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# COMBINING REPELLENT AND ATTRACTIVE AROMATIC PLANTS TO ENHANCE BIOLOGICAL CONTROL OF THREE TORTRICID SPECIES (LEPIDOPTERA: TORTRICIDAE) IN AN APPLE ORCHARD

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# Abstract

Non-host plants that are intercropped with crops can either repel or attract pests, and in some cases achieve pest management. Three aromatic plant species, ageratum (Ageratum houstonianum L.; Asterales: Asteraceae), French marigold (Tagetes patula L.; Asterales: Asteraceae) and summer savory (Satureja hortensis L.; Lamiales: Lamiaceae), were intercropped in ratios of 1:1 or 1:1:1 in an organic apple orchard to investigate the community characteristics and dynamic changes in densities of the Tortricidae species Adoxophyes orana Fisher, Spilonota lechriaspis Meyrick and Acleris fimbriana Thunberg and their natural enemies in 3 vertical strata of the orchard. Intercropping aromatic plants in the orchard increased the densities of natural enemies belonging to the Trichogrammatidae, Ichneumonidae and Braconidae and reduced the rates of increase and the densities of the tortricid species relative to the control. A correlation analysis of the densities of pest species and the densities of parasitoids indicated that the reduction in some tortricid species was related to the densities of certain natural enemy species. We concluded that intercropping with aromatic plants in apple orchards can increase the biological control of tortricid pests.

Key Words: aromatic plants, Tortricidae, natural enemy, spatial distribution, Ageratum houstonianum, Satureja hortensis, Tagetes patula

#### RESUMEN

Las plantas no hospederas intercaladas con los cultivos pueden repeler o atraer las plagas y en algunos casos lograr un manejo de plagas. Tres especies de plantas aromáticas, ageratum (Ageratum houstonianum L.; Asterales : Asteraceae), caléndula francesa (Tagetes patula L.; Asterales: Asteraceae) y ajedrea de verano (Satureja hortensis L.; Lamiales: Lamiaceae), se intercaló en proporciones de 1:1 ó 1:1:1 en un huerto de manzana orgánica para investigar las características de la comunidad y los cambios dinámicos en las densidades de las especies de Tortricidae, Adoxophyes orana Fisher, Spilonota lechriaspis Meyrick y Acleris fimbriana Thunberg y sus enemigos naturales en 3 estratos verticales de la huerta. El cultivo intercalado de plantas aromáticas en el huerto aumentó la densidad de enemigos naturales que pertenecen a las familias Trichogrammatidae, Ichneumonidae y Braconidae y redujo la tasa de crecimiento y las densidades de las especies de tortrícidos con respecto al control. El análisis de correlación de las densidades de especies plaga y las densidades de los parasitoides indicó que la reducción en algunas especies de tortrícidos esta relacionado con la densidad de ciertas especies de enemigos naturales. Llegamos a la conclusión de que el cultivo intercalado con plantas aromáticas en los huertos de manzana puede jugar un papel en el control biológico de plagas tortrícidos, lo que se puede explicar por los efectos químicos repelentes de plantas aromáticas y por la hipótesis de enemigos naturales. Las plantas aromáticas a través de una acción evasiva de químicos pueden influir en la distribución espacial de las especies de tortrícidos y sus enemigos naturales, de manera que la interacción entre ellos se traduce en un mejor control biológico, es decir, una combinación decendente con una regulación ascendente de la densidad de población de las especies de tortrícidos.

Palabras Clave: plantas aromáticas; Tortricidae; enemigo natural; distribución espacial, Ageratum houstonianum, Satureja hortensis, Tagetes patula

Tortricidae is one of the largest families of microlepidopterans and comprises approximately 10,000 described species worldwide (Regier et al. 2012). Tortricid species have become the major pests of apple (Malus spp.; Rosales: Rosaceae) and pear (Pyrus communis L.; Rosales: Rosaceae) tree species (Voudouris et al. 2011). Adult tortricids lay eggs on foliage and on the fruit surface; the larvae spin silk used to curl the leaf, which then serves as a nest. Some tortricid species mine tunnels in the bark, leaves and trunks of fruit trees and seriously endanger the growth of the fruit tree, thereby affecting fruit production. Traditionally, tortricids are controlled primarily by chemical insecticides. However, the extensive use of chemical insecticides has led to serious insecticide resistance problems and environmental contamination (Landis et al. 2000; Tilman et al. 2002; Poveda et al. 2008). Therefore, conservation biological control has been increasingly applied to enhance natural enemy densities and the diversity of arthropod communities in orchard ecosystems by habitat management (Landis et al. 2000; Alteriet al. 2005). The hypothesis is that predatory insects and parasitoids are more effective at controlling populations of herbivores in diverse plant systems than in monocultures (Russell 1989). Increased plant diversity can provide shelter, nectar, pollen or alternative hosts as required by natural enemies (Alteriet al. 2005; Fréchetteet al. 2008), thereby increasing the numbers of predatory and parasitic arthropods. Enhanced densities of natural enemies inevitably reduce pest densities, reduce excessive use of insecticides and ultimately contribute to effective biological pest control (White et al. 1995; Rieuxet al. 1999; Nichollset al. 2001). For example, increasing the genotypic diversity of a population of a dominant old-field goldenrod plant species, Solidago altissima L. (Asterales: Asteraceae) was reported to influence arthropod diversity and community structure and to increase net primary production (Crutsingeret al. 2006).

