Damage Patterns, Monitoring, and Management of Procontarinia mangiferae (Diptera: Cecidomyiidae) in Pakistan

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Damage Patterns, Monitoring, and Management of *Procontarinia mangiferae* (Diptera: Cecidomyiidae) in Pakistan

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Abstract

Since 2005, mango has been damaged by a group of new pests, the gall midges, in all mango-growing areas of Pakistan. Little is known about these pests in Pakistan. In this report, we present information on the occurrence, damage patterns, methods for monitoring, and management of *Procontarinia mangiferae* (Felt), a mango gall midge found in the country. At the study site (Rahim Yar Khan, a district of the province Punjab, Pakistan), the pest was active from January/February to April as eggs, larvae, and adults on mango inflorescence buds, branches (axillaries), and immature fruits. Females of *P. mangiferae* oviposited in inflorescence tissues, and larvae, after feeding on plant tissues, dropped to the soil under the mango trees for pupation from February to April. Mango trees in commercial orchards were more heavily damaged by *P. mangiferae* than were isolated trees in farmer fields (66.7%). The adults of *P. mangiferae* were captured on sticky traps of all tested colors, and were in flight from January to May. Captures per trap were highest on yellow traps, followed by green, blue, and clear traps. Control of mango gall midge was effectively provided by the synthetic insecticide bifenthrin (Talstar 10 EC) and also by application of neem seed kernel extract on the tree canopy if integrated with raking the soil under the mango tree canopy.

Gall midges (Diptera: Cecidomyiidae) include phytophagous and gall-making species (Barnes 1948, Hill 1987). More than 20 species of midges are pests of mango, *Mangifera indica* L., in Brazil, China, Guadeloupe, Hawaii, India, Iran, Japan, Kenya, Mauritius, Oman, Philippines, Réunion, South Africa, Taiwan, United Arab Emirates, and many other parts of the world (De Villiers 1998, Gagné 2004). Most species of gall midges attacking mango belong to *Dasineura* or *Procontarinia*. These gall midges cause damage by feeding on flowers and fruit tissues and making galls on the leaves of mango trees (Uechi et al. 2002, Askari and Bagheri 2005, Kolesik et al. 2009). Gall midge damage promotes the development of epidemics of anthracnose, *Colletotrichum gloeosporioides* Penzig and Saccardo (Uechi et al. 2002). Fruit losses due to anthracnose range from 2 to 39% in India (Prakash and Srivastava 1987).

Gall midges were not reported as pests of mango in Pakistan until recently, and little knowledge exists of gall midge management on mango in the region. By 2006, they were, however, ranked as the number one pest of mango in Pakistan (Anonymous 2006). Gall midge attacking mango inflorescence in Pakistan proved to be a species (*Procontarinia mangiferae* (Felt)) new to the region but known in other countries, including Australia, where it is found on mango throughout the year (Amouroux et al. 2013). In India, it emerges in January, peak in abundance in March, and adults no longer present by April (Prasad 1971). Consequently, we initiated studies on identification, damage patterns, biology, and ecology of *P. mangiferae* to develop management strategies for this and other gall midges associated with mangoes in Pakistan.

Procontarinia mangiferae has been found in all the areas surveyed in Punjab (Pakistan). It has spread widely from north-eastern India (its native range), reaching Africa (Kenya, South Africa), the Caribbean (Guadeloupe and other parts of the West Indies), South America (Brazil), other parts of Asia (Iran, Java, Indonesia, Thailand), and some Oceanic islands (Mauritius, Réunion Island; De Villiers 1998, CABI 2004). Synonyms of *Procontarinia mangiferae* are *Erosomyia mangiferae* (Felt) 1911, *Mangodiplosis mangiferae* Tavares 1918, *Rhabdophaga mangiferae* Mani 1938, and *Erosomyia indica* Grover 1965 (Gagné and Medina 2004, Amouroux et al. 2013).

Although little is known about *P. mangiferae*, the biologies of other two mango-feeding gall midges, *Procontarinia matteiana* Kieffer & Cecconi and *Dasinura amaramanjarae* Grover, have been reported (Rehman et al. 2013a,b, 2014). *Procontarinia matteiana* damages mangoes by forming solitary or grouped galls on the upper and lower surfaces of the leaves. It is active from February/March to November, with two peaks of population, the first in March/April and the second in September/October, when galls are the most abundant. The phenology of two parasitoids, *Closterocerus pulcherrimus* (Kerrich) (Hymenoptera: Eulophidae) and *Synopeas temporale* Austin (Hymenoptera: Platygasteridae), reared from galls of *P. matteiana* was well-synchronized with that of their host (Rehman et al. 2013a).

