

International Conference on Food, Agriculture and Natural Resources, IC-FANRes 2015

Multiple Release Of Two Different Hymenoptera Parasitoid Of Leafminer Fly, Synergy Or Competition

Hari Purnomo^{a*} and Denis J. Wright^b

^aFaculty of Agriculture University of Jember Jalan Kalimantan 37Jember 68121

^bImperial College London at Ascot Buchurst Road Ascot Berkshire SL5 7PY UK

Abstract

Leafminer fly *Liriomyza huidobrensis* Blanchard (Diptera, Agromyzidae) is a key pest on potato plantation. Due to of negative impact of calendar based of pesticide application on environment and farmer health, Biological control by releasing hymenoptera parasitoid is promising as an alternative control to solve the outbreak of leafminer fly. There are two important species of hymenoptera parasitoid in leafminer population *Hemiptarsenus varicornis* (idiobiont ectoparasitoid) and *Diglyphus isaea*. (idiobiont ectoparasitoid). The research objective was to determine whether multiple releasing of two parasitoid in leafminer population had significant control of leafminer fly compared than single release. The results showed that multiple release caused competition between two different behaviors of Hymenoptera parasitoid. Mortality of leafminer fly was caused by *H. varicornis* higher than *Opius* sp. In multiple or single release. The present results suggest that field releases of *D. isaea* would not have a detrimental effect on the indigenous species, *H. varicornis*, and that *D. isaea* may be a “good” candidate to supplement biological control of *L. huidobrensis*.

© 2016 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the organizing committee of IC-FANRes 2015

Keywords, *Hemiptarsenus varicornis*, *Diglyphus isaea*, multiple release, synergy

1. Introduction

Classical biological control is essentially a process for building or rebuilding natural enemies communities (Ehler, 1990). Parasitoids or predators are collected, reared and the released with the aim of regulating pest

* Corresponding author. Tel.: +62-331-334054

E-mail address: liriomyza@gmail.com

populations below economically damaging levels (van Driesche and Bellows, 1996). Biological control strategies should take account of the existing local natural enemy communities; in particular the possibility that introduced species may out-compete and eliminate indigenous natural enemies (Murphy and La Salle, 1999). Competition for host resources may lead, for example, to displacement or host shift of insect parasitoids, and can be an important consideration in biological control (Reitz and Trumble, 2002).

Competition can be categorised as either exploitation or interference (Reitz and Trumble, 2002). Exploitation occurs when individuals, by using resources, deprive others of benefits to be gained from those resources (Denno et al., 1995). Interference competition occurs when individuals harm one another directly through fighting and killing or by the aggressive displacement of territory. In a symmetric competition, the superior competitor will displace the inferior through physical attack, by physiological superiority or by both mechanisms (Godfray, 1994). In the case of physical attack, which often occurs among endoparasitoid larvae, the parasitoid with more rapid rate of development often wins (Krijger et al., 2001). Physiological suppression in competition often occurs when older endoparasitoid larvae eliminate younger larvae.

The key questions in classical biological control are how many parasitoid species should be introduced and released and how does competitive interactions between them influence regulation of the pest population. Some authors believe that introducing more than one natural enemy species leads to greater suppression of pests (Huffaker et al., 1976), others have suggested that competitive interactions may reduce or disrupt the effectiveness of biological control (Rosenheim et al., 1995; Murdoch et al., 1998). Intra- and interspecific competition between parasitoids has been observed in a number of biological control programmes. Urbaneja et al. (2003) reported in his work that *Quadraticus* sp. (Hymenoptera, Eulophidae), an exotic parasitoid that was augmented and released to control citrus leafminer *Phyllocnistis citrella* (Lepidoptera, Gracillariidae) was an intrinsically superior competitor against the native parasitoid *Cirrospilus brevis* (Hymenoptera, Eulophidae). The competitive asymmetric has been found in the biological control program against the coffee berry borer *Hypothenemus hampei* (Coleoptera, Scolytidae) in which *Chephalomonia stephanoderes* (Hymenoptera, Bethyliidae) were more successful compared two others parasitoids *C. hyalinipennis* (Hymenoptera, Bethyliidae) and *Prosops nasuta* (Hymenoptera, Bethyliidae) (Lachaud et al., 2002). Shi et al. (2004) have found Multiparasitism between *Cotesia plutellae* (hymenoptera, Braconidae) and *Diadegma semiclausum* (Hymenoptera, Echnemonidae), which are two major endoparasitoids of diamondback moth (DBM) *Plutella xylostella*. Moreover, Shi et al. (2004) also found that *C. plutellae* was superior to *D. semiclausum* and that larva of *C. plutellae* can attack and eliminate larva *D. semiclausum* when present in the same host.

