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Impact of a natural enemy overwintering refuge and its interaction with the surrounding landscape

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Abstract 1. Egg parasitoids in the genus *Anagrus* (Hymenoptera: Mymaridae) are important mortality factors for grape leafhoppers (*Erythroneura elegantula*; Homoptera: Cicadellidae) in California vineyards, yet must overwinter in habitats external to these vineyards. Existing evidence suggests that French prune trees, which harbour the overwintering host *Edwardsiana prunicola*, planted adjacent to vineyards may enhance early-season abundance of *Anagrus*.

2. *Anagrus* overwintering in French prune tree refuges were labelled with the trace element rubidium in four separate experiments. Rubidium-labelled *Anagrus* were captured in adjacent vineyards in two of the experiments, confirming that French prune trees contribute to early-season *Anagrus* populations. *Anagrus* from refuges were captured at the most distant sampling positions, 100 m from refuges.

3. Use of rare element labelling has, for the first time, enabled the relative contribution of different sources to early-season colonization by this parasitoid to be quantified. Refuges contributed 1% and 34% of *Anagrus* colonizing two of the experimental vineyards, respectively. The remainder originated from overwintering habitats external to the French prune/vineyard system.

4. The spatial patterns of *Anagrus* originating from external overwintering habitats suggest that the French prune trees are generating a 'windbreak effect'. *Anagrus* dispersing within the windstream colonized vineyards at a higher-than-average rate immediately downwind of refuges.

5. The amount of colonization by *Anagrus* from external overwintering habitats was apparently related to the distance to presumed overwintering habitats. These findings demonstrate that both the number of natural enemies emerging from a refuge and the composition of the surrounding landscape are important in determining the impact of local, small-scale habitat manipulations.

Key words. Agricultural landscape, habitat management, refugia, rare element labelling, colonization, dispersal, egg parasitoid, Mymaridae, leafhoppers, grapes.

Introduction

A major factor influencing arthropod populations in crops is the vegetational landscape of the agroecosystem (Perrin, 1980). The agricultural landscape consists of: (1) the agricultural field (consisting usually of a single crop and any weeds present, but sometimes including additional crops or a cover); (2) any native and/or weedy vegetation that may be present on its borders; (3) the surrounding agricultural fields; and (4) the vegetation occurring in native or uncultivated habitats in the surrounding area. The composition of the agricultural landscape determines the presence of overwintering sites and the ability of an insect to locate appropriate habitats and food resources over the course of its lifetime (Margolies & Kennedy, 1985; Wratten & Thomas, 1990; Landis & Haas, 1992). The tendency over recent decades has been towards simplification of the agricultural landscape by: (1) planting large, monocultural fields; (2) removing native and weedy vegetation on field borders; (3) planting multiple fields in the same region to the same crop; and (4) removal, development, or cultivation of native habitats within agricultural areas. In addition to the benefits with respect to ease of agronomic management, this simplification of the landscape has, in certain cases, been for the explicit purpose of managing arthropod pests by, for example, removing overwintering habitats (Perrin, 1980; Herzog & Funderburk, 1989). It has been forcefully argued in recent years, however, that simplification of the agricultural landscape has also resulted in decreased abundance and activity of natural enemies as a result of the removal of critical food resources and overwintering sites

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(Fye, 1972; Dempster & Coaker, 1974; Mayse, 1983; Russell, 1989; Herzog & Funderburk, 1989; Pickett *et al.*, 1990; Wratten & Thomas, 1990; Andow, 1991; Landis & Haas, 1992; Pickett & Bugg, 1995).

The planting of vegetation adjacent to agricultural fields that provides overwintering sites for entomophagous arthropods has been proposed as a means of increasing local natural enemy abundance and thereby overcoming the effects of landscape simplification. Evaluating the impact of overwintering refuges, however, is difficult. Studies comparing natural enemy activity in plots with and without an overwintering refuge can establish whether there is an overall effect (e.g. Murphy, 1994), but cannot always by themselves identify the mechanisms behind the effect. Two factors are of particular importance in evaluating the impact of refuges on natural enemy abundance on an adjacent crop. Firstly, natural enemies colonizing the crop may originate either from the overwintering refuge or from other overwintering sites in the surrounding agricultural landscape, i.e. 'external' sources (e.g. Bishop & Reichert, 1990). The relative contribution of these sources will determine the importance of the refuge with respect to colonization by the natural enemy. The origin of natural enemies is not necessarily apparent, unless they: (1) have low mobility and (2) exhibit strong spatial patterning relative to the refuge. Such a result has been found, for example, for ground beetles dispersing from weedy strips in which they had overwintered (Thomas *et al.*, 1991) and for predatory mites dispersing into cotton from strips of alfalta (Corbett *et al.*, 1991). For highly mobile natural enemies such spatial patterning is not necessarily expected (Corbett & Plant, 1993), and identifying the origin of individuals colonizing the crop must be accomplished in some other way. Secondly, the impact of a refuge is dependent on the spatial extent of its influence on natural enemy abundance, which is determined by the distance to which natural enemies disperse into the crop following spring emergence. Again, since natural enemies in the crop may have originated from the refuge or from external sources, it is necessary to identify their origin in order to assess the spatial distribution of individuals originating from the refuge.

This paper addresses these issues through rare element labelling of the egg parasitoid *Anagrus* spp. (Hymenoptera: Mymaridae) overwintering in a refuge using the trace element rubidium (Corbett *et al.*, 1996), and subsequent recapture of labelled individuals in an adjacent crop. The use of rubidium as a chemical label to study insect dispersal has been reviewed by Akey *et al.* (1991). Due to its chemical properties, rubidium behaves as a chemical analogue to potassium and therefore readily replaces potassium in biological tissues. Rubidium occurs in plant and insect tissues at fairly low and uniform levels (across geographic areas); therefore treating vegetation with relatively low concentrations of Rb can result in levels in insects that are detectable above the natural background concentration for a given species, resulting in a reliable label. As a result of these favourable properties of rubidium, it has been used successfully to mark and study the dispersal of a variety of entomophagous and herbivorous insects through application of rubidium-enriched solutions to the host plant (Akey *et al.*, 1991). In the work presented here, rubidium has been applied to vegetation of an overwintering refuge, resulting in individual *Anagrus* spp. emerging in the spring with detectable rubidium labels.