Dasinura amaramanjarae adults are active from January/February to April, with a peak of abundance in March. Females lay eggs on flowers, and larvae, after feeding, drop to the soil under mango trees for pupation and diapause (Rehman et al. 2013b). Application of bifenthrin (Talstar 10EC) and seed kernel extracts (neem seed kernel extract, NSKE), the latter together with raking of soil under trees, proved best for control of this pest (Rehman et al. 2014).

Farmers rely on the use of pesticide sprays to control mango insect pests in Pakistan (Saifullah et al. 2007). However, the intensive use of pesticides may cause resistance, leave unwanted residues, and kill natural enemies (Zadocks 1993, Dent 1995). To reduce pesticide use, monitoring tools and management tactics for the early detection of this pest are needed. Early detection could also allow the grower to use non-chemical measures like biological control and cultural measures more efficiently (Zijlstra et al. 2011).

Insects show differential responses to colors in selection of host plants (Prokopy and Owens 1983). Sorghum gall midge, *Stenodiplosis sorghicola* Coquillett, females prefer yellow to green, red, or blue traps and respond more quickly to yellow, followed by red, green, and blue traps (Sharma and Franzmann 2001). Determination of color preferences of mango gall midges may be helpful in developing traps for monitoring and management. Yellow sticky traps have been used for the monitoring of the blueberry gall midge *Dasineura oxycoccana* Johnson and the mango pest *D. amaramanjarae* (Plažanin et al. 2012, Rehman et al. 2014).

In India, pesticide application on mango trees and the soil underneath, at peak abundance of mango gall midge adults, provides effective control of *P. mangiferae* and other mango inflorescence gall midges (Grover 1985). However, botanical pesticides and the use of some cultural practices have potential to control these midges and are more environmentally friendly. Botanical pesticides may reduce the development of pest resistance, the need of pesticide applications, and the cost of pest management (Regnault-Roger 1997, Khorram et al. 2011). Neem products have value in pest management due to their selectivity toward phytophagous insects and minimal toxicity to many beneficial insects (Naumann and Isman 1996, Bhanukiran and Panwar 2000). In Nigeria, neem seed extracts suppressed African rice gall midge, *Orseolia oryzivora* Harris and Gagné, populations (Ogah and Ogbodo 2012). In India, cleaning, manuring, irrigating, and hoeing soil under mango trees helps control mango gall midges (Prasad 1966, Grover and Prasad 1966, Grover 1985).

The goals of this study were to 1) conduct a regional survey in Pakistan for the presence of *P. mangiferae*, 2) compare the severity of infestation in commercial mango orchards with that in isolated mango trees intercropped with other crops such as wheat or cotton, 3) determine the seasonal abundance of the gall midge, 4) evaluate the relative effectiveness of different colored sticky traps in monitoring *P. mangiferae* adults, and 5) compare the efficacy of various control methods for managing the pest.

Materials and Methods

Regional Surveys for *P. mangiferae*

Surveys were conducted for *P. mangiferae* on mango trees in seven commercial orchards, divided among three regions of Punjab: 1) Tranda Sway Khan (District Rahim Yar Khan, two commercial orchards), 2) Regional Agricultural Research Institute (District Bahawalpur, two commercial orchards), and 3) near Bahaudin Zakaria University at Bosan Road (District Multan, three commercial orchards), from 2008 to 2010. At each site, 10 inflorescences each from 10 to 12 mango tree were examined at least once a month. This was done as a regional survey to gather data about the presence of the midge within 450 km of the Punjab province of the Pakistan (Fig. 1).

At an additional site in District Rahim Yar Khan, 10 inflorescences (having galls on leaves and damaged small fruits) were sampled weekly from February to April of each year. Leaves bearing galls or gall midge–damaged inflorescences and small fruits (as determined by the presence of galleries and black spots) were randomly selected on mango trees and brought to the laboratory. These plant parts were placed individually in ventilated plastic jars (36 cm in height and 8 cm in diameter) and held at 25°C temperature and 70% relative humidity. The base of each part was dipped in water in a small pan. When the larvae reached the final instar and emerged from the plant tissue, they were collected with a camel hair brush and for pupation were moved into small round jars (12.7 cm and diameter 5 cm) containing a thin layer of moist soil. Adult gall flies emerging from these pupae were collected by using an aspirator, preserved in 70–75% ethanol, and deposited as voucher specimens at CABI – Central and West Asia, Rawalpindi, Pakistan. Species were

