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Varietal Preferences and Within-Orchard and Tree Distribution of Newly Recorded Gall Midges, *Dasineura amaramanjarae* and *Procontarinia mangiferae* (Diptera: Cecidomyiidae), From Commercial Mango Cultivars in Pakistan

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Abstract

Gall midges (Diptera: Cecidomyiidae) damage mango by feeding on flowers and fruit tissues, inducing galls on leaves, and providing inoculums of anthracnose. *Dasineura amaramanjarae* Grover and *Procontarinia mangiferae* (Felt), two gall midges that damage flowers in all mango-growing areas of the world, have recently been recorded in Pakistan, and studies were conducted in 2011 and 2012 on the within-tree and orchard distribution patterns and cultivar preference of both species in Pakistan at one location (Rahim Yar Khan). Both gall midge species were found on all mango cultivars examined (Chaunsa, Fajri, Dusehri, Surkha, Sindhri, and Anwar Ratul), with the most damage occurring to Surkha and Dusehri. Research on midge distribution patterns in different parts of mango orchards (central, southern, northern, eastern, and western sides) showed these species to be found in all areas, with the greatest numbers in the central and southern regions. In addition, both species were most abundant on the lower parts of the mango tree canopy.

Key words: *Dasineura amaramanjarae*, *Procontarinia mangiferae*, anthracnose, mango, gall midge

The mango industry in Pakistan is facing a number of problems including diseases and insect pests (Mahmood and Gill 2002, Ishaq et al. 2004). About 250 insect pests affecting mango have been recorded worldwide (Peña and Mohyuddin 1997). Gall midges, thrips, leafhoppers, caterpillars, and mites are the major pest groups damaging mango (Peña et al. 1998).

Mango gall midges are cosmopolitan in their distribution on mango trees. They have been recorded feeding on flowers and fruit tissues, making galls on the leaves of mango, and their injury promotes the spread of anthracnose infections (Uechi et al. 2002, Askari and Bagheri 2005, Kolesik et al. 2009). Therefore, studies were initiated on mango gall midges in Pakistan. Two midges, *Dasineura amaramanjarae* Grover and *Procontarinia mangiferae* (Felt) (*Erosomiya mangiferae* Felt), are part of the gall midge complex damaging mango in Pakistan, and these species have been found in all mango-growing areas surveyed in Punjab, Pakistan. As per our review of literature, these pest species are reported for the first time from this region. *Dasineura amaramanjarae* feeds on flower buds. Female lays eggs near the stamens, larvae feed inside the flower buds, and full-grown larvae drop to the soil for pupation. Severe infestation leads to failure of the fruit and ultimately crop (Grover and Prasad 1966; Prasad 1966; Rehman et al. 2013a, 2014). *Procontarinia mangiferae* larvae feed on axillaries (branches) of inflorescences and immature fruits (Gagné 2010, Rehman et al. 2016).

There is little research on host plant resistance in mango and its integration with other pest management options. However, some studies report screening mango cultivars for comparative resistance to insect pests. For example, mango has some resistance to *Noorda* sp. (Crambidae: Lepidoptera), *Idioscopus* sp. (Cicadellidae:

Hemiptera) (Bagle and Prasad 1984, Cunningham 1989), and *Sternochetum mangiferae* (F.) (Hansen 1993) (Curculionidae: Coleoptera), and different degrees of susceptibility toward *Anastrepha oblique* Macquart (1835) (Tephritidae: Diptera) (Carvalho et al 1996). In the Philippines, a wild mango, *Mangifera altissima* Blanco (Family: Anacardiaceae) has been found to be resistant to insects of Cicadellidae, Noctuidae, and Curculionidae (Angeles 1991). In South Africa and India, various mango cultivars showed differing levels of susceptibility to *Procontarinia matteiana* Kieffer and Cecconi (Githure et al. 1998). Similarly, differences in susceptibility of mango cultivars against the *Procontarinia mangicola* (Shi) have also been reported in Pakistan (Muhammad et al. 2013). It was noted that the susceptibility of different mango cultivars to *P. matteiana* was associated with certain terpenes emitted by newly flushed mango foliage (Augustyn et al. 2010). Most of this research, however, requires further evaluation.

Insect pest species are distributed in a habitat with a characteristic pattern according to their predisposed genetic behavior and environmental conditions. Understanding the pattern of this spatial distribution provides information about the structure of a population, which affects the precision of estimating population parameters in sampling and targeted management (i.e., insecticides). Moreover, the distribution of pests also governs the distribution of their natural enemies, as these usually follow similar patterns to their host (Kumari 2003).