Anagrus and French prune tree refuges

Anagrus spp. are minute (*c.* 0.5 mm in length), solitary egg parasitoids that attack eggs of various leafhopper species associated with both agricultural and native habitats in California. They are proovigenic, relatively short-lived parasitoids. *Anagrus* spp. are an important mortality factor for the grape leafhopper, *Erythroneura elegantula* (Homoptera: Cicadellidae), and to a lesser extent for the variegated leafhopper, *E. variabilis*, which are important pests of grapes in the western United States (Settle & Wilson, 1990). Parasitism of grape leafhopper by *Anagrus* spp. can reach levels in excess of 90% by the end of the season. Traditionally, *Anagrus* spp. associated with leafhoppers in grapes have been placed in the species *Anagrus epos* Girault. Recent work, however, has suggested that *Anagrus* spp. in vineyards consists of a complex of species and biotypes, including a new species *A. erythroneurae* (Trjapitzin & Chiappini, 1994; Trjapitzin *et al.*, 1995). For simplicity, the *Anagrus epos/A. erythroneurae* species complex is hereafter referred to simply as *Anagrus*.

Anagrus cannot overwinter in grapes because it requires an overwintering host egg and the grape and variegated leafhoppers overwinter as adults. As a result, *Anagrus* must colonize vineyards from external overwintering sites which are sometimes far removed in the extensive agricultural production of the San Joaquin Valley. Doutt & Nakata (1973) established that *Anagrus* overwinters naturally in eggs of a native leafhopper that overwinters as eggs in blackberries, which occur commonly in riparian habitats. *Anagrus* reared from blackberry leafhopper eggs also attacked and successfully emerged from grape leafhopper eggs (Doutt & Nakata, 1973; Williams, 1984), confirming that the same *Anagrus* are capable of exploiting leafhoppers in both riparian and vineyard habitats. Thus the numerous riparian corridors passing through the San Joaquin Valley are considered to be a major overwintering site for *Anagrus* attacking grape leafhoppers.

Kido *et al.* (1984) established that the prune leafhopper, *Edwardsiana prunicola* (Homoptera: Cicadellidae), which occurs in significant numbers on French prunes, can serve as an overwintering host for *Anagrus*. Subsequent studies (Pickett *et al.*, 1990) provided preliminary evidence that the presence of French prune trees adjacent to grape vineyards may increase the abundance of *Anagrus* in grapes and result in enhanced natural control of the grape leafhopper. Murphy (1994) has recently completed a rigorous evaluation of the effectiveness of French prune tree refuges in increasing control of the grape leafhopper. Results from this study indicate that there is a consistent and significant pattern of higher parasitism in grape vineyards with adjacent prune tree refuges than in vineyards lacking refuges.

Through assessing the rubidium content of *Anagrus* captured in vineyards adjacent to treated French prune tree refuges, it is intended to answer the following questions concerning the role of refuges: (1) Are French prune tree refuges directly contributing to *Anagrus* colonizing adjacent vineyards early in the season? (2) If so, what proportion of early season colonizers originate from adjacent refuges, and what proportion originate from overwintering habitats external to the French prune/vineyard system (e.g. riparian habitats)? (3) What is the spatial distribution within vineyards of *Anagrus* that originated from refuges and those that originated from external overwintering habitats?

Materials and Methods

Study sites. Anagrus was labelled in two French prune tree refuges, adjacent to vineyard blocks, during each of two seasons. For study of *Anagrus* emergence in spring of 1992, two sites were used on the E. & J. Gallo Ranch near Livingston, Merced Co., California. The 'Hayes' site consisted of sixty French prune trees arranged in two east-west oriented rows, parallel to vinerows, within a vineyard block with no separation between refuge and vineyard on either side. The site was a few hundred metres south of an extensive riparian habitat along the Merced River. There were ten vinerows planted to the north of the refuge; land within a few kilometres south of the study site was planted almost exclusively to grape vineyards. The second site, 'D20', consisted of fifty trees planted in a single east-west oriented row, parallel to vinerows. Vinerows were immediately adjacent to the refuge to the south, and separated by 4 m from the refuge to the north by a dirt road. The site was 2.5 km south of the Merced River. Land up to the Merced River to the north of the study site and within a few kilometres to the south was planted almost exclusively to grape vineyards. Winds in this region come primarily from the north and northwest during April and May, the primary period of emergence of overwintering *Anagrus*.

For spring emergence in 1993 the D20 site was used again and a French prune tree block at Chalone Vineyards, 20 km east of Soledad, Monterey Co., California. The Hayes site was not used for 1993 due to disease problems with the trees. The fact that the same site was used in two consecutive years does not present a problem with respect to rubidium contamination across years. The rubidium contained in the prune foliage would have been deposited primarily in the soil directly beneath the prune canopy; if there were any effect it would have been to increase the overall labelling achieved in prunes the second year (in which all trees were treated).

The Chalone site consisted of forty-eight French prune trees planted in three east–west oriented rows. The adjacent vineyard block was separated by 15 m from the refuge to the south by a dirt road. Vinerows at this site were oriented along a north–south transect, perpendicular to the refuge orientation. There were buildings and bare ground immediately to the north of the refuge and an additional vineyard block to the north. Chalone Vineyards is a relatively small operation isolated from other grape vineyards. This site, unlike the Merced Co. sites, is located in a hilly region and is surrounded primarily by grasslands, with some wooded habitats in nearby canyons. The nearest major riparian habitats exist along the Salinas River, more than 10 km to the west. Winds at the Chalone Site come primarily from the north during April and May.

Elemental labelling of Anagrus. The methodology employed for labelling *Anagrus* overwintering in French prune tree refuges is described in detail in Corbett *et al.* (1996). To obtain labelled *Anagrus* emerging in the spring, prune foliage was sprayed three to four times during September and October of the previous year with 5000 ppm solutions of Rb. The Rb applications were timed to coincide with the development of the final generation of prune leafhopper nymphs; adults of this generation would be ovipositing overwintering eggs. In the 1992 study, one-half of the trees in a refuge were treated with rubidium; in 1993 all trees in a refuge were treated. Treatments resulted in greatly increased rubidium concentrations in prune foliage, prune leafhopper adults, and prune leafhopper eggs (Corbett *et al.*, 1996). *Anagrus* emerging from treated trees the following spring had an average rubidium content 3.9 times that of individuals collected from untreated control vineyards (0.205 ng [$n = 77$] per individual versus 0.052 ng [$n = 498$]). The distributions of Rb content in treated and control *Anagrus* overlap, but there is a pronounced tail of high Rb content in the treated group (Fig. 1a). The difference in these two distributions has been used to interpret the Rb content in *Anagrus* collected from grape vineyards adjacent to treated refuges, as will be discussed in detail below.

Sampling and analysis of Anagrus. Anagrus were collected in adjacent grape vineyards in the spring on 25.4 × 25.4 cm yellow vinyl cards coated with petroleum jelly. Cards were suspended from trellis wires within the vine canopy using 3/4 inch binder clips. Since the vine canopy fills in quickly early in the season, captures on cards are more likely to represent individuals that were foraging within the canopy in the immediate vicinity of the card than individuals being intercepted during windborne dispersal or being attracted to the card from a distance.