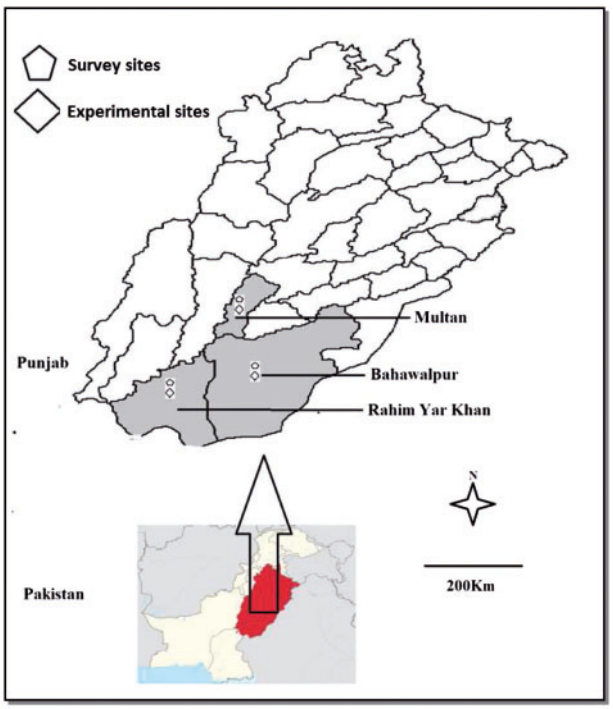

Fig. 1. Survey and experimental sites at different mango-growing areas of Punjab, Pakistan, used for the study of the mango gall midge, *Procontarinia mangiferae*.

identified by Dr. Nigel Wyatt at the Natural History Museum, Cromwell Rd, London SW7 5BD, United Kingdom.

Damage Levels in Commercial Orchards Versus Isolated Mango Trees

The severity of *P. mangiferae* infestations was determined in a commercial orchard from each of the three regions from where exploratory searches were conducted (Rahim Yar Khan, Bahawalpur, and Multan), and also in isolated mango trees of three small-holding farmers in the same regions in 2010 and 2011. In March of each year, at each of these orchards, we randomly selected four trees and collected 15 inflorescences per tree. A tree containing 15 samples (inflorescences) was considered as a replicate. The inflorescences in samples that had symptoms of damage (black spots with holes on branches of inflorescence and small fruits) were considered infested.

Phenology of *P. mangiferae* Larvae

At one commercial mango orchard at Rahim Yar Khan, in each of three years (2009–2011), we sampled larvae of *P. mangiferae* from initiation of flowering to fruit maturity (February to April). For this purpose, five trees of a commercially important variety of mango (Chaunsa) were selected and on each tree, we cut two twigs (at about 1.5 m above the ground) with inflorescences weekly and brought them to the laboratory. The lower ends of these inflorescences were placed in small jars (10 cm in height and 4 cm in diameter) with water to support tissues and keep them from drying out (one inflorescence per jar). They were then placed in large plastic jars (36 cm in height and 8 cm in diameter) covered with muslin cloth held in place with rubber bands. Emergence of *P. mangiferae* was recorded daily and pooled on a weekly basis. After the fruiting season reached a point when inflorescences were no longer available, leaves were sampled instead. From May to the end of January, 50 galled leaves were collected weekly and held until adult emergence, using the methodology described for inflorescences above (Rehman et al. 2013a).

Effect of Trap Color on Catch of *P. mangiferae* Adults

Three colors of sticky traps (10 by 10 cm; yellow, green, and blue) were compared for capture of *P. mangiferae* adults from January to April in 2011 and 2012 at one orchard in Rahim Yar Khan. There we selected four mango trees and on each tree, placed six traps of each color in horizontal arrangement (18 total). Each tree was considered a replicate. Six clear sticky traps were also placed as a control on each of the same four trees. Traps were left in place on trees for 24 h in a given week and then brought into the laboratory, where *P. mangiferae* adults were counted using a magnifying glass. This process was repeated weekly for the 4 mo of the study.

Efficacy of Chemical or Cultural Control of *P. mangiferae*

Trials on management of *P. mangiferae* were conducted at Rahim Yar Khan in plots laid out in a randomized complete block design (RCBD) in 2011 and again in 2012. Six treatments were assessed: (T_1) application of NSKE (10% sol.) on the soil under mango trees; (T_2) application of NSKE (10% sol.) on the canopy of mango trees; (T_3) application of NSKE on mango tree canopy with raking (hoeing) of soil under the tree; (T_4) raking (hoeing) of soil under the tree; (T_5) application of Talstar, 10 EC (Bifenthrin, FMC United group, Lahore, Pakistan) on the canopy with a tractor-mounted boom sprayer at 125 ml/250 liters (12.5 g/ha) of water for one hectare of commercial formulation; and (T_6) an untreated control. A water solution of an NSKE was prepared by grinding and mixing 10 g of seeds in100 ml of distilled water for 3-4 h in a beaker. The mixture was then filtered through a muslin cloth (Singh and Singh 1998).