Rauf and Shepard (1999) have reported that *H. varicornis*, an idiobiont ectoparasitoid is the primary parasitoid attacking leafminer fly in Indonesia. However, the suppression of LMF by *H. varicornis* in the field was low due to the high pesticide use. The classical biological control programme commenced when the government of Indonesia gave approval to import five exotic parasitoids of leafminer fly from Hawaii including *Ganaspidum utilis* (Hymenoptera, Eucolidae), *Diglyphus intermedius* (Hymenoptera, Eulophidae), *D. begini*, *C. oscinadus* and *Halticoptera circulus* (Hymenoptera, Pteromalidae) (Rauf et al., 2000) but so far the effects of such multiple introductions on biological control of leafminers are unknown.

In the present study, preliminary competition experiments were conducted to examine how *H. varicornis*, and a second idiobiont ectoparasitoid, *D. isaea* interact within and between species.

2. Materials and Methods

2.1. Insects and host plants

The culture of host plants (*P. vulgaris*) and of *L. huidobrensis* and its parasitoids were described below The *L. huidobrensis* population used was obtained from the Central Science Laboratory (Defra, Sand Hutton, York). Ten potted bean plants were placed in an oviposition cage (70 cm L x 55 cm W x 45 cm H) containing about 150 adult *L. huidobrensis* (mixed sexes). Cotton wool soaked in a 30% w/v honey/water solution was placed in a 5 cm dia. Petri Dish to provide a food source for adult flies. After 24 h exposure to ovipositing females, the bean plants were placed in holding cages (similar dimensions) for 6 days to allow the leafminers to develop to the third larval instar.

Infested plants were then divided to two groups. One group was used to maintain leafminer populations, the other group was used for rearing *H. varicornis*.

For pure leafminer cultures, the insects were allowed to develop to the pupal stage. Infested leaves were then removed and any pupa that remained on the surface of the leaves were removed by gentle brushing. The pupae were placed into Petri dish (10 cm dia.) with ventilated lids. Adult emergence started after one week and the newly emerged adults were transferred to an oviposition cage. The cultures were maintained in a control temperature (CT) room at $20 \pm 1^{\circ}\text{C}$ and $60 \pm 10\%$ RH (16, 8 L, D photoperiod).

Hemiptaresenus varicornis (Hymenoptera, Eulophidae) was collected from the highland vegetable growing are in Mount Bromo, East Java, Indonesia in 2000 and identified (Konishi, 1997; Noyes, 1998). *Liriomyza huidobrensis* was reared to the third instar as described in Section 3.2. Five pots containing bean plants infested with third instar larva of *L. huidobrensis* were placed in a parasitoid oviposition cage (38 cm L \times 38 cm W \times 38 cm H). Thirty percent w/v honey solution streaked on the inside roof of the cage provided a carbohydrate source for adult insects. Parasitoids were allowed to parasitize *L. huidobrensis* larvae for 24 h. The plants were then placed in a holding cage (70 x 55 x 45 cm) for 10 days to allow parasitoids develop to adults. Emerging adults were collected daily with an aspirator and transferred to parasitoid oviposition cages. The cultures were maintained in a CT room (Section 3.2).

D. isaea as Digsure (i), commercially available parasitoids wasps s provided by Biological Crop Protection Ltd (Occupation Rd, Wye, Ashford, Kent) were used in all experiment. *D. isaea* was sexed based using the method of Dong et al. (2000); Konishi, (1997); Minkenberg and van Lenteren, (1986). *D. isaea* was cultured using the procedures used for *H. varicornis*.

