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SPIDERS (ARANEAE) SURVEYED FROM UNSPRAYED CITRUS ORCHARDS IN FAISALABAD, PAKISTAN AND THEIR POTENTIAL AS BIOLOGICAL CONTROL AGENTS OF *DIAPHORINA CITRI* (HEMIPTERA: LIVIIDAE)

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ABSTRACT

Spiders and Asian citrus psyllid (ACP), *Diaphorina citri*, a pest of citrus and potential prey for spiders, were sampled in unsprayed kinnow mandarin and sweet orange orchards at two research sites in Faisalabad Pakistan from March 2011 to April 2013. Spiders in citrus were collected by weekly tap sampling of foliage and passive interception with a Malaise trap. The weekly percentage of citrus flush growth infested with ACP eggs, nymphs, and adults provided estimates of pest phenology and relative abundance over time. Spider count data and measures of ACP abundance in orchards were assessed together to determine if spider populations were likely to be present at times when ACP was available as prey. From this study, 15 families, 26 genera and 16 species were determinable from tap sampling, while in Malaise trap samples, 14 families, 28 genera and 21 species were identified. Adult spiders were likely too large to attack any ACP life stage. However, immature jumping spiders (Salticidae) and a yellow sac spider, *Cheiracanthium insulanum* may be potential predators of ACP.

Keywords: Asian citrus psyllid, *Diaphorina citri*, Malaise trap, Phenology, Predators, Tap-sampling

INTRODUCTION

Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a notorious pest of citrus because it vectors a phloem-dwelling plant pathogenic bacterium, *Candidatus* Liberibacter asiaticus Jagoueix, Bové and Garnier (α-Proteobacteria) (Jagoueix *et al.*, 1994), that causes a lethal citrus disease, known as huanglongbing (HLB) or citrus greening (Halbert and Manjunath, 2004; Bové, 2006; Grafton-Cardwell *et al.,* 2013). ACP and HLB are serious invasive species that cause immense economic losses in countries with important citrus industries (e.g., Florida [USA] and Brazil [Hodges and Spreen, 2012; Salifu *et al*., 2012]). It is likely that ACP and HLB are native to the Indian subcontinent and part of this native range includes Pakistan (Hussain and Nath, 1927), a country with a commercially viable citrus industry which also suffers economic losses from HLB (Ahmad, 1961; Chohan *et al*., 2007). Despite this impediment to production, citrus is an important crop in Pakistan. Over the 2010-2011 growing season, citrus production in Pakistan covered 194,500 ha on which 1,982,200 tons of fruit was produced of which 493,000 tons was exported with a net worth of ~$232 million ($USD) (Anon., 2011). Citrus production is concentrated in Punjab and accounts for 184,200 ha (~95% of production area) from which 1,912,000 tons (~96%) of total citrus fruit is produced (Anon., 2012).

In August 2008, ACP was detected for the first time in southern California, USA and this pest is now widespread in urban areas where it infests citrus (Rutaceae) and curry leaf trees (*Murraya koenigii* [Rutaceae]) in residential gardens. In March 2012, HLB was detected for the first time in a single residential citrus tree in Los Angeles County, California (Leavitt, 2012). In response to the ACP invasion, a classical biological control program was initiated with foreign exploration efforts focusing on natural enemies, in particular nymphal parasitoids, associated with ACP in Punjab, Pakistan (Hoddle, 2012; Hoddle and Hoddle, 2013). In invaded areas such as Florida, predators, especially coccinellids, have been documented as being important natural enemies of ACP (Michaud, 2001; Michaud, 2004). Other generalist predators that could attack ACP, such as spiders, have received

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relatively little research attention.
In support of the classical biological control program in California targeting ACP, a project to better understand the natural enemy complex associated with this citrus pest in Punjab Pakistan was undertaken. This was achieved through weekly surveys for ACP on two different types of citrus, kinnow mandarin (*Citrus reticulata*) and sweet orange (*C. sinensis*), across two different study sites in Faisalabad over March 2011 to April 2013. During this time period, predators on citrus, in particular spiders, were sampled using two different methods, passive interception with a Malaise trap that was set at one study site, and tap sampling of citrus foliage on experimental trees at both study sites. The results of these surveys to document the diversity and abundance of spider species and their potential relationship with ACP in unsprayed citrus orchards in Faisalabad Pakistan are reported here.

