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Rearing of *Bactrocera zonata* (Diptera: Tephritidae) for parasitoids production and managing techniques for fruit flies in mango orchards

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Abstract. The effectiveness of rearing fruit flies (Diptera: Tephritidae) for parasitoid production and pest control need to be improved. In these studies, the effects of dietary protein ingredients on the biological parameters of the peach fruit fly *Bactrocera zonata* (Saunders) for parasitoid production were investigated. Among the various food ingredients, torula yeast and brewer's yeast were found to be more promising than Nu-Lure® and protein hydrolysate for rearing of *B. zonata*. To reduce the infestation of this pest in mango *Mangifera indica* L. orchards, biological control agents and the male annihilation technique were evaluated. Significantly fewer fruits were infested with fruit flies in the treated blocks compared with fruits in the untreated block. The field efficacy of the hymenopteran larval and pupal parasitoids *Aganaspis (Trybliographa) daci* (Weld) and *Dirhinus giffardii* Silvestri, respectively, plus the male annihilation technique in controlling fruit flies was significantly high than that of biological control agents and male annihilation as separate treatments. The release of parasitoids as agents of biological control can play a major role in the suppression of fruit fly populations and parasitoids also pose a minimal non-target risk. Furthermore, combining control tactics could be a very important aspect of an action plan for fruit fly population suppression.

Key words: *Bactrocera zonata*, peach fruit fly, mango, rearing, diet, integrated pest management, parasitoid, biological control, male annihilation technique

Introduction

Fruit flies (Diptera: Tephritidae) of the subfamily Dacinae are a miscellaneous and swiftly evolving group with more than 700 described species, which are a constant threat to tropical agricultural production (Metcalf, 1990). One dacine species, the peach fruit fly *Bactrocera zonata* (Saunders), is native to tropical Asia, but is spreading to other regions of the world. This pest is known to attack more than 50 fruit host plants, e.g. mango *Mangifera indica* L., peach, guava, apricot and citrus, and vegetable host plants (El-Akhdar and Afia, 2009; Sarwar *et al*., 2013). Mohyuddin and Mahmood (1993) reported that mango fruits in central Punjab are attacked in July; the maximum injury occurs in August when 35% of the fruits are damaged by *B. zonata* or the oriental fruit fly *Bactrocera dorsalis* (Hendel).

Current control methods for this pest rely heavily on the aerial application of pesticides. These chemicals have a negative impact on the environment, specifically on beneficial organisms. Thus, environmentally friendly methods of control are much in need (Roessler, 1989). Biological control

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is increasingly being recognized as a safe and economical means of fruit fly control, and its importance is growing as pesticide use is becoming more restricted. Consequently, recent studies have used various approaches to select biological control agents that are safe and effective.

Several infestations of *Bactrocera* species have been eradicated using a combination of control methods including the male annihilation technique, foliar and soil pesticide treatments, and fruit removal (Marwat *et al.*, 1992). The male annihilation technique is based on males being attracted to methyl eugenol, a component of the female pheromone on which males feed usually only once in their lifetime (Shelly, 2000).

Wong *et al.* (1984) contended that fruit flies are still very serious pests of a wide range of fruits and vegetables. They suggested that inundative releases of laboratory-reared parasitoids might be an appropriate control option. These parasitoids belong to the families Braconidae, Chalcididae and Eulophidae. Ovruski *et al.* (2000) discussed the diversity of fruit fly parasitoids (Hymenoptera) and provided information on the following: parasitoids attacking flies, analysis of parasitoid guilds, parasitoid assemblage size, fly host profiles, distribution patterns, taxonomic status, biological control of pestiferous tephritids, the most pressing needs related to fruit fly biological control, and the effectiveness of the chalcidid *Dirhinus giffardii* Silvestri (Podoler and Mazor, 1981).

To successfully rear fruit flies (and their parasitoids), the rearing technique must be improved by developing a low-cost diet. The ingestion of protein has been reported to increase the egg production of various insects (Cangussu and Zucoloto, 1995; Khan *et al.*, 1999). Furthermore, in sterile insect releases of this pest, to ensure good-quality biological parameters, mass-rearing of insects with effective ingredients and production of competitive adults that successfully suppress the field population are prerequisites (Saha *et al.*, 2007).