2.2. Competition experiments (Petri dish)

A Petri dish (14 cm dia.), with a nylon mesh-ventilation hole (5 cm diameter) in the lid, was used as the competition arena. Experiments were conducted in a CT room at $20 \pm 1^{\circ}\text{C}$ and $60 \pm 10\%$ RH (16, 8 L, D photoperiod). Treatments were as follows (*H. varicornis* and *D. isaea* were denoted as hv and di, respectively), **(1) hv then di**. A leaf containing 30 – 100 *L. huidobrensis* L3 was placed on moistened filter paper in a Petri dish and a single 48 h-old mated female hv was introduced. After 24 h hv was replaced in the arena by a single 48 h-old mated di. After a further 24 h, the leaf was examined under a binocular microscope and the number of live *L. huidobrensis* larvae, and cases of host killing, host feeding, multiparasitism, and oviposition were recorded; **(2) hv**. As treatment 1 except that only Hv was added to the test arena (48 h); **(3) hv+hv**. As treatment 1 except that two 48 h-old mated of *H. varicornis* (hv) were introduced inside the arena (24 h); **(4) di then hv**. As treatment 1 but in reverse order for parasitoids; **(5) di**. As treatment 2; **(6) di+di**. As treatment 3.

All experiments were replicated six times. ANOVA (1 factor, 6 levels of treatment) was used to detect differences between treatments (Statistica, 1999, Statsoft inc., Tulsa, USA). Tukey's test (HSD) was used for mean separation at $P < 0.05$. If necessary, data were arcsine-transformed (Gomez and Gomez, 1984) before the analysis to meet the assumption of normality and homogeneity.

2.3. Competition experiment (cage)

Perspex cages (70 x 55 x 45 cm) with nylon mesh fronts were used. Experiments were conducted in a CT room at $20 \pm 1^{\circ}\text{C}$ and $60 \pm 10\%$ RH (16, 8 L, D photoperiod). There were three treatments, **1/2. hv alone or di alone**. A potted dwarf bean plant with two leaves infected with a total of 30 to 100 *L. huidobrensis* L3 was placed inside a cage and 10 pairs of 48 h-old mated male and female parasitoids were introduced into cage. After 24 h, the plant was transferred to a parasitoid-free cage, and a new plant with infested leaves placed in the cage with the parasitoids. This procedure was repeated every 24 h for a total of 7 days; **3. hv + di**. As treatments 1 and 2 except that five pairs of both parasitoid species were introduced into the cage.

For all treatments, leaves were examined under a binocular microscope after 5 days to check for presence of prepupae or pupae of either *H. varicornis* (white or light brown in colour) or *D. isaea* (green in colour) and for dead larvae of *L. huidobrensis* larvae (caused by either multiparasitism or host feeding).

All experiments were replicated four times. Repeated measurement (7 days) ANOVA (1 factor, 3 levels) was used to determine differences among treatments Statistica version 1999 (Statsoft, 1999). Tulsa, USA). Tukey's test

(HSD) was used for mean separation at $P < 0.05$. If necessary, data were arcsine-transformed (Gomez and Gomez, 1984) before the analysis to meet the assumption of normality and homogeneity.

3. Results

3.1. Competition experiments (Petridish)

Superparasitism and multiparasitism occurred during this experiment although the mean rate was always low (up to 1.4 (Figure 1)). *Hemitarсенus varicornis* were able to oviposit on the same host larvae when they were exposed to larvae either alone or with another female of the same species, thus leading to either self or conspecific superparasitism. Only conspecific superparasitism was found for *D. isaea*. When two species were released sequentially in the same host, multiparasitism was only found in the treatment where *D. isaea* was introduced first (Figure 1). However, the differences between treatments were not significant (ANOVA, $F(5,30) = 0.62$, $P = 0.685$).

Oviposition and host killing rates (Figure 2 and 3, respectively) did not differ significantly between treatments (oviposition, ANOVA, $F(5,30) = 0.94$, $P = 0.47$; host killing total (host feeding + dead by sting), ANOVA, $F(5,30) = 0.87$, $P = 0.51$).