In recent years, studies have demonstrated that the use of intercropped repellent plants or trap plants can be effective alternative methods to reduce pest pressure on the primary crop (Morleyet al. 2005; Tanget al. 2013; Song et al. 2013). The volatile organic compounds that are emitted from plant tissues could directly affect herbivore physiology and behavior due to their potentially toxic, repellent or deterrent properties (De Moraes et al. 2001; Kessler & Baldwin 2001; Aharoni et al. 2003). Alternatively, plant-derived compounds may be emitted by plants in response to herbivore damage and attract the natural enemies of herbivores. For example, Rasmann et al. (2005) found that an insect-induced below ground plant signal, (E)-b-caryophyllene, strongly attracts an entomopathogenic nematode.

Aromatic plants possess both aromatic and medicinal properties and spontaneously release odorous volatile organic compounds (VOCs) that can have insecticidal, antifeedant and repellent effects on insect arthropods (Catherine 1997; Songet al. 2010). Intercropping with aromatic plants as an alternative method for reducing browsing pressure has attracted research attention. Bennisonet al. (2001) found that rosemary (Rosmarinus officinalis L.; Lamiales: Lamiaceae) has repellent effects on Frankliniella occidentalis (Pergande) (Thysanoptera: Thripidae). Additionally, Tanacetum vulgare L. (Asterales: Asteraceae), Chrysanthemum maximum Ramon (Asterales: Asteraceae), Aster tongolensis Franch (Asterales: Asteraceae)and Achillea millefolium L. (Asterales: Asteraceae) can attract and contribute to maintaining predator and parasitoid arthropods in a Quebec apple orchard system (Bostanianet al. 2004). Our previous studies showed that intercropping with aromatic plants significantly reduced the abundances of scarab beetles (Serica orientalis Motschulsky; Scarabaeidae), the spirea aphid (Aphis citricola Van der Goot; Aphididae) and natural enemies, such as Coccinella septempunctata L. (Coleoptera: Coccinellidae), Phytoseiulus persimilis Athias-Henriot (Mesostigmata: Phytoseiidae), and Chrysoperla sinica (Tjeder) (Neuroptera: Chrysopidae) in pear and apple orchard ecosystems (Song et al. 2013). However, very little is known about the effects of mixed intercropping with aromatic plants on Tortricidae and their natural enemy communities at different spatial levels in an apple orchard.

In this study, we selected 3 aromatic plant species, Ageratum houstonianum L., Tagetes patula L. and Mentha canadensis L. (Lamiales: Lamiaceae). These species were intercropped in ratios of 1:1 or 1:1:1 in an organic apple orchard to test the hypothesis that intercropping aromatic plants results in the biological control of Tortricidae pests, primarily through repellency effects. We hypothesized that 1) intercropping aromatic plants would reduce the annual cumulative number and abundance of Tortricidae; 2) intercropping aromatic plants would increase the annual cumulative number and abundance of parasitoid natural enemies of Tortricidae; and 3) intercropping aromatic plants would play a role in the control of pests in orchards.

# MATERIALS AND METHODS

Study Site

This study was conducted in an organic apple orchard northwest of Beijing, China (N 39° 10' E 116° 3'), an area with a warm continental monsoonal climate. The monthly mean temperatures typically range from -4 °C to 25.89 °C, and the mean annual precipitation is approximately 556

mm. The soil type is a light sandy loam. The total area of the orchard was 3.5 ha. Pest management in the orchards was primarily dependent on manual and physical control measures, and the insecticides that were used complied with organic certification standards.

# Experimental Design and Sample Plots

The experiment was conducted from Mar to Nov in 2010, 2011 and 2012. Each year at the end of Mar seeds of ageratum, summer savory, and French marigold were sown between the rows of apple trees (Malus domestica Borkh. cv. 'Fuji') Malus prunifolia (Willd.) Borkh.) and then covered with plastic film to promote germination and growth. The treatments (Tr1, French marigold and summer savory; Tr2, ageratum and French marigold; Tr3, French marigold, summer savory and ageratum; Tr4, ageratum and summer savory; and CK, the control consisted of natural grasses) were based on a randomized block design with 5 treatments and 3 replicates, giving a total of 15 plots. Plot sizes were 18 m × 40 m and were spaced 12 m apart. The intercrops were thinned and retained at an interplant spacing of 0.2 and 0.3 m at the end of Apr. During the growing season, intercropped vegetation was weeded by a weeding machine once each month and mowed at the end of Jul and in the middle of Oct. The clippings were left in place for natural degradation. Management in natural vegetation cover plots was the same as that in intercropped plots except for weeding, which was not performed in the control.