This study was designed to determine the varietal preference of two midges, *D. amaramanjarae* and *P. mangiferae*, on commercially grown varieties of mango and their distribution pattern on various parts of the mango tree and orchards in Punjab, Pakistan. The specific objectives of this study were to record populations of *D. amaramanjarae* and *P. mangiferae* on commercially grown varieties of mango to determine the distribution patterns of both gall midge species in different areas of the mango orchard with respect to direction, and to determine the within-tree distribution of these gall midges.

Materials and Methods

Experimental Site

All experiments were conducted in a mango orchard in Rahim Yar Khan (28.3° N, 65.23° E) in 2011 and 2012. The experimental orchard consisted of about 302 mango trees of different varieties, including Anwar Ratul, Chaunsa, Dusehri, Fajri, Lahotia, Langra, Late Chaunsa, Saroli, Sindhri, Surakh, and Tota Puri. Most of the trees were about 30–40 yr old, while a few were 5–15 yr old. Trees were irrigated 8–13 times a year, cultivated and manured twice a year, and no crop was sown inside the orchard. Blooming in these cultivars usually starts at the same time in February and fruit setting in April in this region (Rehman et al. 2013b). Cotton (May to November) and wheat (November to May) are cultivated outside the orchard. Sampling for all experiments was done from 15 February to 15 April in both years.

Varietal Preferences of Midges

Adults and larvae of *D. amaramanjarae* and *P. mangiferae* were recorded on six commercially grown cultivars of mango: Chaunsa, Dusehri, Fajri, Sindhri, Anwar Ratul, and Surkha, with four trees of similar size and age (18–20 yr) of each of the six cultivars. Four trees (each from a cultivar) were considered as replicate block. Adults of gall midges were counted as numbers trapped on a yellow sticky band (10 by 10 cm) attached to flowering branch tips 2 m above the ground on each tree, with one trap per replicate tree. Traps were deployed for two consecutive days a week for the entire period (15 February to 15 April), each year. The number of adults of each gall midge species was recorded from each trap with a magnifying glass (10×).

Larval abundance was recorded as the number of mature larvae dropping from each of the four bagged inflorescences of 35–40 cm (one on each replicate tree). Plastic bags (45 cm long) with pinholes for ventilation were placed on the tree over the inflorescence for 24 h during each week of the sampling period. Larvae were sampled from the same trees on which traps for adults were deployed. After 24 h, the plastic bags were removed and brought to the laboratory and larvae were separated into both the species. Larvae of *D. amaramanjarae* are reddish, whereas those of *P. mangiferae* are golden yellowish (personal observation). Moreover, larvae of both midges were reared, and further identification was confirmed from adults (Rehman et al. 2013b, 2016). A different inflorescence was chosen for each week (not the ones used before).

Distribution Patterns Within the Orchard and Trees

Directional Part of Orchard

To determine the distribution patterns within the study orchard, eight trees were selected from each of the five regions (central, southern, northern, eastern, and western sides of the mango orchard), for 40 trees. Each sampling region was 5.26 ha. An inflorescence from each of the eight sample trees in each region was covered with a plastic bag for 24 h at weekly intervals to record gall midge larval populations. Mature larvae that fell into the bags were collected and were taken to the laboratory, where they were reared to adult and separated by species and counted.

Within-Tree Distribution

To determine within-tree midge distribution patterns, five mango trees were selected randomly in the orchard. Four inflorescences from each of the southern, northern, eastern, and western sides of both the lower (1–3 m) and upper (3–6 m) parts of the tree canopy were covered with plastic bags for 24 h per week (for a total of 20 flowers for each direction and height combination). Gall midge larvae that dropped into the plastic bags were collected, identified by color, and reared to adults for further confirmation of their identification (Rehman et al. 2013b, 2016). During the study, the orchard's owner applied Talstar, 10 EC (Bifenthrin, FMC United group, Lahore, Pakistan) to control sucking pests of mango (Saifullah et al. 2007) at 125 ml/250 liters of water per hectare on 15 March, 2011 and 2012. However, no insecticide was applied in the orchard against mango gall midge during the study period.