MATERIALS AND METHODS

Study sites and study duration

Two study sites at the University of Agriculture Faisalabad (UAF) were selected for monitoring studies. Site one, Square 9 (N31°25'50.4"; E73°03'40.2"; elevation 190 m), is 10 ha of which 6 ha is citrus comprised of 4 blocks of kinnow, 2 blocks of sweet oranges, 1 block of grapefruit (*Citrus* x *paradisi*), 2 blocks of sweet limes (*C. limetta*), 1 block of *C. reticulata* cv. Feutral's Early, and 1 block of germplasm stock comprised of various Rutaceae. Two blocks, 1 sweet orange and 1 kinnow block, comprised of ~30-40 trees each, were selected for sampling studies. Within each block 15 trees were randomly selected and flagged for weekly monitoring. Site 2, PARS (N31°23'35.20"; E73°01'27.0"; elevation 210 m), also part of the UAF campus, is located 12 km from Square 9. This site is 182 ha of which 90 ha is divided into two widely separated sections of citrus. This study was conducted in the 30 ha citrus section that is comprised of 7 blocks of citrus; 2 kinnow, 1 sweet orange, 2 grapefruit, 1 Feutral's Early, and 1 block of sweet lime. For studies at PARS, 15 kinnow (all in one block) and 15 sweet orange trees were randomly selected and flagged in appropriate blocks for monitoring. The studies conducted at these research sites spanned 26 March 2011 to 20 April 2013, inclusive, for the Malaise trapping and tap sampling was performed from 11 February to 15 September in 2012 and 24 February to 12 April in 2013. No pesticides were applied to any trees during this time.

Tap sampling and Malaise trap monitoring of spiders

The tap method (Hall and Hentz 2010) or tap sampling, was used to sample spiders inhabiting citrus foliage. Each experimental tree was divided into four quadrants and branches within a quadrant were hit 5 times to dislodge spiders. A white plastic tray (38 cm long x 27 cm wide) was placed under the foliage being tap-sampled and spiders landing on the tray were counted and recorded per quadrant for each tree. Spiders were captured and preserved in labeled vials containing 95% ethanol. Samples were kept in a freezer until identified. Numbers of spiders from tap-sampling were calculated for both study sites. A Townes style Malaise trap (http://www.santetraps.com/ catalogue product 1) was set up at Square 9 for the duration of this study. After the first 12 months, the original trap was replaced with a new trap. All insects and spiders captured in the collecting container were preserved in 95% ethanol. The trap was cleared weekly and captured specimens were stored in labeled bags. Spiders were sorted, separated, and kept in labeled vials in a freezer for identification.

Identification of spiders

Spiders were identified to the lowest taxonomical level possible. In general, the spider fauna of the Indian subcontinent is poorly known. Although many species have been described, there are few revisionary works summarizing the known body of knowledge. This is probably due to the fact that research on the spider fauna of the Indian subcontinent is still in its early descriptive stages. Additionally, published taxonomic research is handicapped by the description of only one sex and/or the rudimentary caliber of the genitalic illustrations which do not allow for species-level identifications to be made confidently. Consequently, the validity of some of the identifications presented in this paper should be viewed with caution.
The most useful resource for identifications was Song *et al*. (1999) who provided extensive documentation, mostly of genitalia illustrations, of the spiders of China. Once taxa were tentatively identified, additional searches for individual description papers allowed for identification to species. Jumping spider (Salticidae) identifications were aided by examining genitalic images on an international salticid website (Prószynski, 2013) as well as Loganov (2007) and requesting assistance from G. B. Edwards at the Florida State Collection of Insects, a US expert on salticids. Books on the spider fauna of India provided additional pathways to species or genus level identifications (e.g., Sebastian and Peter, 2009), although sometimes this was achieved only through matching pictures of dorsal views of spiders to specimens which were collected as part of this study. Tikader (1982) was used to identify araneid spiders even though this resource often only illustrated females. The *Cyclosa* specimen was identified by consulting Tanikawa (1992). Levi (1967) and Saaristo (2006) were used to identify theridiid spiders. Linyphiid spiders were identified to species using Locket (1982) and Tu and Li (2004). The yellow sac spider of the genus *Cheiracanthium* was verified with Song *et al*. (1990). Sparassid spiders of the genus *Olios* were identified with Sethi and Tikader (1988). *Oxyopes pandae* was tentatively identified using Gabje (2008). However, despite this extensive work by Gabje (2008), the genus *Oxyopes* would benefit from greater research attention and more detailed genitalia drawings. The mimetid spider, *Ero*, was verified using Thaler *et al*. (2004). Experts who were sought for assistance are listed in the acknowledgments.