For the experiment, we selected five mango trees (Chaunsa variety) for each treatment (total of 30 trees in five blocks). Each tree was considered as a replicate. The experiment was conducted twice (2011 and 2012), and in each year, hoeing and applications of NSKE were carried out from December to April (for 151 d) at fortnightly intervals, while bifenthrin was applied only once, in March. A plastic sheet (1 by 1 m) was spread under the canopy of each tree, and each day from February to April (in both 2011 and 2012), we counted the number of *P. mangiferae* larvae that dropped onto the sheets over a full 24 h.

Data Analysis

To analyze rates of damage among the three regions and between the two orchard types, the percent infestation of inflorescences was calculated and converted to means with standard errors (mean ± SE). For each year's data, Student *t*-tests were used to compare the population of pests between the commercial orchards and the trees grown by small farmers. Analysis of variance (ANOVA) was used to compare infestations of *P. mangiferae* on mango trees at the three localities (Rahim Yar Khan, Bahawalpur, and Multan) among the commercial orchards and the trees grown by small farmers. As *P. mangiferae* infestation approached 100%, arcsine transformation was used to validate assumptions of ANOVA, i.e., the normal distribution of population.

To analyze the effect of trap color on adult gall fly catch, we analyzed the data with ANOVA and differences among the treatments were separated by the least significant difference (LSD) test (Rehman et al. 2014). The same approach was used to analyze counts of larval dropping from trees in the efficacy trial.

Results

Regional Surveys for *P. mangiferae*

The survey confirmed the presence of *P. mangiferae* in the three regions.

Damage Levels in Commercial Orchards Versus Isolated Mango Trees

Infestations of *P. mangiferae* were observed on inflorescence buds, small leaves, branches (axillaries), and small fruits. Larvae galled inflorescence buds, tunneled into branches (axillaries), and fed on small fruits. In cases of severe damage, branches of inflorescences were bent at a right angle (Fig. 2). The infested leaves, inflorescences, and fruits usually withered and dropped from the mango tree. Mature larvae subsequently dropped to the soil for pupation.

In both years (2010 and 2011), damage levels were not significantly different among the three study locations (Rahim Yar Kahn, Bahawalpur, and Multan) among commercial orchards (2010: $F = 1.57$; df = 2, 11; $P < 0.28$ and 2011: $F = 2.35$; df = 2, 11; $P < 0.17$) or among isolated trees in small farms fields (2010: $F = 1.99$; df = 2, 11; $P < 0.21$ and 2011: $F = 0.90$; df = 2, 11; $P < 0.45$). In contrast, commercial orchards had significantly higher infestations of *P. mangiferae* than isolated trees at Rahim Yar Khan (2010: $t = 48.18$; df = 3; $P < 0.003$ and 2011: $t = -11.22$; df = 3; $P < 0.001$), Bahawalpur (2010: $t = 17.19$; df = 3; $P < 0.0004$ and 2011: $t = 8.42$; df = 3; $P < 0.003$), and Multan (2010: $t = -19.9$; df = 3; $P < 0.0003$ and 2011: $t = -8.4$; df = 3; $P < 0.003$; Fig. 3).