# **Arthropod Sampling**

From Apr to Nov of 2012, arthropods were sampled during 10 a.m.-3 p.m. at approximately 10-day intervals. The detailed methods of investigation were as follows.

Apple Tree Canopy Survey: within each plot, 4 adjacent apple trees of approximately similar vigor were selected as sampling points. On each sampling date, each tree was investigated from 4 directions. On each side of the tree, three 30 cm twigs from the high, middle and low levels were chosen to check for the presence of tortricids and their natural enemies. The sampled twigs were covered with nylon sweep nets (30 cm diam; 50 cm depth) to collect highly mobile arthropods, which were brought back to the laboratory for identification. Any larvae that were collected were reared in the laboratory in Petri dishes (10 cm diam; 2) cm depth) under 13:11 h L:D photoperiod until they reached an identifiable stage. The room temperature fluctuated between 20 and 23 °C, and the RH was 60% ± 10%. Once emerged, the insects were identified to the species level.

Arthropod Survey on Ground Vegetation: The "sweep" was defined as a rapid movement of the net through the aromatic vegetation. Each plot selected for 5 samplings of a 2 m long area, and each strip received 5 sweeps, and the number of larvae that were captured on each strip was recorded. All of the collected adult tortricids and parasitoids as well as other arthropods were taken to the laboratory for identification and counting according to a diagnostic classification guides (Lee 1987; Zheng & Gui 1999; Barnard 1999). Lastly, the numbers of adults and larvae were summed.

# Arthropod Survey in Orchard Space

Five traps (plastic basins, each 12 cm diam, 20 cm high) in each treatment plot were filled to one quarter volume with a mixed liquid containing sweet and sour ingredients in water (vinegar, brown sugar, white wine, and water at a ratio of 2:1:1:20, respectively). The traps were hung 1.2 m above the ground on the eastern side of the trees and replaced every 10 days. The heights of the tree trunk and aromatic plants were 0.7-0.8 m and 0.3-0.5 m, respectively. To prevent the basins from overflowing during rainy weather, it was necessary to temporarily cover them with plastic film.

Stratification of the Apple - Aromatic Plant Intercropping System

According to the special structural features of orchard and ground vegetation, the space of the apple - aromatic plant intercropping system was divided into upper, middle and lower sections (Fig. 1).

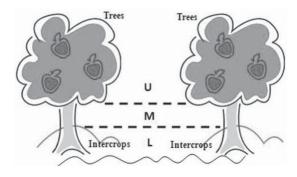


Fig. 1. The stratification model of an ecological system consisting of apple trees in an organic apple orchard that was intercropped with mixed species of aromatic plants. U represents the upper stratum, M represents the middle level stratum and L represents the lower stratum.

Statistical Analysis

Data from the 3 sampling methods were pooled, collated and entered into a relational database along with supplementary details (feeding guild, development mode, feeding location and taxonomic information). The data for each date for the 3 sampling years were averaged before the following values were calculated:

- (1) Richness, i.e., the numbers of pest species or natural enemy families (*S*),
- (2) Abundance, i.e., the number of each pest species or of each natural enemy family (N).

The relative abundance of each tortricid species and each family of natural enemies were calculated. We used one-way ANOVA with Duncan's shortest significant range (SSR) test to compare the differences among the treatments at P = 0.05 and P = 0.01, respectively. Microsoft Excel 2003 and SPSS v.17.0 were used for the data analysis.

# RESULTS

Number of Tortricid Species and Number of Each Family of Natural Enemies

This study mainly focused on 3 tortricid pest species: *Adoxophyes orana* Fisher, *Spilonota lechriaspis* Meyrick and *Acleris fimbriana* Thunberg, and the main natural enemies of Tortricidae, i.e., species of Trichogrammatidae, Ichneumonidae and Braconidae.

Intercropping with various mixtures of aromatic plants significantly influenced the cumulative number of each tortricid species and their parasitic natural enemies (Table 1). The abundances of the A. orana and Spilonota lechriaspis in the CK and Tr1 were considerably larger than in the other treatments, while the abundances of A. orana and S. lechriaspis were the least dense in Tr3 (A. orana Fisher:  $F_{4,14} = 20.064$ ; P < 0.0003; S. Lechriaspis:  $F_{4,14} = 27.846$ ; P < 0.0001). For Acleris fimbriana the cumulative number was greatest in the natural grass plots and significantly smaller

in all of the other treatments ( $F_{_{4,14}}=37.000$ ; P<0.0001). The relative abundance of Trichogrammatidae species was greatest in Tr2, where it was 28.6% larger than in the CK. The relative abundance of Ichneumonidae species was greatest in Tr4, while it was smallest in the CK ( $F_{4,14}=8.227$ ; P=0.006), however, the density in the CK was not significantly different than in Tr1. For the Braconidae species, the cumulative number was the smallest in Tr1 ( $F_{4,14}=4.533$ ; P=0.03), but the numbers in Tr2, Tr3 and Tr4 did not differ significantly from those of CK.