Statistical Analysis

For experiments to determine varietal preference, the mean number of adults (from traps) and larvae (from plastic bags) of both species captured on each cultivar was calculated and analyzed with analysis of variance (ANOVA; following randomized complete block design, i.e., variety and block as factors) for significance of differences among cultivars, with mean separation based on LSD at the 5% level of significance (Gomez and Gomez 1984). The same tests were used to compare numbers of both midge species from second experiment, i.e., to determine distribution patterns within the orchard (different regions) and within trees (directional parts of single mango trees), and for directional parts of single mango trees using Software Statistix 8.1.

Table 1. Mean numbers of larvae and adults of *D. amaramanjarae* in 2011 and 2012 on different mango varieties in Rahim Yar Khan

Mango cultivars	Adults		Larvae	
	2011	2012	2011	2012
Chaunsa	13.5 ± 2.8	6.7 ± 1.1	1.5 ± 0.6b	1.2 ± 0.6bc
Dusehri	17.0 ± 3.0	5.5 ± 1.3	4.5 ± 0.6a	2.5 ± 0.3b
Fajri	9.2 ± 1.3	3.5 ± 0.2	3.2 ± 1.4ab	0.2 ± 0.1c
Sindhri	8.5 ± 1.7	4.0 ± 2.2	0.7 ± 0.5b	1.5b ± 0.6c
Anwar Ratul	11.5 ± 5.6	6.0 ± 1.2	1.0 ± 0.4b	0.2 ± 0.2c
Surkha	17.0 ± 2.6	7.0 ± 0.9	5.5 ± 0.8a	4.0 ± 0.4a
LSD	N S[a]	N S	2.6	1.4

[a] Nonsignificant.

In each column, numbers with different letters are significantly different at the 0.05 level.

Table 2. Mean numbers of larvae and adults of *P. mangiferae* in 2011 and 2012 on different mango varieties in Rahim Yar Khan

Mango cultivars	Adults		Larvae	
	2011	2012	2011	2012
Chaunsa	179.75 ± 27.4	303.5 ± 16.2	0.5 ± 0.5	15.7 ± 1.7bc
Dusehri	214.0 ± 26.9	350.7 ± 15.0	2.2 ± 0.7	13.0 ± 0.8bc
Fajri	185.0 ± 14.5	334.7 ± 14.0	2.7 ± 1.7	12.5 ± 1.9bc
Sindhri	186.25 ± 19.6	320.2 ± 30.1	0.2 ± 0.2	12.0 ± 1.7c
Anwar Ratul	188.75 ± 37.1	357.0 ± 11.9	0.7 ± 0.4	18.2 ± 2.0b
Surkha	291.0 ± 57.2	357.0 ± 14.5	3.7 ± 1.0	32.2 ± 4.0a
LSD	N S[a]	N S	N S	6.2

[a] Nonsignificant.

In each column, numbers with different letters are significantly different at the 0.05 level.

Results

Varietal Preferences of Midges

For *D. amaramanjarae*, there was no significant difference in number of adults among the six varieties sampled in either year ($F_{2011} = 1.34$; df = 5, 23; $P < 0.3$ and $F_{2012} = 1.35$; df = 5, 23; $P < 0.29$). For larvae of this species, however, there was a significant difference among varieties ($F = 5.19$; df = 5, 23; $P < 0.001$) in 2011. More larvae were recorded on Surkha and Dusehri than on Chaunsa, Anwar Ratul, or Sindhri, but the number of larvae on these two varieties did not differ significantly from that on Fajri in 2011. In 2012, for larvae of *D. amaramanjarae*, significant differences were observed among varieties ($F = 8.79$; df = 5, 23; $P < 0.001$). The number on Surkha was significantly higher than on all other varieties (LSD at $\alpha = 0.05$). However, the density of this gall midge on Dusehri did not differ significantly from that on Chaunsa in 2012 (Table 1).

For *P. mangiferae*, there were no differences in numbers of adults among the six varieties sampled in either 2011 or 2012 ($F_{2011} = 1.56$; df = 5, 23; $P < 0.22$ [2011]; $F_{2012} = 1.32$; df = 5, 23; $P < 0.30$ [2012]). For larvae of *P. mangiferae*, there were no significant differences in number among tested varieties ($F = 2.22$; df = 5, 23; $P < 0.10$). However, in 2012, there were significant differences in the number of *P. mangiferae* larvae among different varieties ($F = 13.8$; df = 5, 23; $P < 0.001$). Surkah had significantly higher infestations than all other varieties. Number of *P. mangiferae* adults and larvae were higher than were those of *D. amaramanjarae* on most varieties (Table 2).