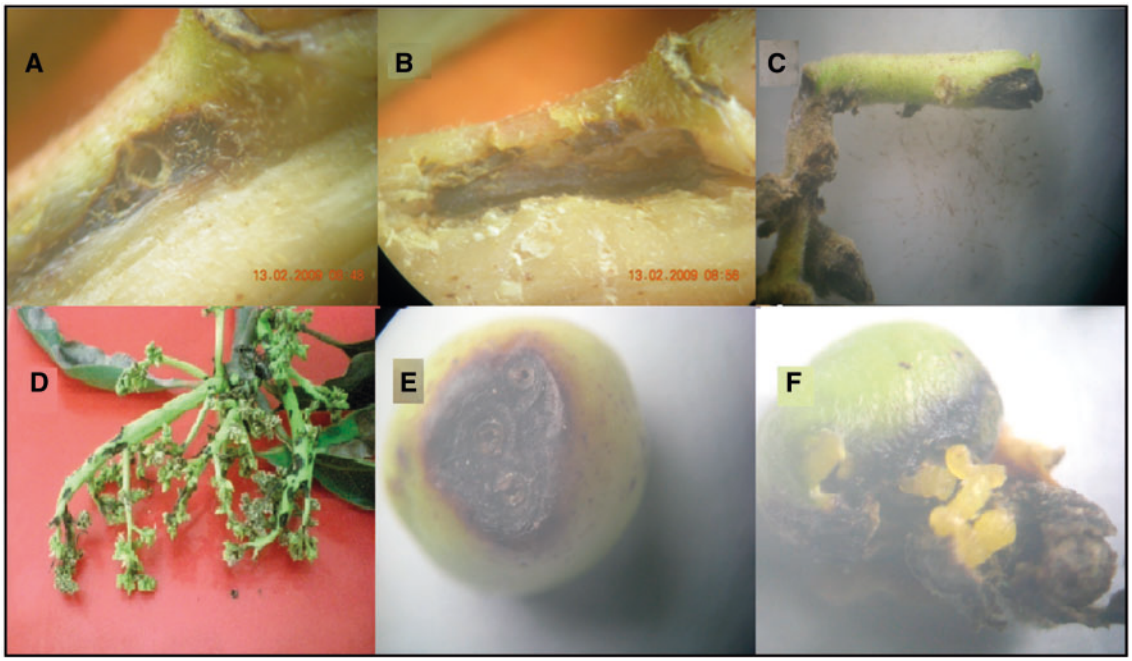

Fig. 2. Infestation of *Procontarinia mangiferae*: (**A**) exit holes, (**B**) gallery, (**C**) mango branch bent at a right angle, (**D**) damaged inflorescence, and (**E** and **F**) damaged small sized mango fruits.

Phenology of *P. mangiferae* Larvae

Monitoring of *P. mangiferae* larvae at Rahim Yar Khan (February to April) for 3 yr (2009 to 2011) showed the insect's phenology to correspond with mango flowering in February and March. Larvae were first seen in February and were most abundant in mid-March (from second to third week, with the number of larvae ranging from 118.4 to 192.25 per inflorescence). Populations declined to zero by the third week of April and were not found thereafter in inflorescences. Larvae were not seen again until the next flowering season (May to January; Fig. 4).

Effect of Trap Color on Catch of *P. mangiferae* Adults

Monitoring of *P. mangiferae* adults, carried out in a mango orchard at Rahim Yar Khan from January to April in 2011 and 2012, found that colored sticky traps caught flies from January to April with three peaks: the first in the last week of February (58.12 ± 4.7 per tree), the second in mid-March (57.5 ± 3.4 per tree), and the third in start of April (120 ± 7.2 per tree; Fig. 5).The difference in captures due to trap color was significant ($F = 57.60$; df = 3, 15; $P < 0.001$), with yellow catching the most flies, and control traps catching significantly fewer flies than all colors (Fig. 6).

Efficacy of Chemical or Cultural Control of *P. mangiferae*

Experiments to evaluate the efficacy of chemical and mechanical management options at Rahim Yar Khan during the mango flowering season (in 2011 and 2012) scored efficacy based on the number of *P. mangiferae* larvae dropping onto plastic sheets under the mango trees. The number of larvae differed among the treatments ($F = 125.98$; df = 5, 29; $P < 0.001$). The best two treatments (not different from each other) were bifenthrin and NSKE sprayed on the tree canopy combined with raking of the soil. In order of decreasing effectiveness following these two treatments were 1) raking of soil alone, 2) NSKE applied alone on tree canopy, and 3) application of NSKE on the soil under mango tree. The highest numbers of larvae were collected from the control (Fig. 7).

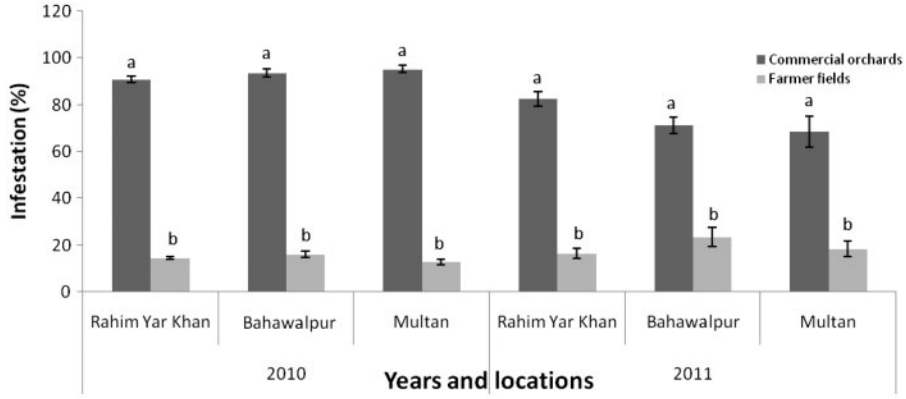

Fig. 3. Percent infestation of *Procontarinia mangiferae* on trees in commercial orchards and farmer fields at Rahim Yar Khan, Bahawalpur, and Multan, Pakistan, in 2010 and 2011.