Spatial Distribution of Tortricid Species and their Natural Enemies

The spatial distribution of tortricid species and their natural enemies varied significantly across aromatic plant treatments (Table 2). Adoxophyes orana and Acleris fimbriana were present in the upper and middle strata of the apple trees. However, Spilonota lechriaspis was present only in the upper stratum of the trees (Table 2). All of the tortricid species were absent in the lower stratum. Although the effects of intercropping with aromatic plants on the tortricid species in different strata were diverse, all of the aromatic plant treatments significantly reduced the total number of tortricid individuals in the upper and middle strata of the apple trees (upper stratum:  $F_{414} = 43.873; P < 0.0001$ ), but in the middle stratum the total number of tortricids was reduced only in treatments Tr2, Tr3 and Tr4 (Middle  $F_{4.14}$ = 27.885; P < 0.0001).

The total number of natural enemies in the upper stratum was significantly greater in Tr2, Tr3 and Tr4 ( $F_{4.14}$ = 9.947; P = 0.003) than in Tr1 or in the CK. Parasitoids belonging to the 3 families were found in the upper stratum. In the upper stratum, the densities of trichogrammatids were increased in Tr3 and Tr4, but not significantly; and the densities of braconids and ichneumonids were significantly increased in all of the treatments except Tr1, In the middle stratum, the densities of trichogrammatids were not differ signifi-

TABLE 1. EFFECTS OF INTERCROPPING MIXED AROMATIC PLANT SPECIES IN AN ORGANIC APPLE ORCHARD ON THE ABUNDANCES OF THE 3 DOMINANT TORTRICIDAE SPECIES AND THE 3 DOMINANT FAMILIES OF NATURAL ENEMIES.

| Species               | Tr1                   | Tr2                   | Tr3                   | Tr4                   | $_{ m CK}$           |
|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|
| Adoxophyes orana      | $47 \pm 6 \text{ a}$  | 40 ± 1 b              | $31 \pm 5$ c          | $30 \pm 3$ c          | 45 ± 4 a             |
| Spilonota lechriaspis | $79 \pm 6 a$          | $70 \pm 6 \text{ b}$  | $58 \pm 4 \text{ c}$  | $66 \pm 3 \text{ b}$  | $84 \pm 9 \text{ a}$ |
| Acleris fimbriana     | $20 \pm 3 \text{ b}$  | $20 \pm 2 \text{ b}$  | $19 \pm 1 \text{ b}$  | $18 \pm 1 \text{ b}$  | $31 \pm 4$ a         |
| Trichogrammatidae     | $26 \pm 1 c$          | $35 \pm 3 \text{ a}$  | $33 \pm 4 \text{ ab}$ | $29 \pm 3 \text{ bc}$ | $25 \pm 3$ c         |
| Ichneumonidae         | $32 \pm 2 \text{ bc}$ | $37 \pm 5 \text{ ab}$ | $36 \pm 2$ ab         | $39 \pm 2 \text{ a}$  | $29 \pm 1 c$         |
| Braconidae            | $26 \pm 1 \text{ b}$  | $29 \pm 3 a$          | $30 \pm 2 \text{ a}$  | $31 \pm 3 \text{ a}$  | $31 \pm 4 \text{ a}$ |

Tr1 was French marigold and summer savory; Tr2 was ageratum and French marigold; Tr3 was French marigold, summer savory and ageratum; Tr4 was ageratum and summer savory; and CK (control) was natural grasses.

Table 2. Stratification of three dominant tortricid species and species of their dominant natural enemy families in various mixed intercropping plots IN AN ORGANIC APPLE ORCHARD