At the Hayes and D20 sites cards were deployed at multiple distances both upwind (north) and downwind (south) of refuges, with five cards at each distance. At the Hayes site in 1992 cards were placed in the following vinerows, with the refuge at zero and positive values being downwind: −10, −5, −3, −1, 1, 3, 5, 10, 17 and 25. Cards were placed in the same vinerows at the D20 site in 1992 with the exception of the absence of the −10 vinerow position. At the D20 site in 1993 cards were placed in the following vinerows: −5, −3, −1, 1, 3, 5, 10, 20 and 30. At the Chalone site all cards were placed downwind along five vinerows spaced ten vinerows apart. In each of these vinerows, cards were placed within vines at the following number of vines from the northern end of the row: 1, 10, 20, 30, 40, 60 , 80, 100, 120 and 140 vines. Cards were deployed weekly from 4 April to 10 May in 1992 and from 30 March to 2 June in 1993. In 1992, cards were suspended for 3 days at a time each week; in 1993, cards were left suspended in the vineyard for a full week.

Cards were examined under a dissecting microscope to find all *Anagrus*. *Anagrus* were removed from cards, rinsed in hexane to remove petroleum jelly (to facilitate subsequent chemical analysis), and placed individually in vials. Individuals were digested by a wet-oxidation procedure using concentrated nitric acid and hydrogen peroxide, then diluted to obtain a final sample volume of 50 µl. Rubidium content was determined using flame atomic emission spectroscopy in which readings were maximized by consuming the complete 50 µl sample in a single reading. Resulting measurements were in nanograms of Rb per individual wasp. Details on the digestion and spectroscopy methods are provided in Corbett *et al.* (1996). A total of 1825 *Anagrus* were analysed for Rb content over the 2 years of this study; 904, 140, 686 and 95 at the Hayes and D20 sites in 1992 and at the D20 and Chalone sites in 1993, respectively.

Interpreting rubidium content of Anagrus. The conventional approach that has been used to interpret rubidium content is to identify an individual as 'labelled' if its Rb content is greater than three standard deviations above the mean native (i.e. naturally occurring or 'endogeneous') rubidium content (VanSteenwyk, 1991). This is appropriate if the native distribution is normal and

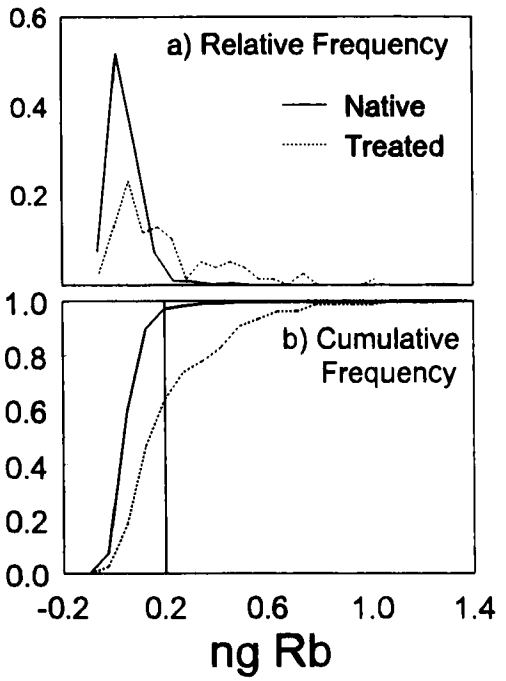

Fig. 1. Relative (a) and cumulative (b) frequency distribution of rubidium content in *Anagrus* collected from untreated grape vineyards (n = 498) and from rubidium-treated French prune trees (n = 77).

if there is little or no overlap in Rb content of insects from the treated vegetation and from the endogeneous population. This approach results in a probability of 0.001 of incorrectly classifying an individual as labelled, and a negligible probability of not detecting an individual that belongs to the treated group. There are numerous aspects of this study that suggest this approach to be inappropriate (see Fig. 1a): (1) neither the native nor the treated distributions of Rb content are normal, (2) there is a large degree of overlap between the treated and native distributions, and (3) a small number of specimens from the native population produced high measurements of Rb content ($p[Rb > 1.2] \leq 0.0041$).

Rather than attempting to categorize each individual as either labelled or unlabelled, a statistical approach has been used in interpreting the Rb content of *Anagrus*. Specifically, all individuals captured across a 2 or 3 weeks sampling period at a site are grouped into a single sample. For each of these samples of *Anagrus* we ask: what is the likelihood that the sample was drawn from the native population? If this likelihood is small then we can reject the 'null hypothesis' that all *Anagrus* are native, and can instead conclude that the Rb-treated French prune trees contributed to the *Anagrus* obtained in the sample. We answer the question 'what is the likelihood that the sample was drawn from the native population?' by executing the following steps for each sample: (1) calculate an appropriate parameter from the sample; (2) obtain a sampling distribution of the parameter by simulating (on computer) a large number of samples from the native population; and (3) determine where the sample parameter falls within the simulated sampling distribution.

A new sample parameter Δ, which is based on the cumulative distribution of Rb content of *Anagrus* in the sample is used. Δ is defined as the two-dimensional area bounded by (1) $x = x_c$, (2) $y = 1.0$, and (3) the cumulative distribution curve of the sample, where x_c = 0.21 ng Rb. 97% of the native population falls below x_c whereas only 65% of the treated population falls below this value (Fig. 1b). The parameter Δ was chosen over other possible parameters such as the mean, mode, or 90th percentile because it contains more information regarding the difference between the treated and native populations; it therefore should provide more power with which to detect samples unlikely to have been drawn from the native population.

Consider a statistical population of *Anagrus* residing within a vineyard adjacent to a rubidium-treated refuge. A sample of n individuals is taken from this population and Δ is calculated from the cumulative distribution curve for Rb content of individuals in the sample. If Rb-treated French prune trees are contributing a significant proportion of the *Anagrus* in the vineyard, then a value as large or larger than Δ should be 'unlikely' to have been obtained from a sample of size n drawn from the native population. In order to quantify this likelihood, Δ must be compared with an appropriate sampling distribution, specifically, the Δ_N s obtained from a large number of samples of size n drawn from the native population. We represent the 'native population' using our sample of 498 adult *Anagrus* collected from grape vineyards near Livingston, California, in June of 1992 and near Lodi and Davis, California, in July 1993 (Fig. 1; see Corbett *et al.*, 1996). The likelihood that all of the *Anagrus* in the sampled population originated from the native population is defined as the probability of obtaining $\Delta_N \geq \Delta$ in a sample of size n. In the Appendix we describe in detail how we calculate Δ for samples of *Anagrus* and how we obtain the sampling distribution for Δ_N.

