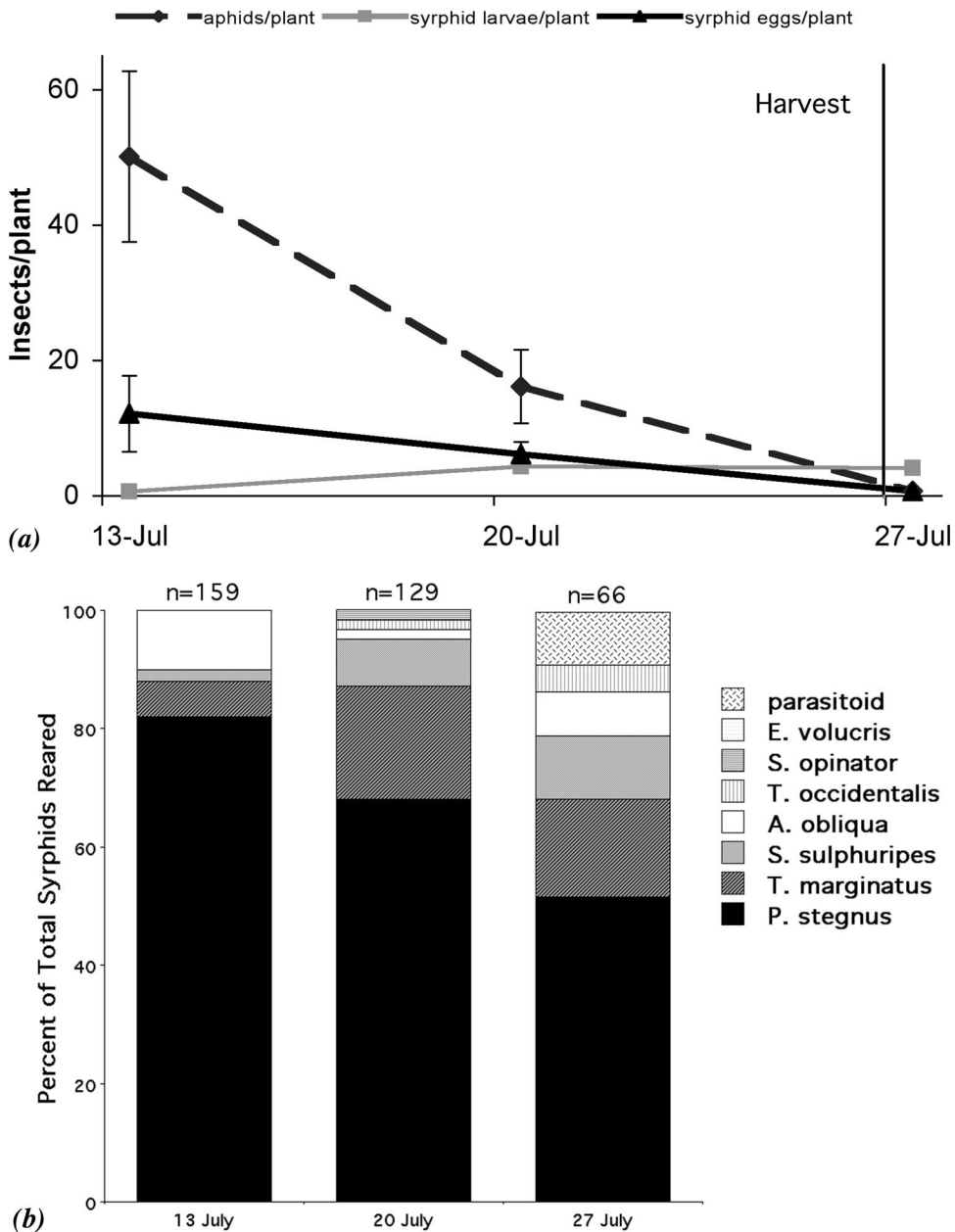


Fig. 4. Aphid and syrphid populations at a high-aphid density site (Aromas). (a) Average \pm SEM per plant densities of aphids, syrphid eggs, and syrphid larvae. Planted, 30 May; harvested, 27 July. $n = 20$ romaine plants per sample date. (b) Syrphid species composition and percentage of parasitism of reared syrphids from each sample date.

ing two or fewer aphids per plant (Figs. 1a and 2a). Average \pm SEM per plant aphid infestation at the moderately infested Gilroy site peaked at 18.5 ± 4.9 1 wk before harvest (Fig. 3a). The Aromas and Salinas fields were highly infested. Average per plant aphid density was 49.6 ± 12.58 at the Aromas site 2 wk before harvest (Fig. 4a), and 88.5 ± 20.62 at the Salinas site 1 week before harvest (Fig. 5a). The Greenfield site was planted with *Nasonovia*-resistant romaine, and the

few aphids present were *M. euphorbiae*. The San Ardo and Gilroy sites contained a mixture of *N. ribisnigri* and *M. euphorbiae*. The two highly infested sites were predominantly *N. ribisnigri*, but they also contained *M. euphorbiae* and *A. solani*.