|   |  | $\mathrm{Upp}$         | Upper stratum (U)     | (U)                   |                             |  | Middl                 | Middle stratum (M)    | α (M)                       |                       |  | Lowe                 | $Lower\ stratum\ (L)$                                     | n (L)                |             |
|---|--|------------------------|-----------------------|-----------------------|-----------------------------|--|-----------------------|-----------------------|-----------------------------|-----------------------|--|----------------------|---|----------------------|-------------|
| Species                                 | Tr1  | Tr2                    | Tr3                   | Tr4                   | CK                          | Tr1  | Tr2                   | Tr3                   | Tr4                         | CK                    | Tr1  | Tr2                  | Tr3   | Tr4                  | CK          |
| Adoxophyes orana                        | 25 ± 3 a                                   | 24 ± 1 a               | 17 ± 3 b              | $17 \pm 1 \mathrm{b}$ | 28 ± 2 a                    | $22 \pm 3 \text{ a}$ $15 \pm 1 \text{ bc}$ $14 \pm 2 \text{ cd}$ $13 \pm 2 \text{ d}$ $18 \pm 3 \text{ b}$ | $15 \pm 1 \text{ bc}$ | $4 \pm 2 \text{ cd}$  | 13 ± 2 d 1                  | 18 ± 3 b              | 1  |                      |   |                      | 1           |
| Spilonota lechriaspis 79 $\pm$ 6 a      | $79 \pm 6a$                                | $70 \pm 6 \mathrm{b}$  | $58 \pm 4 c$          | $99 \pm 99$           | $84 \pm 9a$                 | I  | I                     | I                     | I                           | I                     | I  | I                    |   | I                    | I           |
| Acleris fimbriana                       | $10 \pm 2 \mathrm{b}$                      | $11 \pm 2 \text{ b}$   | $10 \pm 2 \mathrm{b}$ | $11 \pm 1$ b          | $18 \pm 2a$                 | $10 \pm 2b$ $8 \pm 1bc$ $9 \pm 1b$ $7 \pm 1c$ $13 \pm 2a$  | $8\pm 1\;\mathrm{bc}$ | $9 \pm 1  \mathrm{b}$ | $7 \pm 1 c$                 | 13 ± 2 a              | I  | I                    |   | I                    |             |
| $\Sigma$ # of Tortricids                | $108 \pm 10 \text{ b} 102 \pm 8 \text{ b}$ | $102 \pm 8 \mathrm{b}$ | $81 \pm 8 d$          | $93 \pm 3 c$          | $93 \pm 3 c$ $125 \pm 11 a$ | $30 \pm 4$ a $23 \pm 2$ b $23 \pm 2$ bc $19 \pm 2$ c $30 \pm 4$ a  | $23 \pm 2 \text{ b}$  | $3 \pm 2 \text{ bc}$  | 19 ± 2 c 5                  | $30 \pm 4 a$          |  |                      |   |                      |             |
| Trichogrammatidae                       | $11 \pm 1$ ab                              | 11±1ab 11±1ab          | $13 \pm 3 a$          | $12 \pm 2a$           | $9 \pm 1  \mathrm{b}$       | $7 \pm 1$ ab $9 \pm 2$ a $5 \pm 1$ bc $4 \pm 1$ c $8 \pm 2$ a  | $9 \pm 2 a$           | $5 \pm 1 \text{ bc}$  | $4 \pm 1 c$                 | $8 \pm 2 a$           | $8 \pm 1 \mathrm{c}$   | 18 ± 2 a             | $8 \pm 1 c 18 \pm 2 a 15 \pm 2 ab 13 \pm 2 b$ $9 \pm 2 c$ | $13 \pm 2  \text{b}$ | $9 \pm 2 c$ |
| Ichneumonidae                           | $13 \pm 2 \mathrm{b}$                      | 18±3a                  | $18 \pm 1 a$          | $17 \pm 1 a$          | $15 \pm 1 ab$               |  | 8±3a                  | $6 \pm 1$ ab          | 6±1ab 8±3a 6±1ab 7±1ab 4±1b | $4 \pm 1  \mathrm{b}$ | $13 \pm 1$ b $12 \pm 2$ b $12 \pm 1$ b $16 \pm 1$ a $10 \pm 1$ c   | $12 \pm 2 \text{ b}$ | $12 \pm 1  \text{b}$                                      | $16 \pm 1  a  1$     | $0 \pm 1 c$ |
| Braconidae                              | $13 \pm 1 \mathrm{bc}$                     | $15 \pm 2 ab$          | $17 \pm 1 a$          | $18 \pm 2 a$          | $11 \pm 3 c$                | I  |                       | I                     |                             | I                     | $13 \pm 1  b  14 \pm 2  b  12 \pm 1  b  13 \pm 3  b  20 \pm 1  a$  | $14 \pm 2  \text{b}$ | $12 \pm 1  \text{b}$                                      | $13 \pm 3 b$         | $20 \pm 1$  |
| $\Sigma$ # of natural enemy individuals | $37 \pm 1 \mathrm{b}$                      | 45 ± 4 a               | 48 ± 4 a              | $47 \pm 2 a$          | $35 \pm 4 \mathrm{b}$       | $13 \pm 2$ b $17 \pm 3$ a $11 \pm 1$ b $11 \pm 2$ b $12 \pm 2$ b   | 17±3a1                | $(1 \pm 1  b)$        | $11 \pm 2  \text{b}$ ]      |                       | $33 \pm 2  b  43 \pm 2  a  40 \pm 4  a  42 \pm 6  a  39 \pm 1  ab$ | 13 ± 2 a 4           | 10±4a   | 12±6a§               | 39 ± 1 a    |