Monitoring ACP eggs, nymphs, and adults on citrus flush growth

ACP females preferentially oviposit on flush growth. Colonies of nymphs develop exclusively on this young plant material and this growth stage is highly preferred by adult ACP (Hall *et al*., 2008). Consequently, over March 2011 to

June 2013, the presence of flush was estimated each week by randomly selecting 10 branches around each experimental tree at shoulder height and assessing them for the presence or absence of flush growth (i.e., immature soft green leaves at various stages of expansion) which was then recorded by tree, site, and date. In comparison, non-flush consisted of mature leaves that were stiff and had dark green coloration. Sampled flush growth was examined in the field for the presence of ACP eggs, nymphs, and adults and the number of flushes infested with each life stage was recorded. These data were used to determine percentage flush growth infested by each ACP life stage (i.e., number of branches with flush growth infested with ACP life stage /[(number of ACP infested and non-infested flush growth branches + non flush growth branches)*100]) per tree at each sampling site and date. Percentage leaf flush data were used to determine the phenology of ACP eggs, nymphs, and adults, and their relative abundance over time.

RESULTS AND DISCUSSION

From this study, 43 distinct spider species were recognized in 17 families and 38 genera with 23 identifiable to a specific species or near species level (Table 1). In the tap samples, there were 15 families, 26 genera and 16 specific species or near species identifications. In Malaise trap samples, 14 families, 28 genera, and 21 specific species or near species were identified. There was an overlap of 12 families, 18 genera, and 14 species between the two collecting methods (i.e., tap sampling and Malaise trap).

Tap sampling

Tap sampling provided a realistic representation of potential spider species attacking ACP as this approach was performed directly on citrus trees where spiders and ACP were co-occurring. In contrast, the Malaise trap was intercepting spiders moving either aerially or on the ground and these species may not normally be in association with ACP even though they inhabit citrus orchards. The most dominant spider taxa recorded were the jumping spiders (Salticidae), followed by orb weavers (Araneidae), and combfooted spiders (Theridiidae) (Table 1, Fig. 1). The remainder of the most common spiders encompassed the cursorial hunters; *Cheiracanthium insulanum* (Miturgidae), *Olios tener* and *O. stimulator* (Sparassidae) and the ambusher *Thomisus* cf. *pugilis* (Thomisidae) (Table 1).

Immatures were the most common stage of all spiders that were sampled. In examining guilds of spiders by phenology, cursorial hunters were the most frequently collected spiders tap sampled citrus branches with jumping spiders being present in the samples every month (Fig. 2A). Other cursorial spider species were present throughout the year at lower densities (Fig. 2A). The web-spinning guild was less abundant than the cursorial spiders and were most frequently collected in August and September (Fig. 2B) with two species of *Neoscona* (Araneidae), along with *Eriovixia excelsa* (Araneidae) and *Coleosoma blandum* (Theridiidae) comprising the majority of captured specimens.

The phenologies of several single species are presented with all stages of development in Figs. 3A-D. Adults of the cursorial yellow sac spider, *Cheiracanthium insulanum*, were captured sporadically throughout the summer (Fig. 3A) although it was more commonly collected in the Malaise trap (Table 1, also see below). The orbweaver, *Eriovixia excelsa* (Araneidae), was captured in greatest numbers toward the end of summer, a finding typical for this family of spiders (Fig. 3B). Jumping spiders (Salticidae), were represented with the greatest diversity in this study with several species (*Phintella* sp. #1 [Fig. 3C], *Thyene imperialis* [Fig. 3D], *Epocilla* sp. #1, *Pseudicius admirandus*) being frequently collected (Table 1).