A complex of syrphid species was reared from each site, with three species present at the lower density sites, and four to seven species present at the moderate- and high-density sites (Figs. 1b–5b). The species

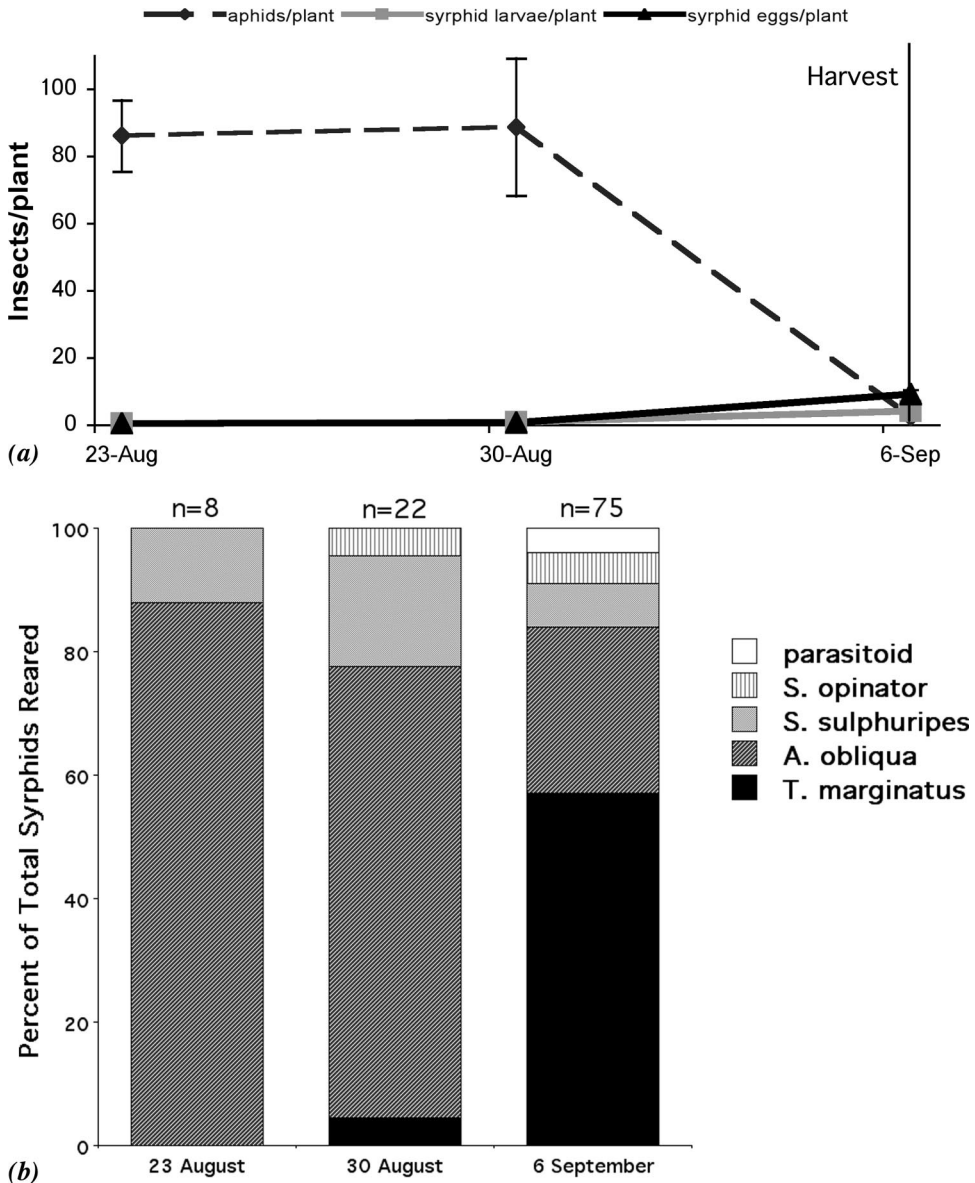


Fig. 5. Aphid and syrphid populations at a high aphid density site (Salinas). (a) Average \pm SEM per plant densities of aphids, syrphid eggs, and syrphid larvae. Planted, 10 July; harvested, 6 September; $n = 20$ romaine plants per sample date. (b) Syrphid species composition and percentage of parasitism of reared syrphids from each sample date.

mixture was different at each site. *T. marginatus* was the only species reared from each site. *T. marginatus* tended to be the predominate species at the low- and moderate-density sites, making up 47–79% of the syrphids reared (Figs. 1b–3b).