Tr1 was French marigold and summer savory; Tr2 was ageratum and French marigold; Tr3 was French marigold, summer savory and ageratum; Tr4 was ageratum and summer savory; and CK (control) was natural grasses cantly from the control in Tr1 and Tr2, but they significantly less than in the control in Tr3 and Tr4; the densities of ichneumonids were significantly increased only by Tr2, and braconids were absent in this stratum. In the middle stratum, the densities of all of the natural enemies were significantly greater in Tr2 ( $F_{4,14}=6.673; P<0.05$ ) than in the other treatments. In the lower stratum, all treatments except Tr1 significantly increased the densities of trichogrammatids ( $F_{4,14}=6.673; P<0.05$ ), and all of the treatments significantly increased the densities of ichneumonids, but significantly reduced the densities of braconids relative to the control. However, the total numbers of the 3 families of natural enemies in the lower stratum were not significantly increased by any of the treatments.

Dynamics of the Three Tortricid Species in Relation to Intercropping Treatments

The responses of A. orana, S. lechriaspis and A. fimbriana and the total number of all 3 tortricid species to the various treatments were dynamic (Fig. 2) at various developmental stages of the apple trees. Generally, the densities of A. orana in the 5 treatments strongly trended upward between early Apr and early Jun, peaked near Jun 25, then declined fairly sharply through Jul and subsequently declined slowly until the end of Oct. The numbers of *A. orana* in the treatments with aromatic plants were significantly smaller than in the natural grass plots, and the peak populations were the smallest in Tr3 and Tr4 (Fig. 2A). Spilonota lechriaspis displayed 2 peaks, the first on 5 Jun and the second on 5 Aug. The numbers of *S. lechriapsis* in the aromatic plant treatments were significantly smaller than in the natural grass plots, and the peak S. lechriapis population was smallest in Tr 3 and TR 4 (Fig. 2B) Trends of the densities of A. fimbriana in the 5 treatments differed in the various phenophases of the apple trees. Thus the trend line of A. fimbriana density in some treatments slowly ascended, then rapidly declined, then rapidly ascended and finally rapidly declined (Fig. 2C); and peak populations were the smallest in Tr2 and Tr4. The total number of all 3 tortricid species in plots intercropping with aromatic plants was significantly smaller than in natural grass plots (Fig. 2D); and peak populations were the smallest in Tr3 and Tr4.

Dynamics of the Three Families of Natural Enemies of the Three Tortricid Species  $\,$ 

Generally, the numbers of Trichogrammatidae, Ichneumonidae and Braconidae and the total number of wasps of these 3 families slowly ascended from early Apr to mid Jun, peaked near Jun 20 and subsequently slowly declined until early

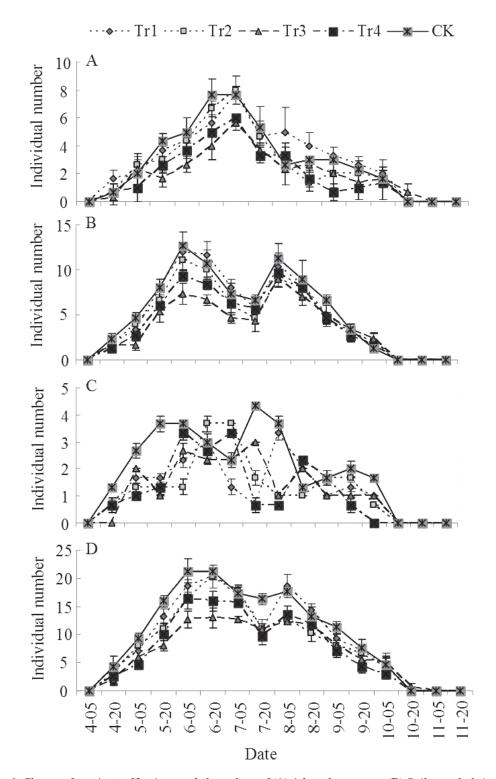


Fig. 2. Changes from Apr to Nov in sampled numbers of (A) Adoxophyes orana, (B) Spilonota lechriaspis, (C) Acleris fimbriana and (D) the total number of these 3 dominant Tortricidae species in different mixed intercropping plots in an apple orchard. Tr1 was French marigold and summer savory; Tr2 was ageratum and French marigold; Tr3 was French marigold, summer savory and ageratum; Tr4 was ageratum and summer savory; and CK (control) was natural grasses.

Oct. Moreover, densities of the trichogrammatids and ichneumonids in the plots intercropped with aromatic plants were significantly greater than those in the natural grass plots (Figs. 3A and 3B). The peak densities of trichogrammatids were the greatest in Tr2, Tr3 and Tr4, while the peak densities of ichneumonids were the greatest in Tr4 and Tr3. Seasonal changes in the braconid densities seemed less variable in all of the treatments (Fig. 3C) than those of the trichogrammatids and the ichneumonids. The total numbers of wasps in the 3parasitoid families in plots intercropped with aromatic plants (with the exception of Tr1) were significantly greater than in the natural grass plots (Fig. 3D).