Distribution Patterns Within the Orchard and Trees

Directional Part of Orchard

For *D. amaramanjarae*, significantly more larvae were found on trees in the southern and central parts of the orchard in 2011 ($F = 6.21$; df = 4, 39; $P < 0.001$) and 2012 ($F = 3.11$; df = 4, 39; $P < 0.03$).

For *P. mangiferae*, numbers of larvae were not significantly different among orchard locations in 2011 ($F = 1.5$; df = 4, 39; $P < 0.22$), but were significantly higher in the southern region than other areas in 2012 ($F = 9.65$; df = 4, 39; $P < 0.001$; Table 3).

Within-Tree Distribution

For *D. amaramanjarae*, there were significant differences in numbers of larvae among the four directional quadrants within mango trees in 2011, with larval density being highest on the southern side ($F = 6.41$; df = 3, 39; $P < 0.001$), but there were no significant differences in 2012 ($F = 0.45$; df = 3, 39; $P < 0.72$).

For *P. mangiferae*, there were significant differences in number of larvae among the four cardinal quadrants within trees in 2011, with the southern section having the highest number of larvae ($F = 5.81$; df = 3, 39; $P < 0.001$), while no significant differences were found in 2012 ($F = 2.8$; df = 3, 39; $P < 0.06$; Table 4).

Comparing the upper and lower parts of the canopy, we found that there were higher *D. amaramanjarae* larvae found in the lower half of the tree canopy in 2011 ($F_{2011} = 14.7$; df = 1, 39; $P < 0.001$) and 2012 ($F_{2012} = 9.2$; df = 1, 39; $P < 0.001$). For *P. mangiferae*, considerably higher number of larvae were found in the lower canopy, compared with the upper part, in both 2011 ($F_{2011} = 4.2$; df = 1, 39; $P < 0.04$) and 2012 ($F_{2012} = 8.44$; df = 1, 39; $P < 0.001$; Table 5).

Discussion

Although there were no significant differences among numbers of adults of *D. amaramanjarae* or *P. mangiferae* trapped on the six varieties in either year, *D. amaramanjarae* larvae were more abundant on cultivars Surkha, Dusehri, and Fajri in 2011 and on Surkha in 2012. *Procontarinia mangiferae* larvae were not significantly different among six varieties in 2011 but more abundant on Surkha in 2012. Although mango varieties have been found to have different susceptibility to insect pests in different parts of the world, there is a scarcity of consistent research regarding the resistance of mango cultivars to gall midges. Most of the published research reports only susceptibility and resistance based on the occurrence and abundance of adults or their damage. In South Africa and India, *P. matteiana* caused different levels of damage on various mango cultivars (Githure et al. 1998). Similarly, differences in the susceptibility of mango cultivars to *P. mangicola* have been reported in Pakistan (Muhammad et al. 2013). In this study, variation in the density of *D. amaramanjarae* and *P. mangiferae* was observed in cultivars like Chaunsa, Anwar Ratul, and Sindhri during both years. Chaunsa is the most popular commercial cultivar in Pakistan. Anwar Ratul and Sindhri are the next choice in order of preference for commercial growers. Variation in gall midge density on cultivars might be owing to nutritional levels, and nutrient type, among other factors. (Altieri and Nicholls 2003, Zafar et al. 2010). Susceptibility of mango cultivars to *P. matteiana* has been associated with certain terpenes emitted by recently flushed mango foliage (Augustyn et al. 2010). More generally, the effect of chemical substances on the attraction of *D. amaramanjarae* and *P. mangiferae* adults to mango cultivars and the subsequent populations of these gall midges needs to be explored.

Table 3. Mean numbers of larvae of *D. amaramanjarae* and *P. mangiferae* in 2011 and 2012 at various locations of mango orchard in Rahim Yar Khan

Orientations of mango orchard	*D. amaramanjarae*		*P. mangiferae*	
	2011	2012	2011	2012
South	7.8 ± 1.6a	8.7 ± 1.7a	13.6 ± 5.7	86.7 ± 16.8a
North	2.2 ± 0.9b	3.2 ± 2.1c	2.5 ± 1.4	27.7 ± 8.1bc
East	2.7 ± 0.3b	4.0 ± 0.7bc	6.5 ± 3.7	21.6 ± 3.7c
West	3.2 ± 1.2b	3.2 ± 1.2c	8.6 ± 2.8	24.5 ± 2.7bc
Center	7.5 ± 1.2a	7.6 ± 1.1ab	5.7 ± 1.4	47.5 ± 6.7b
LSD	3.1	4.2	N S[a]	25.3

[a] Nonsignificant.
In each column, numbers with different letters are significantly different at the 0.05 level.