The effective exploitation of host-rearing for parasitoid production as a tool for fruit fly control needs to be improved. Simultaneously, this can be a major contribution to population suppression when using the sterile insect technique. Furthermore, combining effective control tactics could be a very important aspect of a practical action plan for fruit fly population suppression in the field. Therefore, a coordinated project was initiated. In this study, we investigated the effects of various diet ingredients on the rearing of *B. zonata* and also the efficacy in mango orchards of biological control agents and male annihilation in managing this pest that is a widely distributed fruit fly in Pakistan.

Materials and methods

Fruit fly colony

The stock laboratory colony of *B. zonata* at the Nuclear Institute of Agriculture, Tando Jam, Pakistan, was reared at 25 ± 2 °C, 60–65% relative humidity (RH) and 16L:8D photoperiod. The fruit flies were originated from a population collected from infested guava and mango orchards, and rearing has been carried out for several generations to improve laboratory adaptation. Pupae from this colony were used to obtain fresh specimens of adult male and female *B. zonata*. Eggs were collected from adult flies in the colony. Adults were kept in cages (45 × 30 × 35 cm) with nylon mesh covers and given a food supplement containing sugar and yeast placed in Petri dishes. Water was supplied using cotton wicks in conical flasks; the wicks and water were changed regularly to avoid microbial contamination, especially moulds. Before testing, larvae were reared in the laboratory on the standard artificial diet consisting primarily of wheat bran, sugar and yeast and also bacterial and fungal inhibitors. Sawdust, present below larva-holding boxes, was sifted weekly to collect pupae. Pupae were transferred to smaller plastic containers and held until the emergence of flies.

Effects of dietary protein on the rearing of Bactrocera zonata

A total of 30,000 eggs of *B. zonata* were seeded on diets containing proteins in various forms and allowed to develop into larvae, pupae and adults. The dietary protein ingredients – protein hydrolysate, Nu-Lure®, brewer's yeast and torula yeast – were each added separately to the standard diet. The insects were kept in a laboratory under controlled temperature and RH conditions.

The biological parameters of *B. zonata* (pupal recovery, pupal weight, adult emergence, flying ability and egg hatch) were assessed. Two weeks after adult emergence, eggs were collected twice a week using plastic egg-laying receptacles (vials perforated with 50 1 mm-diameter holes). Newly matured larvae were kept individually in small plastic containers until pupation. Newly formed (24 h) pupae were counted and weighed, and subsequent adult emergence was recorded. Adults that emerged from each diet were considered as the F1 generation and kept in similar cages. The flight ability (percentage of fliers) of adults reared on each diet was also recorded. This experiment was extended to quantify pupal quality and adult emergence in the F2 generation.

Effects of biological control agents and other management techniques on the infestation of Bactrocera zonata *in mango orchards*

Measures to manage the flies included, separately and in combination, the male annihilation technique and releases of larval and pupal hymenopteran parasitoids (*Aganaspis (Trybliographa) daci* (Weld) and *D. giffardii*) throughout the mango fruiting season. All treatments, replicated five times, were applied to five separate blocks of mango orchard, each with an area of 2 ha. The field efficacy of the treatments was compared with that of the control.

Parasitoids were obtained from a colony that had been reared at 28 ± 2 °C, 60–80% RH and 16L:8D photoperiod and maintained for several generations in the same laboratory. Parasitoids eclosed from pupae inside cages (25 × 25 × 25 cm). The wasps were fed a solution of honey and 40% sugar. About 500 wasps were held inside each cage until their release. Usually, parasitoids from the cages were transferred to small containers with screen tops (about 1000 parasitoids per container) and placed under host fruit trees. The containers were opened gently and parasitoids allowed to disperse to nearby trees. Parasitoids left inside the containers were removed with a small brush and carefully placed on nearby vegetation. Every 2 weeks, from fruit-setting to ripening, approximately 2470 adults of each parasitoid species were released into each hectare.

The male annihilation technique was applied through traps baited with methyl eugenol at the rate of about 15 traps per ha. These traps were freshly baited every month.

The infestation level and population density of fruit flies were recorded every week. About 100 fruits were collected either from the soil or randomly from the trees and placed in plastic containers. The fruits were held in a laboratory under uncontrolled temperature and humidity conditions until full decomposition. Once a week, larvae and pupae were collected and placed in Petri dishes until adult emergence. The number of larvae or pupae that had been parasitized, and the parasitoid species involved, were recorded.

To measure the density of the fly population, plastic traps baited with chemicals (10% sugar solution, 85% methyl eugenol and 5% insecticide) on cotton wicks inside the traps were hung on mango trees to attract females and males.

Statistical analyses

The data were statistically analysed using a computer package, and an analysis of variance was carried out. Analyses were carried out at the $P < 0.05$ level of significance.