Correlation Analysis of the Tortricidae and its Natural Enemies

The correlation analyses that were conducted in this study (Table 3) indicated that the densities of the natural enemies regulated the densities of tortricid species. The densities of both A. orana and S. lechriaspis were inversely correlated with the densities of parasitoids of the 3 wasp families in all treatments (P < 0.01). The highest coefficients of inverse correlation between A. orana and trichogrammatid density, A. orana and braconid density, and A. orana and ichneumonid density occurred in Tr4, Tr4, and CK, respectively. The highest inverse correlation coefficients between S. lechriaspis density and trichogrammatid density, S. lechriaspis and ichneumonid density, and S. lechriaspis and braconid density occurred in CK, Tr3, and Tr2, respectively. Further, the density of A. fimbriana was inversely correlated with the density of predators belonging to the 3 wasp families in all treatments (P < 0.05), but with of no significant correlation with trichogrammatid density.

#### DISCUSSION

Intercropping with aromatic plants had significant effects on populations of the 3 tortricid species and their primary natural enemies in an organic apple orchard. Tortricids were the main pests that caused severe damage to local apple trees. One important finding of this study was that intercropping with certain aromatic plants in apple orchards significantly increased the abundances of their natural enemies and significantly reduced the numbers of tortricid individuals (Table 1). A decrease in the tortricid population numbers in apple orchards should reduce the likelihood of significant damage to the fruit trees. These anticipated results may be explained by 2 possible mechanisms: the repellency of the emitted aromatic chemicals or the impacts of natural enemies.

The chemicals derived from the aromatic plants can affect pest feeding, host location, oviposition and other behaviors due to their toxicity or repellency (Lu et al. 2007). These chemical compounds could also interfere with pest feeding behavior by affecting their normal physiology, resulting in malnutrition, abnormalities and even death (Lu et al. 2008). Moreover, aromatic plants may interfere with the ability of herbivores to locate a host for oviposition (Xu et al. 2005). Intercropping Vicia faba L. with Ocimum basilicum or Saturela hortensis, for example, significantly reduced the adult population of the sugar beet aphid Aphis fabae Scopoli (Basedow et al. 2006). In addition, Khan (2006) showed that Desmodium uncinatum intercropped in a maize field significantly reduced stem borer infestations by releasing specific volatiles. However, the densities of all of the insect species in our plots did not decrease due to intercropping with aromatic plants, such as the large numbers of A. orana and S. lechriaspis in Tr1. This result indicates that plant volatiles released by non-host plants may not have toxic and repellent effects on all insects. The blend of volatiles can also alter the behavioral responses of some insects (Song et al. 1996). For example, the sulfur butterfly, Colias erate (Lepidoptera: Pieridae), oviposits on the non-host plant Aristolochia debilis (Aristolochiaceae), which contains D-pinitol, an important oviposition stimulant that is involved in host recognition (Honda et al. 2012). Moreover, experienced herbivorous insects may not be repelled by non-host plants. For instance, in laboratory experiments, experienced ovipositing diamondback moth females were attracted to host plants that were treated with extracts from a non-host-plant and laid a greater proportion of eggs on the treated rather than on the untreated host plants (Liu et al. 2005).

The decrease of tortricid populations may have been due to an attraction of natural enemies to aromatic plants. The lush cover that is provided by the aromatic plants can increase the abundance of the natural enemies of pests by offering additional habitats, serving as intermediate hosts, and providing food (pollen and nectar). Increasing the numbers of natural enemies, such as parasitic wasps and flies or predatory mites, can help to control herbivorous pests (Drukker et al. 2000; Heil 2008; Kessler & Baldwin 2001). For example, Mathews et al. (2007) found that additional nectar can improve the performance of Trichogramma minutum in biological control. Shearer & Atanassov (2004) found that the adaptability in the laboratory of *Chrysoperla plorabunda* (Fitch) was enhanced by peach nectar. In this study, natural enemy populations in the plots that were intercropped with aromatic plants were more abundant than in natural vegetation plots; and ultimately they decreased the densities of pests in the orchard. However, the densities of all parasit-