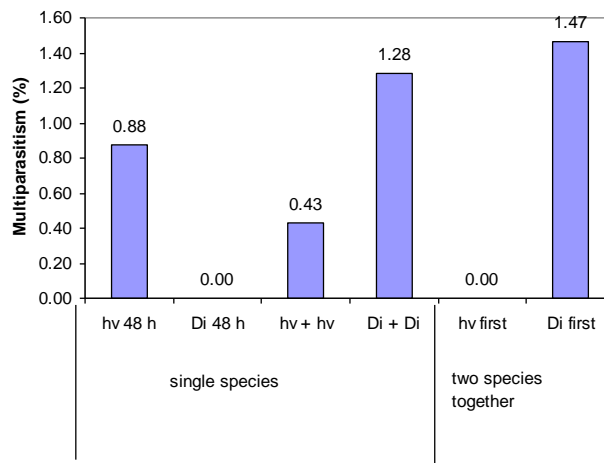


Figure 1. Multiparasitism and superparasitism by single and mixed parasitoid species.

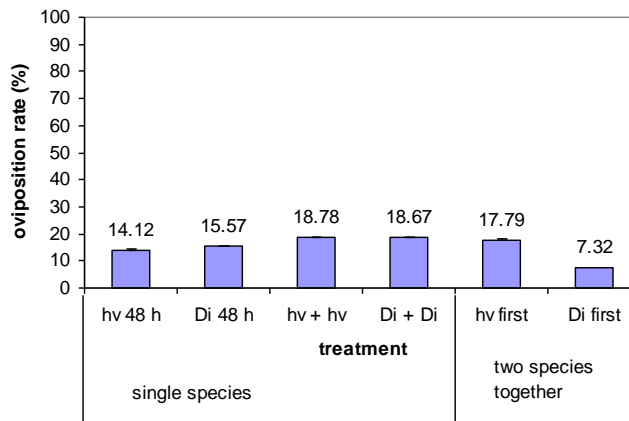


Figure 2. Oviposition rate of single and mixed parasitoid species.

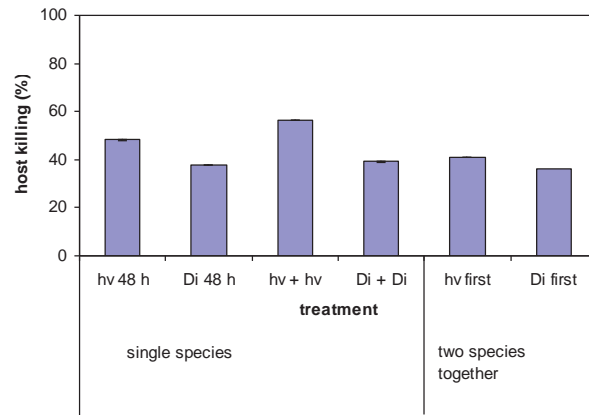


Figure 3. Host killing rate by single and mixed parasitoid species.

3.2. Competition experiments (cage)

Mean total oviposition, host-killing and total parasitism rates (oviposition + host-killing) are shown in Figures 4, 5 and 6, respectively. Oviposition rates (Figure 4) were not significantly different between treatments after 7 days observation (ANOVA, $F_{(2,54)} = 0.86, P = 0.45$) and there was no significant interaction between treatments and time of observation (ANOVA, $F_{(2,54)} = 0.55, P = 0.87$).

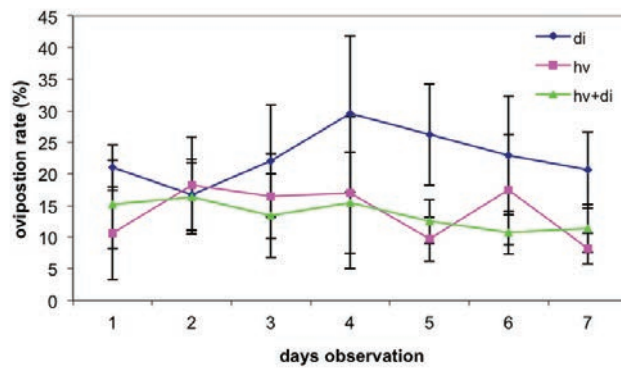


Figure 4. Oviposition rate of single and mixed parasitoid species .

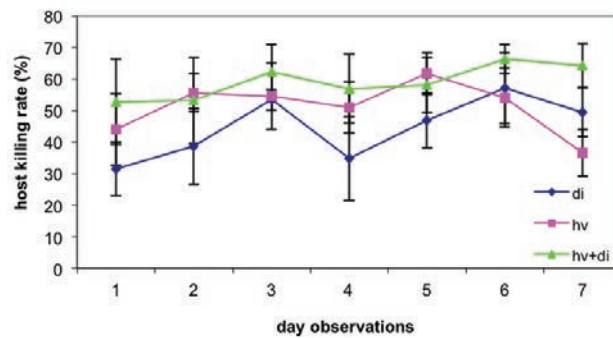


Figure 5. Host killing rate of single and mixed parasitoid species.