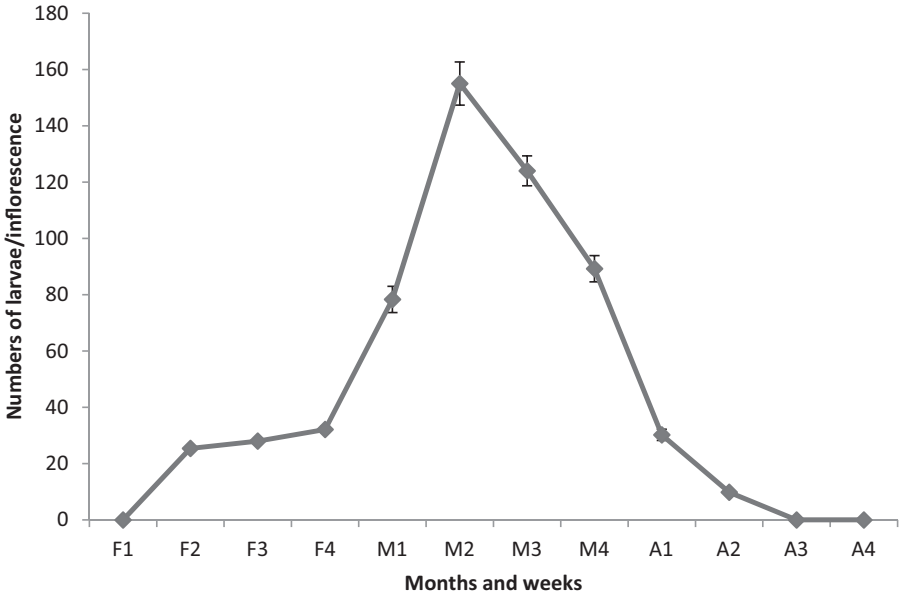

Fig. 4. Mean numbers (mean ± SE) of *Procontarinia mangiferae* larvae per inflorescence at Rahim Yar Khan, Pakistan, in the months of February, March, and April (mean population from 2009 to 2011).

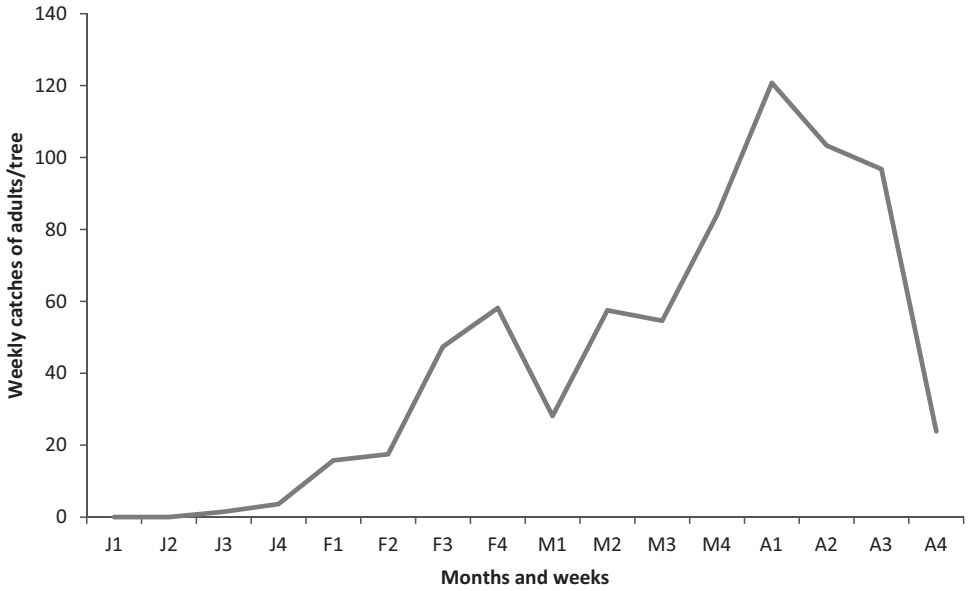

Fig. 5. Monitoring of *Procontarinia mangiferae* with yellow-colored traps at Rahim Yar Khan, Pakistan (mean population from 2011 and 2012).