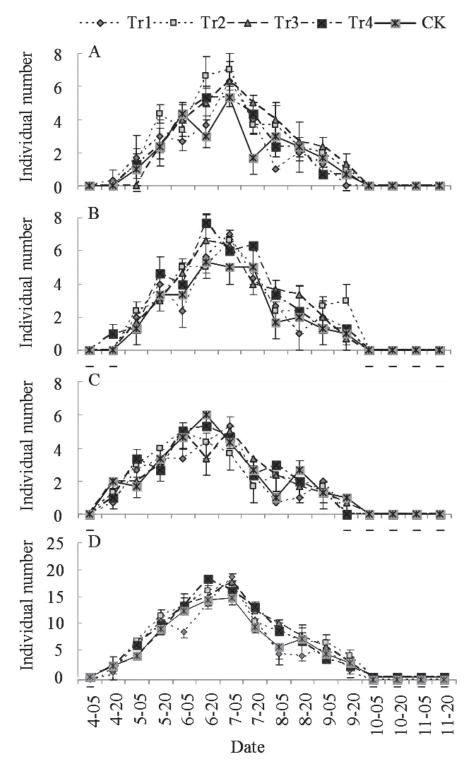


Fig. 3. Changes from Apr to Nov in sampled numbers of (A) Trichogrammatidae, (B) Ichneumonidae, (C) Braconidae and (D) the total numbers of these 3 dominant families of natural enemies in different mixed intercropping plots in an apple orchard. Tr1 was French marigold and summer savory; Tr2 was ageratum and French marigold; Tr3 was French marigold, summer savory and ageratum; Tr4 was ageratum and summer savory; and CK (control) was natural grasses.

Table 3. Correlation analysis of the densities of 3 dominant tortricid species and the densities of species of their dominant natural enemy families in EACH OF THE VARIOUS MIXED INTERCROPPING PLOTS IN AN ORGANIC APPLE ORCHARD.

| T <sub>2</sub> -1  | Dominant                   | Dominant natural enemy raming | 113                        | Treatment  | Dominant                        | Dominant natural enemy family | uly                        |
|--|----------------------------|-------------------------------|----------------------------|--|---------------------------------|-------------------------------|----------------------------|
| TIT  | Trichogrammatidae          | Ichneumonidae                 | Braconidae                 | Tr2  | Trichogrammatidae Ichneumonidae | Ichneumonidae                 | Braconidae                 |
| Adoxophyes orana<br>Spilonota lechriaspis<br>Acleris fimbriana | 0.86**<br>0.73**<br>0.48   | 0.90**<br>0.74**<br>0.56*     | 0.79**<br>0.72**<br>0.56*  | Adoxophyes orana<br>Spilonota lechriaspis<br>Acleris fimbriana | 0.92***<br>0.77**<br>0.84**     | 0.91**<br>0.72**<br>0.83**    | 0.77**<br>0.85**<br>0.57*  |
| Tr3  | Trichogrammatidae          | Ichneumonidae                 | Braconidae                 | Tr4  | Trichogrammatidae               | Ichneumonidae                 | Braconidae                 |
| Adoxophyes orana<br>Spilonota lechriaspis<br>Acleris fimbriana | 0.89**<br>0.80**<br>0.76** | 0.87**<br>0.82**<br>0.78**    | 0.79**<br>0.69**<br>0.78** | Adoxophyes orana<br>Spilonota lechriaspis<br>Acleris fimbriana | 0.95***<br>0.78**<br>0.84**     | 0.92**<br>0.73**<br>0.73**    | 0.92**<br>0.79**<br>0.85** |
| CK   | Trichogrammatidae          | Ichneumonidae                 | Braconidae                 |  |                                 |                               |                            |
| Adoxophyes orana<br>Spilonota lechriaspis<br>Acleris fimbriana | 0.86**<br>0.86**<br>0.65** | 0.94**<br>0.75**<br>0.67**    | 0.88**<br>0.80**<br>0.61** |  |                                 |                               |                            |

Tr1 was French marigold and summer savory; Tr2 was ageratum and French marigold; Tr3 was French marigold, summer savory and ageratum; Tr4 was ageratum and summer savory; and CK (control) was natural grasses. \* P < 0.05, \*\* P < 0.01.

ic species may not increase in the plots that were intercropped with aromatic plants. Thus, the densities of braconids in some treatments with intercropped aromatic plants were less than in the CK, and this was particularly significant in the lower stratum (Tables 1 and 2). However, the results of this analysis indicate that the volatile odors of aromatic plants do not always attract all parasitoid species. In addition, the intermingling of volatiles that are released by host and non-host plants may hamper the searching behavior and reduce the host-finding success of parasitoids (Gols et al. 2005; Perfecto&Vet 2003). For instance, experienced parasitoid females that were attracted to yarrow (Achillea millefolium) odors showed no response when these odors were offered simultaneously with odors of a non-host plant, possibly due to a masking effect (Randlkofer et al. 2007). In addition, the nectar in the CK also attracts parasitic wasps. In this study, there was an inverse correlation between the abundance of tortricids and their natural enemies in all plots (Table 3).

Our results indicate that the biological control of Tortricids in apple orchards by intercropping with aromatic plants is an effective control approach. We believe that the specific mechanisms that are associated with intercropping with aromatic plants require additional in depth research on such aspects as the composition of volatile aromatic oils that possess a repellent effect, and the choice of aromatic plant species that are used for intercropping orchard environments. It is especially important to study the chemical properties of aromatic plants and to understand the interactions between aromatic plants and pest species and to investigate ways to produce synthetic volatiles that have the potential to repel pests in this and related ecosystems.

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