Assuming we reject the null hypothesis that all *Anagrus* in a vineyard originated from the native population, we can then proceed to estimate the proportional contribution by French prune tree refuges. We estimate this proportion as follows. The number in the sample with Rb content greater than 0.21 ng is tallied. This number is then divided by the sample size to estimate the proportion of the population with >0.21 ng Rb. Next, the proportion of the sample that would be expected to exceed 0.21 ng Rb, based on random sampling from the native population, is subtracted from this number. As indicated above, 0.022 of the native population is above this threshold. This gives an estimate of the proportion of 'labelled' *Anagrus* in the population (i.e. those originating *from the refuge* with Rb content >0.21 ng). This proportion is then multiplied by two correction factors for: (1) the proportion of the treated population above 0.21 ng Rb (0.35; see Fig. 1b); and (2) the proportion of trees in the refuge that were treated with rubidium. These steps are summarized as

$$R = \left(\frac{n_c}{n} - 0.022\right)\left(\frac{1}{0.35}\right)\left(\frac{1}{P_t}\right) \qquad (1)$$

where R is the estimated proportion of *Anagrus* from the refuge, n is the total number of individuals in the sample, n_c is the number of individuals in the sample with Rb content greater than 0.21 ng, and P_t is the proportion of trees in the refuge that were treated with rubidium. P_t is 0.5 for 1992 and for 1993 P_t = 1.0. Calculating R in this way results in a conservative estimate (i.e. underestimate) because the full 2.2% should only be subtracted when (n_c/n) approaches zero.

Our estimate of R obviously has some uncertainty associated with it. The largest component of this uncertainty in R is associated with estimating the proportion of the population that was labelled (i.e. emerging from treated trees with Rb content >0.21 ng), our confidence in the proportion labelled being dependent on the sample size and on n_c. To account for this source of uncertainty, 95% confidence limits were calculated for the first term in Eq. 1 using the F distribution (as described in Zar, 1984). Uncertainty in R was then determined by applying the two correction factors separately to the upper and lower 95% confidence limits. Since there are other potential sources of uncertainty in R, we refer to these adjusted confidence limits simply as 'uncertainty limits' on R.

Critical assumptions of our methodology. The validity of our hypothesis test, and of our estimate of proportional contribution by the refuge, is dependent on four critical assumptions. Firstly, we assume that all sampled *Anagrus* are colonizers and therefore did not originate *within* the vineyard; these first-generation *Anagrus* will not be labelled. To satisfy this assumption, sampling must be terminated prior to significant first-generation recruitment of *Anagrus* within the vineyard. There is generally a 4–8 week window following first appearance of *Anagrus* and prior to emergence of adults from grape leafhoppers; we terminated card sampling within 2 weeks of the first observation of emergence holes in grape leafhopper eggs in leaves collected from study vineyards.

Secondly, we assume that we have a valid estimate of the proportion of *Anagrus* emerging from treated prune trees with Rb content >0.21 ng. Obtaining *Anagrus* directly from twigs collected from the Hayes and D20 sites was difficult due to the low density of overwintering *Anagrus* at these sites and to the general difficulty of locating these individuals within the bark and subsequently isolating them at emergence. As a result, our frequency distribution of Rb content for treated *Anagrus* (Fig. 1a) was generated mostly (but not exclusively) from individuals obtained from other French prune tree orchards where prune leafhopper densities were much higher (Corbett *et al.*, 1996). Nevertheless, we accept the value 0.35 (in Eq. 1) as a valid estimate for out study sites because: (1) French prune trees were treated with equivalent amounts and timing of Rb solutions, and (2) Rb treatments resulted in similar elevations in Rb concentrations in foliage and prune leafhoppers at all sites (Corbett *et al.*, 1996).

Thirdly, we assume that *Anagrus* retain elevated levels of rubidium throughout their adult life. A laboratory evaluation of retention of Rb indicates that *Anagrus* emerging with >0.21 ng Rb still have levels well in excess of 0.21 ng for at least 48 h following emergence (Corbett *et al.*, 1996); individuals in this study did not live more than 72 h. Thus a 'cohort' of *Anagrus* emerging from treated French prune trees remains distinguishable from the native population as it ages, with a larger proportion having Rb content greater than 0.21 ng.

Finally, we assume that our sample of the 'native population' (i.e. the 498 *Anagrus* from untreated vineyard; Corbett *et al.*, 1996) is representative of the Rb content of *Anagrus* colonizing vineyards from all sources other than treated French prune trees. There are two notable ways in which this assumption might be violated. Firstly, there may be regional variation in Rb content of *Anagrus*. Our sample of the native population comes from three separate vineyards near Livingston, Lodi and Davis, California. The proportion of *Anagrus* in these samples having Rb content <0.21 ng was 94.7% ($n = 75$), 97.3% ($n = 291$) and 98.5% ($n = 130$), respectively. In contrast, the proportion of *Anagrus* from treated French prune trees having Rb content <0.21 ng was 64.5% ($n = 77$). There appears to be some regional variability in distribution of Rb content, but not enough to be of concern in our study. Secondly, there may be variation in Rb content of *Anagrus* between leafhopper hosts and/or host plants. We cannot address this question directly because we did not collect samples of *Anagrus* emerged from other hosts nor did we collect samples of early-season colonizers of non-treatment vineyards. However, we will use some of our results to indirectly address this problem.

Results

Colonization of vineyards by Anagrus from refuges

The null hypothesis that 'all *Anagrus* are from the native population' was rejected (with $P < 0.005$) during at least one time period at the Hayes site in 1992 and at the D20 site in 1993 (Table 1). This confirms that *Anagrus* overwintering in refuges contributed to early-season populations of *Anagrus* in adjacent grape vineyards at these sites.

Refuges did not contribute detectable numbers of *Anagrus* at the D20 site in 1992 or the Chalone site in 1993 (Table 1). The lack of contribution at the Chalone site is due to the fact that French prune trees at this site were sprayed by the grower with a pesticide (Lorsban) on 14 April 1993 to control an aphid outbreak. This was at the onset of emergence of *Anagrus* and therefore probably resulted in mortality of the vast majority of individuals. The lack of contribution at the D20 site in 1992 is probably due to the low abundance of prune leafhoppers and overwintering *Anagrus* observed at that site in the fall of 1992 and spring of 1992, respectively (pers. obs.). The lack of contribution at these sites has provided useful 'control' samples. The rubidium content of *Anagrus* colonizing the D20 site in 1992 and the Chalone site are consistent with our native sample (Table 1). This suggests that our native sample is generally representative of *Anagrus* colonizing vineyards from sources other than treated French prune trees.

Relative contribution of refuges and external sources

The largest contribution of *Anagrus* from French prune tree refuges was observed at the D20 site in 1993 (Table 2). The refuge was contributing an average of 0.63 individuals per trap during the last two sampling periods, which represented 45% and 22% of all colonizing *Anagrus* during those periods. The Hayes site refuge, in 1992, contributed an average of 0.14 individuals per trap only during the first, 3-week sampling period, which represented 9% of all colonizing *Anagrus* during that period. There was no detectable contribution by refuges for other dates and/or sites.