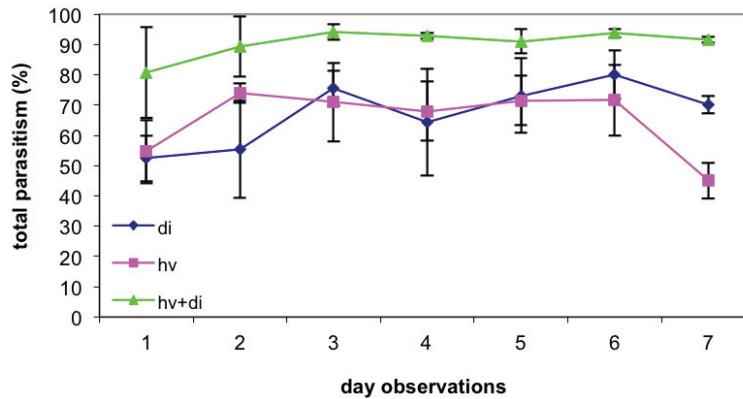


Figure 6 Total parasitism rate by single and mixed parasitoid species.

Similar results were obtained for host killing (Figure 7.5), with no significant difference between treatments (ANOVA, $F_{(2,54)} = 1.1$, $P = 0.36$) and no significant interaction between treatment and time of observation (ANOVA, $F_{(2,54)} = 0.88$, $P = 0.57$).

There was a significant difference in total parasitism (Figure 7.6; oviposition + host killing) rate (ANOVA, $F_{(2,54)} = 5.79$, $P < 0.02$), with this being significantly greater when the two species were together compared with single species treatments ($p < 0.05$, Tukey's HSD test). There was no significant difference between the two single species treatments ($p > 0.05$, Tukey's HSD test).

4. Discussion

The present experiments showed that very low rates of superparasitism and multiparasitism occurred in the Petri dish arenas in any of the treatments, none of which were significantly different, indicating very little if any interaction between *H. varicornis* and *D. isaea*. Wang and Messing (2003) showed that multiparasitism occurred when *Fopius arisanus* (Hymenoptera, Braconidae) and *Diachasmimorpha tryoni* (Hymenoptera, Braconidae) were present in the same host, a tephritid fruit flies *Ceratitidis capitata*. *F. arisanus* was a winner in this intraspecific interaction. The early hatching larvae of *F. arisanus* suppressed the development of conspecific eggs or larvae of *D. tryoni*. In contrast, the present experiments found no evidence that oviposition by *H. varicornis* or *D. isaea* could kill conspecific larvae. These two species of parasitoid also appeared to share the same resource effectively through complementary occurrence in time and space (e.g. Godfray, 1994). This observation implies that biological control will be more successful when both natural enemies of *L. huidobrensis* are present, and is supported by theory (De Bach, 1966; Hudson and Greenman, 1998).

Many factors, such as oviposition experience, the presence of conspecific female parasitoids and the availability of hosts may influence host discrimination (Reitz and Trumble, 2002). For example, Urbaneja et al. (2003) showed that *Quadraticus* sp. (Hymenoptera; Eulophidae) was an intrinsically superior competitor compared with *Cirrospilus brevis* (Hymenoptera; Eulophidae) on a population of a lepidopteran leafminer on citrus. The present, Petri dish, experiments (and the cage experiments) showed no significant competitive interactions occurred between *D. isaea* and *H. varicornis*. This remains to be investigated in the field but since a vegetable production system is a far more complicated system and it would be predicted that any competition between two species would not be as intense as in the laboratory due to factors such as host plant preference, parasitoid host stage range, differential adaptation to temperature, and susceptibility to insecticides (Liu and Jiang, 2003).