Results

Effects of dietary protein on the rearing of Bactrocera zonata

Torula yeast, brewer's yeast and Nu-Lure® were found to be good food sources for rearing *B. zonata*. The highest mean values for all the tested parameters were obtained with the diet containing torula yeast and the lowest values with the diet containing protein hydrolysate. Torula yeast and brewer's yeast were found to be better for rearing *B. zonata* than Nu-Lure® and protein hydrolysate (Table 1). Thus, the inclusion of torula yeast or brewer's yeast provided a good source of protein in the standard larval diet. Brewer's yeast is more economical than torula yeast and is available locally, and therefore brewer's yeast can be used instead of torula yeast, which is an expensive and imported product. In future, additional diet ingredients will be evaluated to standardize the artificial diet for rearing *B. zonata* in the laboratory.

Effects of various biological control agents and other management techniques on the infestation of Bactrocera zonata *in mango orchards*

Based on fruit samples from an untreated orchard, both males and females were found to be present from the first week of fruit-setting during March–April, with the maximum infestation being observed during the ripening stage in July. Infested fruits were found both on trees and on the ground. The low fruit infestation early in the season resulted

Table 1. Effects of diet ingredients on the rearing parameters of *Bactrocera zonata*

Protein ingredients	Pupal recovery (%)	Pupal weight (mg)	Adult emergence (%)	Fliers (%)	Egg hatch (%)
Protein hydrolysate	42.68d	7.48d	39.65c	28.00d	22.45d
Nu-Lure®	49.79c	8.85c	57.10b	44.75c	53.70c
Brewer's yeast	75.68b	10.23b	65.01ab	75.25b	78.50b
Torula yeast	80.10a	11.43a	75.29a	82.00a	84.40a

Mean values within a column followed by the same letter are not significantly different at $P < 0.05$.

Table 2. Fruit infestation by *Bactrocera zonata* in mango orchards under various management techniques

Management techniques	Fruit infestation (%)				
	March	April	May	June	July
Biological control	0	0	1.00b	6.00b	17.00b
Male annihilation	0	0	0.50bc	4.25bc	11.75c
Biological control + male annihilation	0	0	0.20c	2.50c	7.75d
Control	0	0	3.00a	11.75a	27.75a

Mean values within a column followed by the same letter are not significantly different at $P < 0.05$.

from a low adult population, probably due to the low temperature or a very low overwintering adult population. Thus, the mean percentage of fruit infestation per week by *B. zonata* was zero in March and April. There were low captures in traps late in the infestation period because fruits were available to the pest only then.

Fruit infestation was significantly lower in the treated blocks than in the untreated one. In July, fruit infestation was lower in the biological control plus male annihilation treatment block than in either of the separate treatment blocks (Table 2).

The population build-up in April and May was high, possibly due to optimum temperature or to the emergence of adults from overwintering pupae. The population densities of *B. zonata* during April were at a maximum level. From May to July, the population density of fruit flies declined sharply in the biological control plus male annihilation treatment block; this treatment was more effective at controlling the fruit flies than the separate treatment with biological controls (Table 3). Evidently, male annihilation was a more effective control measure than the release of parasitoids (Tables 2 and 3).

The results showed that the male attractant was highly selective for males; for each female captured, 20 males were captured.

Based on samples of mangoes taken after successive releases of parasitoids, the number of *B. zonata* flies emerging in all the treated blocks was found to have decreased. Increases in parasitization from parasitoids and decreases in fruit infestation were observed in all the treated blocks, when compared with the control.

Good pest control was achieved through the biological control plus male annihilation treatment. However, in this trial, the release of parasitoids evidently played only a minor role in pest control, and the major controlling factor was male annihilation (Table 3). Nevertheless, combining biological control with male annihilation reduced the level of fruit damage in July more than that achieved by either control method alone (Table 2).

Discussion

Based on pupal recovery, torula yeast was found to be the best protein ingredient for the mass-rearing of *B. zonata* in the laboratory. Protein hydrolysate led to significantly lower recovery, with high mortality of late-instar maggots, compared with other sources. This is because of the quick depletion of food material or the presence of fewer dietary nutrients in protein hydrolysate required for larval development. Torula yeast and brewer's yeast were more promising than Nu-Lure® and protein hydrolysate. The differences in recovery can be explained by the higher content of protein in torula and brewer's yeasts and consequently the increased number of ovarioles in female flies. In concurrence with this, other workers have observed successful egg production after ingestion of protein food. Saha *et al.* (2007) evaluated the effects of various adult diets on pupal quality, adult emergence, ovariole number and longevity in *Bactrocera cucurbitae* (Coquillett). Pupal quality and percentage of adult emergence were found to be slightly higher in the F2 generation than in the F1 generation. Larval feeding is known to have a significant influence on the ovariole number in adult flies. The total egg production of *B. dorsalis* depends basically on two interdependent variables: the number of ovarioles present in the ovaries and the quality of nutrients consumed by the larvae and adults (Khan *et al.*, 2000). In this species, Shelly *et al.* (2005) found that inclusion of protein in the diet enhanced male mating performance.