Malaise trap sampling

Malaise trap sampling provided a different species composition of spiders in comparison to tap sampling (Table 1). The yellow sac spider, *C. insulanum*, was the most frequently collected species in the Malaise trap. Two species of linyphiid spiders, *Erigone bifurca* and *Gnathonarium gibberum*, were abundant also. These tiny spiders often disperse by ballooning making them vulnerable to interception by a Malaise trap. Salticid and araneid spiders were frequently encountered but were not as common when compared to captures from tap sampling (Table 1).

ACP life stage phenology, abundance, and correlation with spider abundance

Percentage leaf flush infested with ACP eggs, nymphs, and adults provided information on the phenology of these life stages and their relative frequency on citrus flush over March 2011 to April 2013 (Fig. 4). ACP eggs were found on flush growth over June-Oct 2011, Feb-Oct 2012, and again starting in Feb 2013. Percentage flush infested with ACP eggs never exceeded 15% during these surveys (Fig. 4A). ACP nymphs tended to be found most commonly on citrus flush from the end of February through the beginning of April. Over this time period, peak flush infestation reached approximately 20-30% of sampled foliage (Fig. 4B). Smaller infestations of ACP nymphs were observed over July-Oct and percentage flush infested at this time was typically less than 10% of sampled foliage (Fig. 4B). Adult ACP exhibited three distinct periods of activity during this survey. Adult psyllids were found on citrus flush from early April to Sept. 2011 (numbers peaked in early June 2011); early April to late Oct. 2012 (numbers peaked, but not substantially, in early May 2012), and again adult ACP were found on flush growth beginning in early April 2013. Peak percentage flush infested with ACP adults ranged approximately 30-50% (Fig. 4C).

Spiders most likely to attack ACP life stages on flush growth were immature stages of cursorial and ambush hunters, of which salticids were most commonly collected (Fig. 1). Further, salticids were the most commonly collected cursorial hunters in citrus (Fig. 2A), and immature stages of at least two salticid species, *Phintella* sp. #1, and *T. imperialis* (Fig. 3C,D), were present on trees for most of year and would coincide with ACP eggs and nymphs. The phenology of dispersing yellow sac spider, *C. insulanum*, captured in the Malaise trap showed a pronounced population peak in Aug. 2011 (Fig. 5), a time that coincided with ACP egg, nymphs, and adults being present on flush (Fig. 4). This peak was not observed in Aug. 2012, and densities of this spider were

Table 1
Spiders collected from tap sampling and a Malaise trap in two unsprayed citrus groves, Square 9 and PARS, University of Agriculture, Faisalabad, Punjab, Pakistan (Male [adult], Fem = Female [adult], and Imm = immature spider).