Discussion

This is the first report of the distribution, damage patterns, intensity of infestation, biology, monitoring, and management of *P. mangiferae* in mango-growing areas of Punjab, Pakistan. Damage by *P. mangiferae* has been reported in other mango-growing areas of the world, including Iran (Pezhman and Askari 2004, Askari and Bagheri 2005) and India (Prasad 1966), and the damage in Pakistan followed a similar patter as in India, with three generations of *P. mangiferae*, the first generation usually inducing blister-like galls on fleshy leaves surrounding the flower buds, the second generation feeding on inflorescences, and the third generation attacking small fruits.

Cultural practices appear to partially suppress the pest, given that *P. mangiferae* populations were significantly higher on mango trees in commercial orchards than isolated trees at all study locations. Several factors may be responsible for this trend, including the lack of additional resources for the gall midge to feed on in the immediate area of the isolated mango trees on the small farm, the greater use in smallholders' fields of crop rotation, and higher frequencies of soil disturbance (typically10 cultivations and five hoeings annually; Khalil and Amanullah 2002). In India, hoeing of soil under mango trees also reduced gall midge populations (Prasad 1966, Grover and Prasad 1966).

Our findings on gall midge phenology and voltinism were similar to those of Prasad (1971) and Grover (1986), who reported that females of *P. mangiferae* laid eggs on inflorescences, and that larvae after hatching penetrated into various mango tissues, dropped to the ground after feeding in their final instar, and entered the soil, and adults, upon emergence, caused outbreaks on mango during flowering in India. The whole population enters diapause after the flowering season (Prasad 1971). New adults emerge in January, peak in

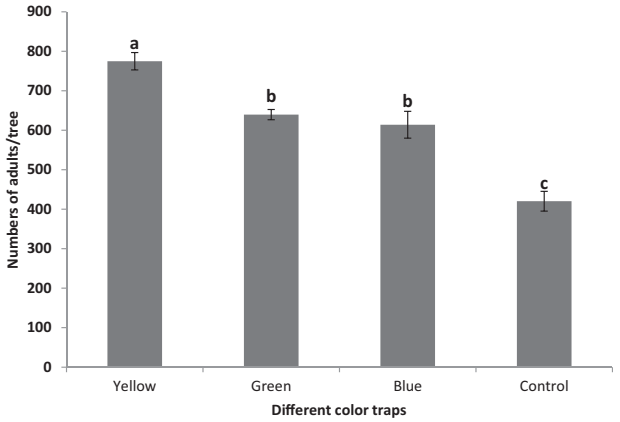

Fig. 6. Comparison of different colored traps (mean ± SE) for monitoring of *Procontarinia mangiferae* at Rahim Yar Khan, Pakistan. Bars topped with different letters are significantly different (LSD test, $\alpha = 0.05$, $P < 0.05$; mean population from 2011 and 2012).

March, and disappear in April. In contrast, on Réunion Island, larvae are found throughout the year, on inflorescences during flowering and on leaves during the vegetative period. Densities of *P. mangiferae* are higher during flowering season from June to October than during the period of vegetative growth from November to May. This longer duration of the active larval population in Réunion may be attributed to the sporadic production of new inflorescences and leaves throughout the year and the ability of *P. mangiferae* to feed on different parts of mango trees under diverse eco-cultural conditions (Amouroux et al. 2013).

Traps for monitoring adult flight are used for many insects, and yellow-colored traps are usually the most effective color (Conway 1984). Trap catches can help to time pesticide applications (Vale 1982, Grieshbach 2011). Yellow traps have been used to monitor sorghum gall midge (*S. sorghicola*), blueberry gall midge (*D. oxycoccana*), and other phytophagous insects. As here, yellow traps are generally more effective than green-, red-, black-, or blue-colored traps (Meyerdirk et al. 1979, Sharma and Franzmann 2001, Plažanin et al. 2012).

It should be noted that weekly trap catches of *P. mangiferae* of the most attractive colored traps were not consistent, but varied with sampling dates in both seasons. Temporal variation toward color preference has been reported for different insects. For example, adults of the blunt-nosed leaf hopper, *Limotettix vaccinii* (Van Duzee) (Hemiptera: Cicadellidae), on cranberry were most attracted to red traps in the early season and then to yellow traps in the late season (Rodriguez-Saona et al. 2012). Similarly, the attraction to color of pirate bugs and honey bees was also influenced by the time of year (Rodriguez-Saona et al. 2012). The variation in color preference of *P. mangiferae* might be due to the changes in the background color behind traps due to the occurrence of various phenological events in mango trees like flushing, flowering, and fruit setting. Temporal variation in trap catch due to trap background changes has also been reported for certain leafhoppers (Saxena and Saxena 1975). In addition to background, winds may have a passive effect on how well the small-sized gall midges (only 2–3 mm in length) adhere to different colored traps. This hypothesis requires further research, however, along with how weather more generally affects the abundance of midges in relation to trap color.