Since *D. isaea* and *H. varicornis* are able to kill leafminer larvae by probing with their ovipositors and feeding on host haemolymph this may lead to intra- and interspecific lethal interference competition if parasitized hosts are fed upon and killed (Collier and Hunter, 2001). In fact, host feeding is a common mechanism of interaction among parasitoids (Godfray, 1994; Collier and Hunter, 2001; Collier et al., 2002). There was no evidence of such

competition in the cage experiments with *D. isaea* and *H. varicornis* and in fact total parasitism (oviposition + host killing) was significantly greater when both species were present.

While the issue of whether single or multiple natural enemy species provide better pest control has been much discussed by biological control practitioners and population biologists, it has been resolved neither theoretically nor empirically (Visser and Rosenheim 1998; Collier et al., 2002; Hassell, 2000; Mills, 2000; Reitz and Trumble, 2002; Lachaud et al., 2002). In nature most hosts support more than one parasitoid species (Mills, 2000) and leafmining insects tend to possess the highest parasitoid load (Hawkins and Lawton, 1987).

The present results suggest that field releases of *D. isaea* would not have a detrimental effect on the indigenous species, *H. varicornis*, and that *D. isaea* may be a “good” candidate to supplement biological control of *L. huidobrensis*. However, to avoid failure in biological control programmes further studies are required, including determination of host range and hyperparasitism (Lynch and Thomas, 2000).

Reference

- Collier, T., Hunter, M., 2001. Lethal Interference Competition In Whitefly Parasitoids *Eretmocerus Eremicus* And *Encarsia Sophia*. *Oecologia* 129, 147-154.
- Collier, T., Kelly, S., Hunter, M., 2002. Egg Size, Intrinsic Competition, And Lethal Interference In The Parasitoids *Encarsia Pergandiella* And *Encarsia Formosa*. *Biol. Control* 23, 254-261.
- De Bach, P., 1966. The Competitive Displacement And Coexistence Principles. *Ann. Rev. Entomol.* 11, 183-212.
- Denno, R.F. McClure, M.S., Ott, J.R., 1995. Interspecific Interaction In Phytophagous Insect, Competition Reexamined And Resurrected. *Annu. Rev. Entomol.* 40, 297-331.
- Dong, C.Z., Lasalle, J., Huang, D.W. 2000. A Review Of The Chinese Diglyphus Walker (Hymenoptera, Eulophidae). *Orient. Insects.* 34, 263-288.
- Ehler, L.E., 1990. Introduction Strategies In Biological Control Of Insects. In Mackauer, M., Ehler, L. E. , Roland, J., (Eds). *Critica Issues In Biological Control*. Pp. 111-134. Intercept, Andover, Uk.
- Godfray, H.C.J. 1994. *Parasitoids Behavioral And Evolutionary Ecology*. Princeton University Press, New Jersey, 520 Pp.
- Hassel, M. P., 2000. *The Spatial And Temporal Dynamics Of Host-Parasitoid Interaction*. Oxford University Press. Oxford,
- Hawkins, B.A., Lawton, J., 1987. Species Richness For Parasitoids Of British Phytophagous Insect. *Nature* 326, 788-790.
- Hudson, P. , Greenman, J., 1998. Competition Mediated By Parasites, Biological And Theoretical Progress. *Tree.* 13, 387-390.
- Huffaker, C.B., Simmonds, F.J., Laing, J.E. 1976. The Theoretical And Empirical Basic For Biological Control. In Huffaker, C. B. , Messenger, P. S. (Eds.) *Theory And Practice Of Biological Control*. Pp. 41-78. Academic Press, New York.
- Konishi, K. 1997. An Illustrated Key To The Hymenopterous Parasitoids Of *Liriomyza Trifolii* In Japan. *Miscellaneous Publication Of The National Institute Of Agro-Enviromental Science No. 22*, Pp. 27-76 (In English).
- Krijger, G.L., Peters, C.L., Seventer, J.G. 2001. Competitive Ability Of Neotropical *Drosophila* Predicted From Larval Development Times. *Oikos* 92, 325-332.
- Lachaud, G.P., Hardy, I.C.W., Lachaud, J.P. 2002. Insect Gladiator, Competitive Interaction Between Three Species Of Bethyloid Wasps Attacking The Coffee Berry Borer, *Hypothenemus Hampei*. *Bio. Control* 25, 231-238.
- Lasota, J.A. , Dybas, R.A. 1991. Avermectins, A Novel Class of Compounds, Implications For Use In Arthropod Pest Control. *Ann. Rev. Entomol.* 36, 91-117.
- Liu, S.S. , Jiang, L.H. 2003. Differential Parasitism Of *Plutella Xylostella* (Lepidoptera, Plutellidae) Larvae By The Parasitoid *Cotesia Plutellae* (Hymenoptera, Braconidae) On Two Host Plant Species. *Bull. Entomol. Res.* 93, 65-72
- Lynch, L.D , Thomas, M.B. 2000. Nontarget Effects In Biocontrol Of Insect With Insects, Nematodes And Microbial Agents, The Evidence. *Biocont. News Inform.* 21, 117-130.
- Mills, N.J. 2000. Biological Control, The Need Realistic Models And Experimental Approaches To Parasitoid Introduction. In Hochberg, M. E. , Ives, M. E. (Eds.). *Parasitoid Population Biology*. Pp 217-234. Princeton University Press. Princeton.
- Minkenber, O.P.J. , Van Lenteren, J.C., 1986. The Leafminers *Liriomyza Bryoniae* And *L. Trifolii* (Diptera, Agromyzidae), Their Parasites And Host Plants, A Review. *Agric. Univ. Wageningen Papers*, No. 86. 50p.
- Murdoc, M.M., Briggs, C.J., Collier, T.R. 1998., Biological Control In Insect, Implications For The Theory In Population Ecology. In Dempster, J. P., Mclean, I.F.G. (Eds.). *Insect Populations In Theory And Practice*. Pp. 167-186. Kluwer Academic. Dordrecht.
- Murphy, S.T., Lasalle, J. 1999. Balancing Biological Control Strategies In The Ipm Of New World Invasive *Liriomyza* Leafminers In Field Vegetables Crops. *Biocont. News Inform.* 20, 91-104.
- Rauf, A. , Shepard, B.M. 1999. Leafminers In Vegetables In Indonesia; Surveys Of Host Crops, Species Composition, Parasitoids, And Control Practices. In Lim, G.S., Soetikno, S.S., Loke, W.H. (Eds) *Proc. Workshop On Leafminers Of Vegetables In Southeast Asia, Tanah Rata, Malaysia 2-3 February 1999*, Pp. 25-34, Cab International Southeast Asia, Serdang, Malaysia.
- Rauf, A., Shepard, B.M. , Jonhson, M.W. 2000. Leafminer In Vegetables, Ornamental Plants And Weeds In Indonesia, Surveys Of Host Crops, Species Composition And Parasitoids. *Int. J. Pest Manag.* 46, 257-266.
- Reitz, , S.R. , Trumble J.T. 2002. Competitive Displacement Among Insects And Arachnids. *Annu. Rev. Entomo.* 47, 435-465.