The largest contribution from external overwintering sources occurred at the Hayes site in 1992, with an average ranging from 1.6 to 9.9 individuals per trap. External sources contributed

Table 1. Summary of rubidium content of *Anagrus* and statistical tests for baseline samples and samples from study vineyards.

Sample source			*n*	No. >*Xc*	% >*Xc*	High values (ng Rb)	Δ	*P*†
Baseline samples								
Untreated vineyards			498	11	2.2	1.37, 1.24, 0.48	0.0066	0.29
Treated French prune trees			77	27	35.0	1.05, 0.73, 0.72	0.0781	<0.0001
Study vineyards								
1992	Hayes	8–18 April	266	10	3.8	1.62, 1.47, 1.25	0.0191	0.0013
		25 April to 10 May	638	22	3.5	2.21, 0.52, 0.43	0.0058	0.4
	D20	8–18 April	34	3	8.8	0.75, 0.25, 0.24	0.0182	0.098
		25 April to 10 May	106	8	7.6	0.45, 0.34, 0.27	0.0055	0.36
1993	D20	7–23 April	93	2	2.2	0.27, 0.26	0.0002	0.89
		30 April to 14 May	182	31	17.0	0.77, 0.58, 0.54	0.0272	0.0005
		21 May to 3 June	411	40	9.7	1.77, 1.04, 0.95	0.0212	<0.0001
	Chalone	7–23 April	17	0	0.0	–	0	0.34
		30 April to 14 May	36	0	0.0	–	0	0.59
		21 May to 3 June	42	3	7.1	0.44, 0.33, 0.28	0.0098	0.18

* *Xc* = 0.21 ng Rb.

† Probability of obtaining the sample from the native population (see Appendix).

smaller numbers of *Anagrus* at the D20 site in both 1992 and 1993. The average contribution at this site ranged from 0.3 to 2.4 individuals per trap. The lowest contribution from external sources occurrred at the Chalone site, with an average contribution less than 0.3 individuals per trap.

Spatial patterning of Anagrus

Anagrus from refuges colonized adjacent grape vineyards throughout the sampling area from 15 m upwind to 100 m downwind of the refuge (Figs 2a, 4b and 4c). The highest concentration of *Anagrus* from refuges was generally within the first five vinerows downwind. Some colonization also occurred immediately upwind of the refuge (Figs 4b and 4c). The relatively large number of *Anagrus* from refuges found at 80–100 m downwind (twenty-five to thirty vinerows) at the D20 site in 1993 (Fig. 4b) suggests that *Anagrus* may colonize grape vines beyond this distance.

Anagrus colonizing grape vineyards at the Hayes and D20 sites were not uniformly distributed within the study vineyards but, rather, exhibited clear spatial patterning (Figs 2–4, 'Total'). The patterning at the Hayes and D20 sites was related to the presence of French prune tree refuges: pronounced peaks in *Anagrus* density occurred consistently at three vinerows downwind of the refuges at these sites during both years. There was no such spatial pattern in *Anagrus* captures observed at the Chalone site (Fig. 5), where the vineyard was separated from the refuge by 15 m. *Anagrus* originating from refuges at the Hayes and D20 sites, as estimated by applying Eq. 1 to samples from each distance, also exhibited peaks in density at the third vinerow downwind of refuges but these peaks were less frequent and less pronounced than those for total *Anagrus* (Figs 2a, 4b and 4c, 'From Refuge'). Pronounced peaks in total *Anagrus* occurred at three vinerows downwind of refuges even when there was no detectable contribution from the refuge (e.g. Figs 2b, 3b and 4a), indicating that these patterns were not caused by *Anagrus* originating from the refuges.

Table 2. Relative contribution of prune tree refuges and external overwintering sources to *Anagrus* in study vineyards.

1992				1993			
% from refuge		No./card/week		% from refuge		No./card/week	
Dates	*R* (UL)*	Refuge	External	Dates	*R* (UL)	Refuge	External
Hayes				D20			
8–18 April	8.6 (2.9–19.2)	0.14	1.56	7–23 April	0.0	0.00	0.62
25 April to 10 May	0.0	0.00	9.91	30 April to 14 May	45.4 (31.6–58.6)	0.61	0.73
				21 May to 3 June	21.5 (15.8–28.5)	0.65	2.39
D20				Chalone			
8–18 April	0.0	0.00	0.26	7–23 April	0.0	0.00	0.10
25 April to 10 May	0.0	0.00	1.82	30 April to 14 May	0.0	0.00	0.22
				21 May to 3 June	0.0	0.00	0.28

* UL = uncertainty limits (see text for explanation).

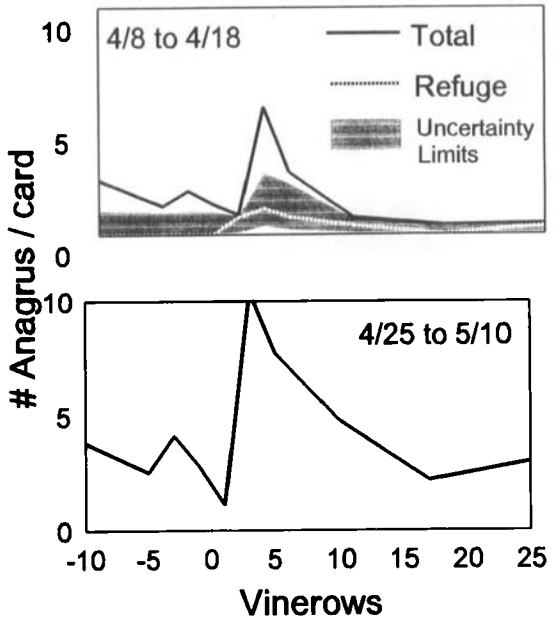

Fig. 2. Spatial distribution of *Anagrus* collected from Hayes site in 1992. French prune tree refuge is located at 0 on *x* axis. The number of *Anagrus* from the refuge is estimated using Equation 1.

Sex ratios of captured Anagrus

Gender was recorded for *Anagrus* collected from the D20 and Chalone sites in 1993. *Anagrus* from the D20 site had a significantly female-biased ratio of 1.6:1 ($n = 250$, $\chi^2 = 1568.00$, $P << 0.001$). *Anagrus* collected at the Chalone site, which we assume to be almost exclusively from overwintering sits external to the refuge/vineyard system, had a heavily female-biased ratio of 7.5:1 ($n = 34$, $\chi^2 = 19.9$, $P < 0.005$).