P. stegnus made up 71% of the syrphids reared from the high-density Aromas site (Fig. 4b). *T. marginatus* contributed 13% at this site, and five additional species comprised the remainder. *S. sulphuripes* and *A. obliqua*, the third and fourth most common species in the general survey, were reared from four of the five sites. Each made a substantial contribution to the syrphid

complex at only one site: *S. sulphuripes* made up 40% of the syrphids at the San Ardo site (Fig. 1b), and *A. obliqua* made up 41% at the Salinas site (Fig. 5b).

Parasitism of syrphid larvae was proportionally higher at the low- and moderate-density aphid infestation sites than at the Aromas and Salinas sites, where densities of aphids and syrphid larvae were higher. Ten percent of the syrphid larvae collected from the low-density sites were parasitized (Figs. 1b and 2b). Fifty-seven percent of the larvae collected on the last sample date from the moderately infested site were parasitized (Fig. 3b). Parasitism at the Aromas and

Table 1. Percentage of romaine plants with syrphids and other natural enemies at five principle survey sites

Site	% plants		Other natural enemies present
	w/syrphid eggs or larvae	w/other natural enemies	
San Ardo	71	60	Dwarf spiders, big-eyed bugs, lacewings, minute pirate bugs
Greenfield	61	22	Ladybird beetles, dwarf spiders, big-eyed bugs, lacewings, minute pirate bugs
Gilroy	85	16	Ladybird beetles, dwarf spiders, minute pirate bugs, lacewings, parasitized aphids
Aromas	97	27	Dwarf spiders, big-eyed bugs, lacewings, predatory thrips
Salinas	60	34	Ladybird beetles, dwarf spiders, aphids killed by fungi, minute pirate bugs, lacewings, parasitized aphids

Salinas sites was 2 and 3%, respectively (Figs. 4b and 5b). Parasitism of syrphid larvae tended to increase with crop maturity (Figs. 1b–5b).

Oviposition. *Toxomerus marginatus* and *S. sulfuripes* were the only species that were commonly reared from romaine plants that had few or no detectable aphids. They also were reared from moderately and highly infested plants. *P. stegnus* was only reared in significant numbers from highly infested plants. *P. stegnus* also demonstrated unusual oviposition behavior, laying eggs in parallel, contiguous clusters. It was not unusual to find clusters of five to seven *P. stegnus* eggs, and we found one cluster of 18 eggs. All other species of syrphid collected from romaine during the course of this survey laid eggs singly or occasionally in groups of two or three.

Of the 290 *T. marginatus* that we reared from the egg stage, almost half (48%) were reared from plants on which we detected no aphids. More than half (55%) of the 31 *S. sulfuripes* reared from the egg stage came from plants on which we detected no aphids. By contrast, only 1% of the 188 *P. stegnus* reared from the egg stage were collected from plants on which we did not detect aphids. Only 4% of the 49 *A. obliqua* reared from the egg stage were collected from plants with no detectable aphids.

The average number of eggs per plant was low for *T. marginatus* (2.14 ± 0.14), *S. sulfuripes* (1.19 ± 0.08), and *A. obliqua* (1.88 ± 0.44). The average number of *P. stegnus* eggs reared per plant was 7.52 ± 2.41 . The average number of aphids per plant on which eggs of the following syrphid species were collected was 7.73 ± 1.78 for *T. marginatus*, 15.08 ± 9.47 for *S. sulfuripes*, 85.72 ± 26.96 for *P. stegnus*, and 102.11 ± 33.28 for *A. obliqua*. It was common to rear more than one species of syrphid from the same romaine plant.

The data set was subjected to logistic regression analysis with whole plant aphid infestation divided into two classes, plants with zero to two aphids, and those with three or more aphids. Variance due to site by date interaction was about one fourth of residual error (0.03653 versus 0.3152). The species by aphid class interaction was significant ($P < 0.0001$). *P. stegnus* and *A. obliqua* were more likely to lay eggs on romaine with three or more aphids (odds ratio, OR = 10.2 and OR = 6.5 respectively). *T. marginatus* was more likely to lay eggs on romaine with two or fewer aphids (OR = 3.5). *S. sulfuripes* did not exhibit a tendency based on our data.

With the exception of the Salinas site, syrphid larval densities were usually lower than syrphid egg densities until near harvest, at which point observed larval densities rose slightly above egg densities (Figs. 1a–4a). The shift in predominance from eggs to larvae was sometimes evident on the species level. For example, at the Aromas site (high aphid infestation), the ratios of eggs to larvae for *P. stegnus* from the 20 plants collected each week were 123:7 (13 July), 45:39 (20 July), and 1:33 (27 July, harvest). Egg-to-larval ratios for *T. marginatus* from the same sample dates were 9:0, 7:17, and 2:9. The tendency of *P. stegnus* to oviposit in clusters of eggs may produce a distribution in the field that is distinct from species that tend to lay eggs singly, such as *T. marginatus*.