Biological control is an important part of integrated pest management (IPM). In the present study, the successful release and establishment of parasitoids resulted in significant parasitization and some suppression of the field population of the fruit fly. As little research has been conducted on the use of *A. daci* and *D. giffardii* for the control of *B. zonata*, little is known about this pest and its natural enemies. In agreement with the present

Table 3. Population density of *Bactrocera zonata* in mango orchards under various management techniques

Management techniques	Population density of *B. zonata* (number per trap per week)				
	March	April	May	June	July
Biological control	242b	399b	360a	261b	249b
Male annihilation	189c	99c	50b	33c	19c
Biological control + male annihilation	183c	82c	45b	29c	13c
Control	327a	461a	419a	543a	531a

Mean values within a column followed by the same letter are not significantly different at $P < 0.05$.

findings in the field, other workers have reported successful control of fruit flies of the *Bactrocera* spp. Waterhouse (1993) commented that, under favourable conditions and with a suitable fruit fly host, parasitization levels of up to 70% can be achieved. Releases of a suite of parasitoids have been found to result in reductions in the populations of fruit flies of up to 95%. Peters (1997) reported that the release of parasitoids was successful, the parasitoids became established on the fruit fly, and the level of parasitization was 78% on most of the favoured fruits. Of the at least 82 species of parasitoids that have been reared from tephritids during exploration programmes, only 44 have been released and only 20 have become established (Wharton, 1989). Several factors might account for past failures to establish parasitoids, including a lack of information on the biology and ecology of the target fruit fly pest and natural enemies associated with it. In the present study, a low population level was observed during the early fruiting stage, whereas at the ripening stage, the level of fruit infestation was higher. The same trend was observed in captures of females and males during all the treatments. Such dissimilar patterns are probably due to climatic variations over time. Environmental factors such as rain and temperature have been reported to affect trap captures (Miranda *et al.*, 2001).

Finally, numerous studies have demonstrated the feasibility of parasitoid augmentation for fruit fly population suppression (Harris *et al.*, 2000). In the present study, fruit damage caused by *B. zonata* was found to be reduced and a biological base for further improvement of an IPM programme was developed. In conjunction with sanitation, protein bait sprays and male annihilation treatments, the release of parasitoids can reduce the fruit fly risk (Vargas *et al.*, 2003).

Conclusion

The techniques described herein for rearing *B. zonata* and its parasitoids are relatively simple. Natural enemies help to some extent to reduce fruit fly populations. It is important to examine further the practical advantages that might be obtained from establishing fruit fly parasitoids that parasitize *B. zonata* and other pest species. Parasitoids that oviposit into the puparium, e.g. *D. giffardii*, and into the larva, e.g. *A. daci*, have been neglected mainly because of sampling difficulties, but they deserve more attention. Nevertheless, there is no evidence that establishing parasitoids in orchards would cause adverse effects. Research on biological control agents should continue, e.g. the major hosts of the target fruit flies, the suitability of the flies for the candidate parasitoids and the level of parasitization already achieved by native or introduced parasitoids. Reduction of pest infestations through parasitoids is likely to be of greatest value to the traditional farmer, but they should not be exclusively relied upon to control a pest population; but complementary control methods should be applied together.

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References

Cangussu J. A. and Zucoloto F. S. (1995) Self-selection and perception threshold in adult females of *Ceratitis capitata* (Diptera, Tephritidae). *Journal of Insect Physiology* 41, 223–227.

El-Akhdar E. A. H. and Afia Y. E. (2009) Functional ultrastructure of antennae, wings and their associated sensory receptors of peach fruit fly, *Bactrocera zonata* (Saunders) as influenced by the sterilizing dose of gamma irradiation. *Journal of Radiation Research and Applied Sciences* 2, 797–817.