NSKE application integrated with raking the soil (for disturbing pupating gall midges under mango trees) was the most effective among the neem treatments, and this treatment was equivalent in efficacy with the conventional pesticide application, bifenthrin. In Pakistan, this insecticide is usually recommended for controlling insect pests of mango (Saifullah et al. 2007). In comparison, bifenthrin is toxic to some hymenopteran parasitoids (Prabhaker et al. 2007), whereas neem-based insecticides are safer for beneficial insects (Caboni et al. 2006, Hasan et al. 1996, Gahukar 2000). Neem extracts integrated with other control measures can provide economical pest control (Gahukar 2000). In Nigeria, neem seed extracts significantly suppressed African rice gall midge populations compared with the control treatment (Ogah and Ogbodo 2012).

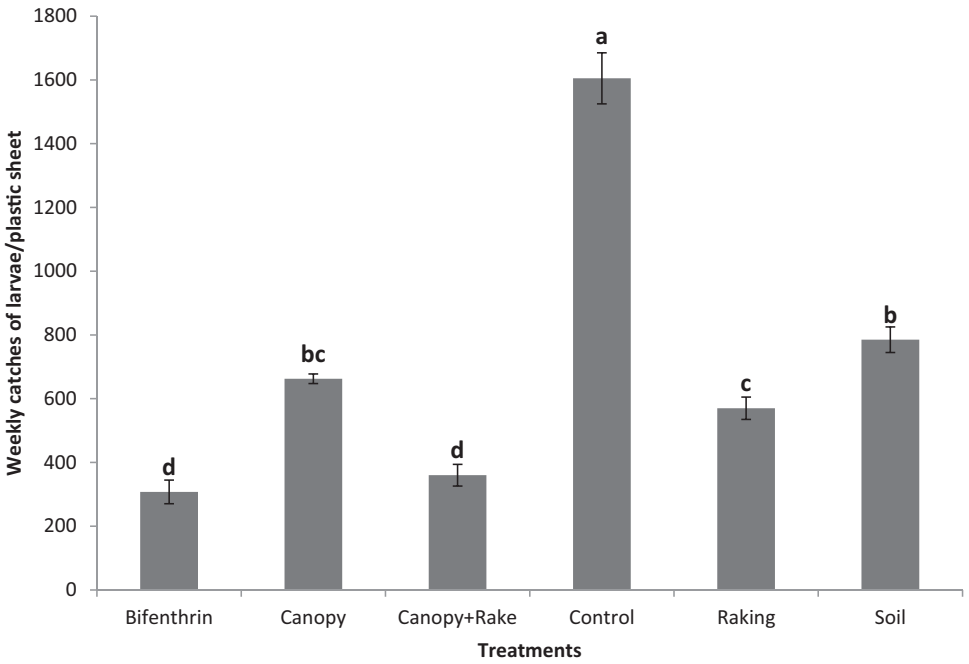

Fig. 7. Means (mean ± SE) of *Procontarinia mangiferae* population after different treatments at Rahim Yar Khan, Pakistan. (1. Soil = spray of neem on soil. 2. Canopy = spray of neem on canopy, 3. Bifenthrin = spray of bifenthrin on canopy, 4. Raking = raking of soil, 5. Canopy + rake = spray of neem on canopy + raking of soil). Bars topped with different letters are significantly different (LSD test, $\alpha = 0.05$, $P < 0.05$; mean population from 2011 and 2012).

In India, soil preparation activities such as cleaning, manuring, irrigating, and hoeing also contribute to control of mango gall midges (Prasad 1966, Grover and Prasad 1966, Grover 1985). The application of NSKE integrated with these other cultural practices is an environmentally friendly management option for long-term and sustainable control of this pest. More research, however, is needed on the development of an economic injury threshold for this pest and on the possible effects of area-wide application of NSKE.

In Pakistan, where the mango industry has been facing the problem of gall midges since 2005, farmers usually rely on pesticides for the management of this pest. This study is an initial attempt to understand the behavior of mango gall midges for developing long-term management strategies on a large scale against the pest.

Acknowledgments

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