Other Natural Enemies. Other natural enemies recovered from romaine at the five principle survey sites included ladybird beetles (Coccinellidae), dwarf spiders (Linyphiidae: Araneae), big-eyed bugs (*Geocoris* sp.: Lygaeidae), minute pirate bugs (*Orius* sp.: Anthrenidae), green lacewings (*Chrysopa* and *Chrysoperla* spp.: Chrysopidae), brown lacewings (*Hemerobius* spp.: Hemerobiidae), and predatory thrips (Thysanoptera) (Table 1). In addition, we found aphids that had been parasitized and killed by fungi. The number of romaine plants harboring natural enemies other than syrphids ranged from 16% at the Gilroy site to 60% at the San Ardo site (Table 1).

Discussion

This survey confirmed that there are several syrphid species involved in suppressing *N. ribisnigri* and other aphid pests of organic lettuce to below economically significant levels on California's Central Coast. *T. marginatus*, *P. stegnus*, *S. sulfuripes*, and *A. obliqua* are the most important syrphid predators of aphids in organic romaine on the Central Coast, but in most commercial organic fields that were studied, combinations of these species as well as rarer species were present. *T. marginatus* was present at each of the five repeatedly sampled sites and predominated at three of them, but at only one site (Greenfield) did it seem to be the sole significant player.

Conservation biological control of *N. ribisnigri* and other aphids in romaine may be successful in part because the syrphid complex includes species that exploit distinct niches. *T. marginatus* and *S. sulfuripes* eggs were frequently found on romaine plants with

few or no detectable aphids, whereas *P. stegnus* was only reared in significant numbers from highly infested romaine. *T. marginatus* and *S. sulfuripes* apparently will oviposit on lettuce that does not present sufficient aphids as food for the completion of the syrphid larval stage. We frequently collected eggs of *T. marginatus*, and to a lesser extent *S. sulfuripes*, from large commercial romaine fields that were essentially free of aphids and other prey. Chandler (1968a,b) demonstrated that some syrphid species (*Platycheirus peltatus* Meigen, *Platycheirus manicatus* Meigen, *Melanostoma scalare* F., and *Melanostoma mellinum* L.) will oviposit on uninfested plants. However, the plants in these experiments were adjacent to plants infested with aphids, which may have produced oviposition stimulants. Several factors may influence syrphid oviposition, including aphid density, the presence of honeydew or cornicle excretions, and host plant characteristics (Peschken 1965, Chandler 1968a, Smith 1969, Chambers 1988, Budenberg and Powell 1992, Barga et al. 1998, Belliure and Michaud 2001, Sutherland et al. 2001).

The survey results did not help explain why syrphids sometimes fail to clean up aphid infestations before harvest, other than to reinforce the notion that syrphid populations can vary significantly from location to location in regard to their species make up. Environmental conditions vary from farm to farm, and it is possible that syrphid predation is inadequate in some fields because natural and insectary floral resources are insufficient to support the necessary level of oviposition by females. Failed control also may be due to timing: it is possible that romaine must be infested early in its cycle to give the syrphids adequate time to build up. Parasitoids may impact the effectiveness of syrphids at some sites. The failure rate is sufficiently low around the Salinas Valley that experienced organic lettuce growers suffer minimal losses. It is noteworthy that although no two organic lettuce producers on the Central Coast follow the same protocol for insectary crops or pest management, they experience similar levels of success in the production and marketing of organic lettuce and similar levels of loss to *Nasonovia ribisnigri* and other aphids.

Other Natural Enemies. Predators of aphids other than syrphids were found at all sites, although usually in low numbers. Entomopathogenic fungi infected aphids at some sites, although evidence of fungal pathogens was more common during the spring rains, before repeated crop sampling was initiated. Parasitized aphid mummies were extremely rare in *Nasonovia* colonies, which form in the inner leaves of the romaine plant, an area inaccessible to most parasitoids. It seems likely that under certain conditions other natural enemies, especially fungal pathogens, may contribute significantly to aphid mortality in organically grown lettuce. It also seems likely that predators such as *Orius* spp., lacewings, and spiders may prey on syrphid eggs or larvae, and could under some conditions enhance aphid survival by suppressing syrphid populations. Competition among natural enemies can produce a variety of outcomes (Rosenheim 1998). The

impact of natural enemies other than syrphids on aphid colonies is not clear and is being investigated in 2006.

Acknowledgments

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