Table 4. Mean numbers of larvae of *D. amaramanjarae* and *P. mangiferae* in 2011 and 2012 in south, north, east, and west quadrants of mango trees in Rahim Yar Khan

Orientations of mango tree	*D. amaramanjarae*		*P. mangiferae*	
	2011	2012	2011	2012
South	60.0 ± 5.8a	3.7 ± 1.6	73.7 ± 7.5a	21.4 ± 4.8
North	30.4 ± 4.0b	1.9 ± 0.7	45.5± 5.4b	10.5 ± 2.4
East	41.0 ± 4.8b	2.5 ± 0.8	52.9 ± 5.5b	12.3 ± 3.3
West	41.8 ± 3.6b	3.2 ± 1.1	47.1 ± 5.8b	9.2 ± 2.3
LSD	13.9	N S[a]	15.5	N S

[a] Nonsignificant.
In each column, numbers with different letters are significantly different at the 0.05 level.

Table 5. Mean numbers of larvae of *D. amaramanjarae* and *P. mangiferae* in 2011 and 2012 on lower and upper parts of mango tree canopies in Rahim Yar Khan

Part of mango tree canopy	*D. amaramanjarae*		*P. mangiferae*	
	2011	2012	2011	2012
Lower	52.8 ± 3.3a	4.4 ± 0.9a	61.14.0a	18.1 ± 2.4a
Upper	33.7 ± 3.4b	1.2 ± 0.4b	48.55.3b	8.6 ± 2.2b
LSD	10.1	2.1	12.4	6.6

In each column, numbers with different letters are significantly different at the 0.05 level.

From a pest management point of view, it is worth noting that the most popular commercial mango cultivars (Chaunsa, Anwar Ratul, and Sindhri) were not the most preferred varieties for these inflorescence gall midges. However, present findings suggest further research on the relationship between gall midge numbers and yield loss on commercial cultivars as well as the yield loss relationship in germplasm, particularly rootstock for developing resistant cultivars as a long-term strategy.

In this study, infestations of both inflorescence gall midge species were higher on the lower (1–3 m) part of the tree canopy than on the upper region (3–6 m). Populations of both *D. amaramanjarae* and *P. mangiferae* larvae were also higher on the southern sides of mango trees in 2011. In another study (Rehman et al. 2013b), we used a funnel ring method to sample larvae to determine their within-tree distribution of *D. amaramanjarae* in the same region in 2009 and 2010. However, the funnel ring method was labor-intensive, as larvae of mango leaf gall midges were also captured in the funnel rings, and time was needed to separate the larvae of leaf gall midge species from those of inflorescence gall midges. However, both methods demonstrated that *D. amaramanjarae* density was higher on the lower and southern sides of trees. Present studies of distribution patterns in mango orchards have found populations to be the most abundant in the southern and central parts of the orchards. In this study, the density of *P. mangiferae* was higher on the southern side of mango tree. Mango gall midges follow different patterns of distribution. In India, *P. matteiana* shows an aggregated pattern of distribution, while *Erosomyia indica* Felt (now synonymized with *P. mangiferae*) tends to follow a random pattern, and neither midge's distribution was significantly different between various horizontal and vertical sections of mango tree (Verghese et al. 1988, Verghese and Rao 1988). Several reasons may account for the greater abundance of midges on the southern sides of trees and of the orchard as a whole with respect to the environment of Punjab. Because gall midges are attracted to light (Kashyap 1986), the pattern of light is likely to influence midge distribution in trees (Lebel et al. 2008, Khan 2010). Because light is more intense on the southern side of trees at the study site, the direction should logically attract more gall midges. The branches of the mango trees bloom in alternate years, i.e., a branch blooming in one season or year will not fully bloom in the next. Therefore, the difference in distribution patterns of *P. mangiferae* and *D. amaramanjarae* from other gall midge species may also be attributed to the unequal distribution of flowers in the mango tree canopy because of changing of flushing and blooming among horizontal and vertical strata of mango tree canopy (Issarakraisila et al. 1991). These findings recommend an extra care to the lower part and southern sides of canopy during insecticide applications.

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