- Rosenheim, J.A., Kaya, H.K., Ehler, L.E., Marois, J.J., Jaffe, B.A. 1995. Intraguild Predation Among Biological Control Agents, Theory And Evidence. *Biol. Control* 5, 303-335.
- Shi, Z. H., Li, Q. B., Li, X. 2004. Interspecific Competition Between *Diadegma semiclausum* Hellen (Hym., Ichneumonidae) And *Cotesia plutellae* (Kurdjumov) (Hym., Braconidae) In Parasitizing *Plutella xylostella* (L) (Lep., Plutellidae). *J. Appl. Entomol.* 128, 437-444.
- Urbaneja, A., Llacer, E., Garrido, A., Jacas, J.A., 2003. Interspecific Competition Between Two Parasitoids of *Phyllocnistis citrella* (Lepidoptera, Gracillariidae), *Cyrtospilus brevis* and Exotic *Quadrastichus* sp. (Hymenoptera, Eulophidae). *Biol. Control* 28, 243-250.
- Van Driesche, R.G., Bellows Jr., T.S. 1996. *Biological Control*. Chapman And Hall. New York.
- Visser, M.E., Rosenheim, J.A. 1998. The Influence Of Competition Between Foragers On Clutch Size Decisions In Insect Parasitoids. *Biol. Control*. 11, 169-174.
- Wang, X.G., Messing, H. 2003. Intra- and Interspecific Competition By *Foriulus arisanus* and *Diachasmimorpha tryoni* (Hymenoptera, Braconidae), Parasitoids of Tephthrid Fruit Fly. *Biol. Control* 27, 251-259.