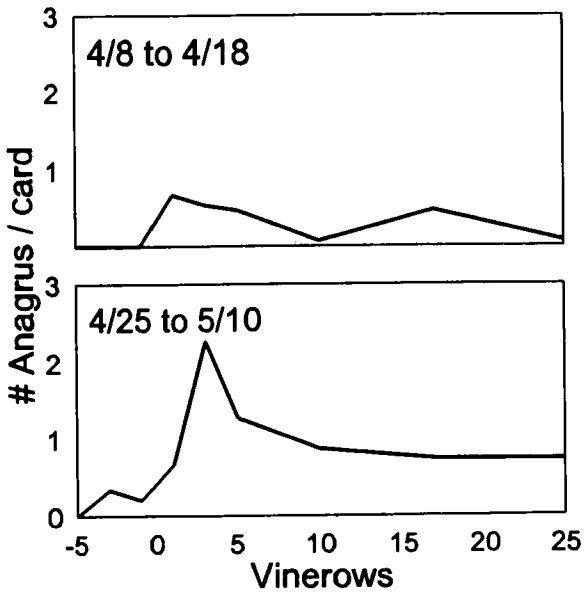

Fig. 3. Spatial distribution of *Anagrus* collected from D20 site in 1992. French prune tree refuge is located at 0 on *x* axis.

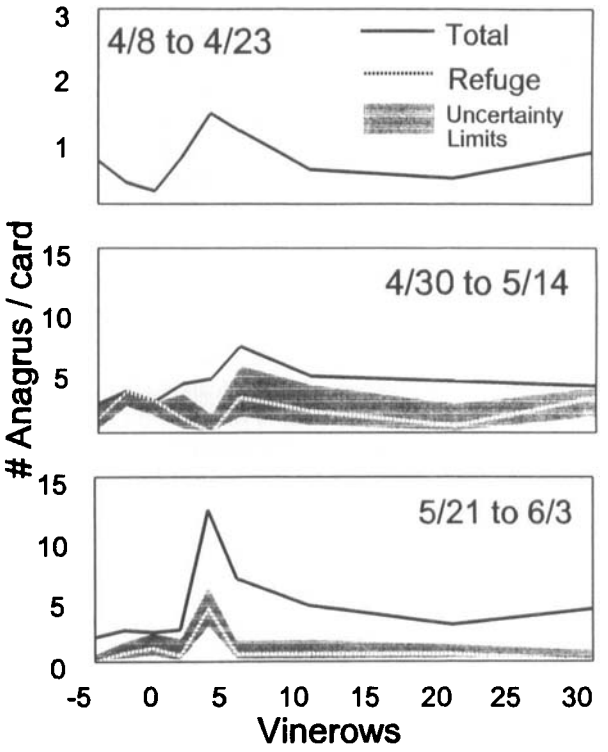

Fig. 4. Spatial distribution of *Anagrus* collected from D20 site in 1993. French prune tree refuge is located at 0 on *x* axis. The number of *Anagrus* from the refuge is estimated using Equation 1.

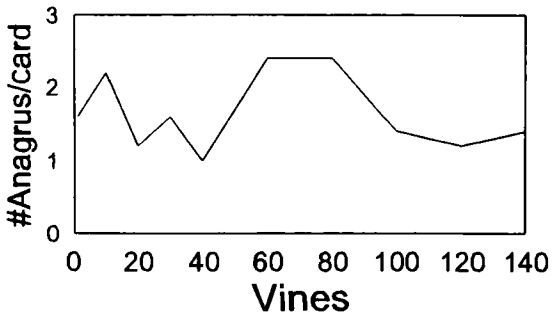

Fig. 5. Spatial distribution of *Anagrus* collected from Chalone site in 1993.

Discussion

French prune tree refuges at two study sites in the San Joaquin Valley have been confirmed as contributing to the abundance of *Anagrus* in adjacent vineyards. During the period of maximum contribution, 9% and 45% of the *Anagrus* colonizing these two vineyard blocks originated from the adjacent overwintering refuge (Table 2). This satisfies a basic requirement for the success of an overwintering refuge: significant numbers of the natural enemy disperse out of the refuge and colonize the adjacent crop prior to and during the initial build-up of the pest population. This result also provides, for the first time, direct confirmation of the impact of a non-crop habitat on the abundance of a natural enemy in an

adjacent crop. Murphy (1994) found that early-season parasitism rate by *Anagrus* was higher in vineyard blocks adjacent to French prune trees versus paired control blocks. The results suggest that these effects can, at least in part, be attributed directly to activity of *Anagrus* having emerged from French prune tree refuges. *Anagrus* originating from refuges were not confined to the area immediately adjacent to the refuge, but rather colonized vines out to 100 m, and perhaps further (Fig. 4b). Thus overwintering refuges for *Anagrus* have the potential to influence early-season abundance over a large spatial scale given sufficient numbers of *Anagrus* overwintering in the refuge.

Most of the *Anagrus* colonizers at the study sites were from overwintering habitats external to the vineyard/French prune system (Table 2). Doutt & Nakata (1973) found that early-season colonization by *Anagrus* was greater in vineyards that were closer to the Kings River (Fresno Co., California) and proposed that the numerous riparian corridors in the San Joaquin Valley are the major overwintering habitat for *Anagrus*. The exact origin of *Anagrus* colonizing from external sources is unknown; however, the results are consistent with Doutt & Nakata's proposal. The estimated capture of colonizers from external sources during 1992 was more than 4 times greater at the Hayes site, which is within 200 m of the Merced River, than at the D20 site, which is 2.5 km from the river (Table 2). Both the Hayes and D20 sites are south of the Merced River. Since prevailing winds are from the north and northwest, *Anagrus* dispersing from riparian habitat along the Merced River would have been carried toward these study sites. Again in 1993 the site nearest to a riparian habitat had the greatest colonization by *Anagrus* from external sources: the estimated capture of colonizers from external sources was more than 5 times greater at the D20 site versus the Chalone site, which is more than 10 km from any significant riparian habitat (Table 2).

Differences in colonization from external sources were partially responsible for the large difference in R estimated for the Hayes site in 1992 and the D20 site in 1993 (Table 2). Thus, the proximity of the vineyard to other external overwintering habitats (i.e. riparian corridors) was an important factor for the importance of refuges to early-season abundance of *Anagrus* at the study sites. Similar patterns were found by Bishop & Reichert (1990) in their studies of spider colonization in experimental plots. Wooded areas adjacent to experimental 'gardens' were rich in spider diversity and abundance. However, the species composition of spiders colonizing adjacent gardens suggested that the vast majority of colonizers originated from other, more distant sources. To the extent that regional-scale dispersal is important for other entomophagous insects (Pedgley, 1982), the composition of the surrounding landscape will probably have a large influence on the impact of any local, small-scale habitat manipulation.