Harris E. J., Bautista R. C. and Spencer J. P. (2000) Utilisation of the egg-larval parasitoid, *Fopius* (*Biosteres*) *arisanus*, for augmentative biological control of tephritid fruit flies, pp. 725–732. In *Area-Wide Control of Fruit Flies and Other Insect Pests* (edited by K. H. Tan). Penerbit Universiti Sains Malaysia, Pulau Pinang, Malaysia.

Khan M., Shahjahan R. M. and Wadud M. A. (1999) Effect of larval dietary protein sources on different aspects of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Bangladesh Journal of Entomology* 9, 39–44.

Khan M., Shahjahan R. M. and Wadud M. A. (2000) Influence of larval feeding and adult diets on ovariole number and egg production of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Bangladeshi Science and Technology* 2, 59–64.

Marwat N. K., Hussain N. and Khan A. (1992) Suppression of population and infestation of *Dacus* spp. by male annihilation in guava orchard. *Pakistan Journal of Zoology* 24, 82–84.

Metcalf R. L. (1990) Chemical ecology of Dacinae fruit flies (Diptera: Tephritidae). *Annals of the Entomological Society of America* 83, 1017–1030.

Miranda M. A., Alonso R. and Alemany A. (2001) Field evaluation of Medfly (Dipt., Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). *Journal of Applied Entomology* 125, 333–339.

Mohyuddin A. I. and Mahmood R. (1993) Integrated control of mango pests in Pakistan. *Acta Horticulturae* 341, 467–483.

Ovruski S., Aluja M., Sivinski J. and Wharton R. (2000) Hymenopteran parasitoids on fruit-infesting Tephritidae (Diptera) in Latin America and the southern United States: diversity, distribution, taxonomic status and their use in fruit fly biological control. *Integrated Pest Management Reviews* 5, 81–107.

Peters A. (1997) Prospects for the use of biological control agents to control fruit flies, pp. 199–204. In *Management of Fruit Flies in the Pacific.* Regional symposium. 28–31 October 1996, Nadi, Fiji (edited by A. J. Allwood and R. A. I. Drew). ACIAR Proceedings No. 76.

Podoler H. and Mazor M. (1981) *Dirhinus giffardii* Silvestri (Hym.: Chalcididae) as a parasite of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Dip.: Tephritidae). 2. Analysis of parasite responses. *Acta Oecologica, Oecologia Applicata* 2, 299–309.

Roessler Y. (1989) Insecticidal bait and cover sprays, pp. 329–336. In *Fruit Flies: Their Biology, Natural Enemies and Control.* World Crop Pests, Vol. 3B (edited by A. S. Robinson and G. Hooper). Elsevier, Amsterdam.

Saha A. K., Khan M., Nahar G. and Yesmin F. (2007) Impact of natural hosts and artificial adult diets on some quality parameters of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). *Pakistan Journal of Biological Sciences* 10, 178–181.

Sarwar M., Hamed M., Rasool B., Yousaf M. and Hussain M. (2013) Host preference and performance of fruit flies *Bactrocera zonata* (Saunders) and *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) for various fruits and vegetables. *International Journal of Scientific Research in Environmental Sciences* 1, 188–194.

Shelly T. E. (2000) Trapping male oriental fruit flies (Diptera: Tephritidae): does feeding on a natural source of methyl eugenol reduce capture probability? *Florida Entomologist* 83, 109–111.

Shelly T. E., Edu J. and Pahio E. (2005) Influence of diet and methyl eugenol on the mating success of males of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). *Florida Entomologist* 88, 307–313.

Vargas R. I., Jang E. B. and Klungness L. M. (2003) Area-wide pest management of fruit flies in Hawaiian fruits and vegetables, pp. 37–46. In *Recent Trends on Sterile Insect Technique and Area-Wide Integrated Pest Management*. Research Institute for Subtropics, Okinawa, Japan.

Waterhouse D. F. (1993) *Biological Control Pacific Prospects.* Supplement 2. Australian Centre for International Agricultural Research, Canberra. 138 pp.

Wharton R. A. (1989) Classical biological control of fruit-infesting Tephritidae, pp. 303–313. In *Fruit Flies: Their Biology, Natural Enemies and Control.* World Crop Pests, Vol. 3B (edited by A. S. Robinson and G. Hooper). Elsevier, Amsterdam.

Wong T. T. Y., Mochizuki N. and Nishimoto J. I. (1984) Seasonal abundance of parasitoids of the Mediterranean and oriental fruit flies (Diptera: Tephritidae) in the Kula area of Maui, Hawaii. *Environmental Entomology* 13, 140–145.