Anagrus colonizing the study vineyards from external sources consistently exhibited a distinct spatial pattern: low abundance in the first vinerow downwind of French prune trees; a large increase at the third vinerow downwind; and, a gradual decline from this peak with increasing distance from the refuge (i.e. Figs 2b and 4c). This spatial pattern is identical to that documented by Lewis and co-workers (Lewis, 1965a, b; Lewis & Dibley, 1970) as a result of the influence of a 'windbreak' on dispersing insects. As air flows over a solid but permeable vertical structure, such as a lath fence, a turbulence zone is generated downwind which causes particles to fall out of the airstream

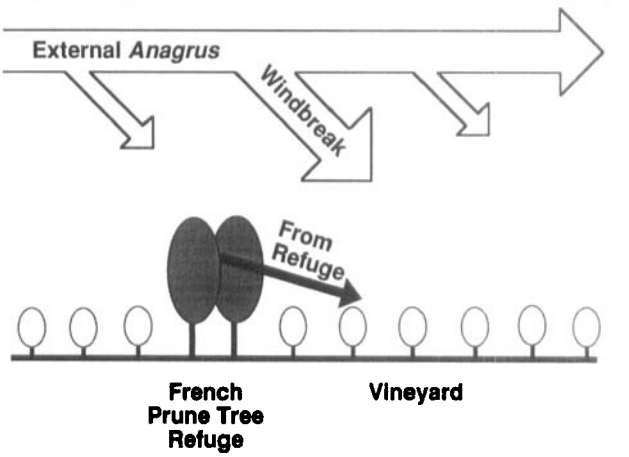

Fig. 6. Hypothesized sources of *Anagrus* colonizing vineyards early in the season. *Anagrus* colonize vineyards from adjacent French prune tree refuges. *Anagrus* also colonize from external overwintering sites. A windbreak effect generated by prune trees causes increased colonization by external *Anagrus* immediately downwind of refuges.

within this turbulence zone (Lewis & Dibley, 1970). It is likely that a 'windbreak' effect is operating in this system: *Anagrus* emerging from overwintering habitats external to the vineyard/French prune system are colonizing at a higher-than-average rate immediately downwind of the refuge as a result of the turbulence generated by French prune trees. French prune tree refuges are thus having two impacts on early-season abundance of *Anagrus*: (1) directly contributing *Anagrus* that have overwintered in the refuge, and (2) increasing the colonization rate by *Anagrus* having overwintered in habitats external to the French prune/vineyard system (Fig. 6). The amount of additional colonization generated by a windbreak effect of refuges is dependent on the proximity and size of external overwintering habitats, because *Anagrus* must be dispersing in large numbers in the windstream for a windbreak effect to cause increased colonization. Thus, refuges that are close to riparian habitats would generate high colonization, whereas refuges that are many kilometres away might generate no noticeable 'windbreak induced' colonization.

Anagrus emerging from overwintering hosts are apparently able to disperse over many kilometres to colonize vineyard habitats. This is consistent with dispersal capabilities exhibited by *A.delicatus*, which attacks eggs of the planthopper *Prokelesia marginata* (Delphacidae: Homoptera) in eastern saltmarshes (Antolin & Strong, 1987). *A.delicatus* were captured on islets off the Florida coast, 0.75 km or more from the nearest possible sources. Antolin & Strong (1987) found that colonizers of these distant islets were predominantly female, indicating that females are more prone to engage in long-distance dispersal. Isolated vineyards in California, such as the Chalone vineyard used in this study, may represent host 'islands' for dispersing *Anagrus*. Colonizers of the Chalone vineyard were 88% female; apparently females are more inclined to or more capable of traversing the 10 km separating this vineyard from the nearest major riparian corridors.

The range of dispersal distances exhibited by newly emerged *Anagrus* is quite large: an individual *Anagrus* might stay within the overwintering habitat, colonizing vines just a few metres from

its overwintering host (as for rubidium-labelled *Anagrus*), or it might colonize a vineyard many kilometres away (as seen at the Chalone site). These dispersal probabilities, together with the size and distribution of overwintering habitats within the landscape, were responsible for the colonization patterns observed in our study. The relative probability of dispersing various distances is unknown for *Anagrus* or for any other entomophagous insect. Dispersal distance is probably dependent on many factors including behavioural forces mediating the initiation and termination of a dispersal event (e.g. Blackmer & Byrne, 1993) and meteorological forces acting on an insect in flight (Pedgley, 1982). Studying these aspects of regional-scale dispersal will be critical to understanding the role of landscape structure on local insect abundance and to developing successful landscape manipulation approaches for the enhancement of entomophagous insects.

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References

Akey, D.H., Hayes, J.L. & Fleischer, S.J. (eds) (1991) Use of elemental markers in the study of arthropod movement and trophic interactions. *Southwestern Entomologist Supplement*, **14**.

Andow, D.A. (1991) Vegetational diversity and arthropod population response. *Annual Review of Entomology*, **36**, 561–586.

Antolin, M.F. & Strong, D.R. (1987) Long-distance dispersal by a parasitoid (*Anagrus delicatus*, Mymaridae) and its host. *Oecologia*, **73**, 288–292.

Bishop, L. & Riechert, S.E. (1990) Spider colonization of agroecosystems: mode and source. *Environmental Entomology*, **19**, 1738–1745.

Blackmer, J.L. & Byrne, D.N. (1993) Flight behavior of *Bemisia tabaci* in a vertical flight chamber: effect of time of day, sex, age and host quality. *Physiological Entomology*, **18**, 223–232.

Corbett, A., Leigh, T.F. & Wilson, L.T. (1991) Interplanting alfalfa as a source of *Metaseiulus occidentalis* (Acari: Phytoseiidae) for managing spider mites in cotton. *Biological Control*, **1**, 188–196.

Corbett, A., Murphy, B.C., Rosenheim, J.A. & Bruins, P. (1996) Labelling an egg parasitoid, *Anagrus epos* (Hymenoptera: Mymaridae), with rubidium within an overwintering refuge. *Environmental Entomology* (in press).

Corbett, A. & Plant, R.E. (1993) Role of movement in the response of natural enemies to agroecosystem diversification: a theoretical evaluation. *Environmental Entomology*, **22**, 519–531.

Dempster, J.P. & Coaker, T.H. (1974) Diversification of crop ecosystems as a means of controlling pests. *Biology in Pest and Disease Control* (ed. by D. P. Jones and M. E. Solomon), pp. 106–114. Blackwell Scientific Publications, Oxford.

Doutt, R.L. & Nakata, J. (1973) The *Rubus* leafhopper and its egg parasitoid: an endemic biotic system useful in grape-pest management. *Environmental Entomology*, **2**, 381–386.

Fye, R.E. (1972) The interchange of insect parasites and predators between crops. *PANS*, **18**, 143–146.

Herzog, D. & Funderburk J.E. (1989) Ecological bases for habitat management and pest cultural control. *Ecological Theory and Integrated Pest Management* (ed. by M. Kogan), pp. 217–250. John Wiley & Sons, New York.

Kido, H., Flaherty, D.L., Bosch, D.F. & Valero, K.A. (1984) French prune trees as overwintering sites for the grape leafhopper egg parasite. *American Journal of Enology and Viticulture*, **35**, 156–160.

Landis, D.A. & Haas, M.J. (1992) Influence of landscape structure on abundance and within-field distribution of European corn borer (Lepidoptea: Pyralidae) larval parasitoids in Michigan. *Environmental Entomologist*, **21**, 409–416.

Lewis, T. (1965a) The effects of an artificial windbreak on the aerial distribution of flying insects. *Annals of Applied Biology*, **55**, 503–512.

Lewis, T. (1965b) The effect of an artificial windbreak on the distribution of aphids in a lettuce crop. *Annals of Applied Biology*, **55**, 513–518.

Lewis, T. & Dibley, G.C. (1970) Air movement near windbreaks and a hypothesis of the mechanism of the accumulation of airborne insects. *Annals of Applied Biology*, **66**, 477–484.

Margolies, D.C. & Kennedy, G.G. (1985) Movement of the twospotted spider mite, *Tetranychus urticae*, among hosts in a corn-peanut agroecosystem. *Entomologia Experimentalis et Applicata*, **37**, 55–61.

Mayse, M.A. (1983) Culture control in crop fields: a habitat management technique. *Environmental Management*, **7**, 15–22.

Murphy, B.C. (1994) Testing a habitat diversity tactic for improving biological control: using prune trees as an overwintering habitat for enhancing the effectiveness of the parasitoid *Anagrus epos* (Hymenoptera: Mymaridae). Ph.D. dissertation, Department of Entomology, University of California, Davis.

Pedgley, D. (1982) *Windborne Pest and Diseases: Meteorology of Airborne Organisms*. John Wiley & Sons, New York.

Perrin, R.M. (1980) The role of environmental diversity in crop protection. *Protection Ecology*, **2**, 77–114.

Pickett, C.H. & Bugg, R.L. (eds) (1996) *Enhancing Natural Control of Arthropod Pests through Habitat Management*. AgAccess, Davis, California.

Pickett, C.H., Wilson, L.T. & Flaherty, D.L. (1990) The role of refuges in crop protection, with reference to plantings of French prune trees in grape agroecosystem. *Monitoring and Integrated Management of Arthropod Pests of Small Fruit Crops* (ed. by N. J. Bostonian and L.T. Wilson), pp. 151–165. Intercept Ltd, Dover.

Russell, E.P. (1989) Enemies hypothesis: a review of the effect of vegetational diversity on predatory insects and parasitoids. *Environmental Entomology*, **18**, 590–599.

Settle, W.H. & Wilson, L.T. (1990) Invasion by the variegated leafhopper and biotic interactions: parasitism, competition, and apparent competition. *Ecology*, **71**, 1461–1470.

Thomas, M.B., Wratten, S.D. & Sotherton, N.W. (1991) Creation of 'island' habitats in farmland to manipulate populations of beneficial insects. *Journal of Applied Ecology*, **28**, 906–917.

Trjapitzin, S.V. & Chiappini, E. (1994) A new *Anagrus* (Hymenoptera: Mymaridae), egg parasitoid of *Erythroneura* spp. (Homoptera: Cicadellidae). *Entomology News*, **105**, 137–140.

Trjapitzin, S.V., Daane, K.M. & Gonzalez, D. (1995) Biological control of the grape-infesting leafhoppers. *Erythroneura* spp., based on a study

of their egg parasitoids. 1994–1995 Research Report for California Table Grapes, Vol. 23. California Table Grape Commission, Fresno, California.

VanSteenwyk, R.A. (1991) The uses of elemental marking for insect dispersal and mating competitiveness studies: from the laboratory to the field. *Use of Elemental Markers in the Study of Arthropod Movement and Trophic Interactions* (ed. by D. H. Akey, J. L. Hayes and S. J. Fleischer), pp. 15–24. *Southwestern Entomologist Supplement*, **14.**

Williams, D.W. (1984) Ecology of a blackberry-leafhopper-parasite system and its relevance to California grape agroecosystems. *Hilgardia*, **52**, 2–32.

Wratten, S.D. & Thomas, C.F.G. (1990) Farm-scale spatial dynamics of predators and parasitoids in agricultural landscapes. *Species Dispersal in Agricultural Landscapes* (ed by R. G. H. Bunce and D. C. Howard), pp. 219–237. Belhaven Press, London.

Zar, J.H. (1984) *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, NJ.

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Appendix

The parameter Δ was calculated for each samples as

$$\Delta = \left(\sum_{k+1}^{n} (x_i - x_{i-1}) \left(\frac{n-i+1}{n} \right) \right) + (x_k - x_c) \left(\frac{n-k+1}{n} \right) \quad \text{(A.1)}$$

where n is the sample size, x_i are the measured rubidium contents of *Anagrus* in the sample (arranged in ascending order with x_1 being lowest and x_n being highest), x_c is the threshold Rb content, in this case 0.21 ng Rb, and k is the index of the first specimen with $x > x_c$.

We simulated a random sample of size n from the native population by obtaining n separate random deviates, each one being selected using a sequence of uniform random deviates (URD). (URDs were obtained using the 'RAN0' procedure from Press *et al.*, 1988.) The first URD was used to determine whether the random deviate was above or below x_c. If the URD was <0.988 an individual with a rubidium content $<x_c$ had been chosen; the sample count was incremented but no further processing was done with the individual. If the URD was >0.988, an individual with a rubidium content $>x_c$ had been chosen; two more URD were subsequently used to specify a rubidium content for this individual. The second URD was used to choose among seven discrete categories. These categories, and their probability distribution, were designed to simulate the observed frequency distribution of the native sample in the range 0.21–1.51 ng Rb (Table A1). The third URD was used to choose a specific random deviate from within the category, with all values within the range having equal probability of being chosen.

Table A1. Frequency distribution used for finding random deviates with Rb >0.21 ng from native population.

Category range	Observed frequency	Observed cumulative frequency	Choose this category if URD < this value and > next lower value
0.21–0.23	2	0.17	0.17
0.23–0.25	0	0.17	0.25
0.25–0.27	1	0.25	0.33
0.27–0.29	3	0.50	0.42
0.29–0.31	0	0.50	0.50
0.31–0.41	1	0.58	0.67
0.41–0.51	3	0.83	0.83
0.51–1.51	2	1.00	1.00

A sampling distribution for Δ_N was obtained for each sample of *Anagrus* as follows. Using the procedure described above, 10,000 samples of n 'individuals' were randomly drawn from the native population, where n is the size of the field sample of *Anagrus*. For each sample of n, Δ_N was calculated using Eq. A.1. The probability, P, that the field sample was drawn from the native population was calculated as the proportion of the 10,000 Δ_Ns having a value greater than the Δ calculated for the field sample of *Anagrus*.

Reference

Press, W.H., Flannery, B.P., Teukolsky, S.A. & Vetterling, W.T. (1988) *Numerical Recipes in C*. Cambridge University Press.