	Tap samples				Malaise trap				
	Male	Fem	Imm	Total	Male	Fem	Imm	Total	Total
Araneidae									
Araneus mitificus	-	2	14	16	4	1	-	5	21
Cyclosa omonaga	-	-	-	0	1	-	-	1	1
?Cyclosa? sp. #1	1	-	-	1	1	-	-	1	2
Cyrtophora cicatrosa	1	-	4	5	-	-	-	0	0
Cyrtophora ?citricola?	-	-	3	3	-	-	1	1	4
Eriovixia excelsa	8	29	107	144	-	-	1	1	145
Hypsosinga sp. #1	-	-	-	0	1	-	-	1	1
Larinia sp. #1	-	-	1	1	1	-	-	1	2
Neoscona polyspinipes	-	-	-	0	3	-	-	3	3
Neoscona theisi	-	-	-	0	4	3	-	7	7
Neoscona immatures	-	-	119	119	-	-	10	10	129
Unidentifiable immatures	-	-	33	33	-	-	-	0	33
Clubionidae									
Clubiona cf. *filicata*	1	4	51	56	2	1	11	14	70
Corinnidae									
Castianeira sp. #1	-	-	-	0	1	-	-	1	1
(Phrurolithine) sp. #1	-	-	-	0	1	-	-	1	1
Dictynidae									
Dictyna sp. #1	1	-	-	1	-	-	-	0	1
Gnaphosidae									
Micaria immatures	-	-	-	0	-	-	23	23	23
Gnaphosid sp. #1	1	1	-	2	-	-	-	0	2
Gnaphosid immatures	-	-	15	15	-	-	6	6	21
Linyphiidae									
Erigone bifurca	-	-	-	0	26	35	23	84	84
Gnathonarium gibberum	2	-	8	10	5	7	85	97	107
Unidentified linyphiids	2	1	3	6	-	1	0	1	7
Lycosidae									
Unidentified lycosids	2	1	2	5	-	-	54	54	59
Mimetidae									
Ero aphana	-	1	-	1	-	-	-	0	1
Miturgidae									
Cheiracanthium insulanum	2	4	90	96	18	30	199	247	343
Oxyopidae									
Oxyopes pandae	-	1	-	1	6	2	-	8	9
Oxyopes (2 species)	-	-	-	0	3	4	-	7	7
Oxyopes immatures	-	-	38	38	-	-	10	10	48
Peucetia immatures	-	-	2	2	-	-	-	0	2
Philodromidae									
Thanatus immature	-	-	-	0	-	-	5	5	5
Tmarus sp. #1	1	3	19	23	-	-	5	5	28
Salticidae									
Bianor albobimaculatus	-	-	-	0	1	-	-	1	1
Epocilla sp. #1	13	15	31	59	-	-	-	0	59
Menemerus sp. #1	1	-	-	1	-	-	-	0	1
Myrmarachne (2 species)	4	1	5	10	2	5	9	16	26
Phintella sp. #1	11	13	93	117	-	-	3	3	120
Phlegra sp. #1	-	-	-	0	-	1	-	1	1
Pseudicius admirandus	2	6	29	37	-	-	5	5	42
Thyene imperialis	15	19	83	117	2	2	10	14	131
Salticid sp. #1	2	11	1	14	3	2	-	5	19

	Tap samples				Malaise trap				
	Male	Fem	Imm	Total	Male	Fem	Imm	Total	Total
Salticid male sp. #2	1	-	-	1	-	-	-	0	1
Salticid male sp. #3	1	-	-	1	-	-	-	0	1
Salticid female sp. #4	-	1	-	1	-	-	-	0	1
Salticid female sp. #5	-	1	-	1	-	2	-	2	3
Salticid immatures	-	-	290	290	-	-	15	15	305
Sparassidae									
Olios stimulator	1	1	-	2	1	1	1	3	5
Olios tener	1	1	-	2	2	-	-	2	4
Olios immatures	-	-	66	66	-	-	33	33	99
Tetragnathidae									
Dyschiriognatha dentata	-	-	-	0	1	-	-	1	1
Theridiidae									
Chrysso pulcherrima	1	1	6	8	2	-	2	4	12
Coleosoma blandum	4	9	68	81	2	1	13	16	97
Euryopis sp. #1	1	-	-	1	-	-	-	0	1
Phycosoma martinae	-	-	-	0	1	1	2	4	4
Theridion cf. *melanostictum*	20	5	-	25	4	-	-	4	29
Theridion sp. #1	3	16	-	19	3	-	-	3	22
Theridion sp. #2	2	1	-	2	-	-	-	0	2
Theridion sp. #3	-	2	-	2	-	-	-	0	2
Theridion sp. #4	-	1	-	1	-	-	-	0	1
Theridion immatures	-	-	52	52	-	-	30	30	82
Thomisidae									
?Diaea? sp. #1	1	-	-	1	-	-	-	0	1
Thomisus cf. *pugilis*	10	3	29	42	31	2	21	54	96
Xysticus immatures	-	-	-	0	-	-	1	1	1
Uloboridae									
Philoponella sp. #1	2	-	-	2	-	-	-	0	2
Unidentifiable	1	-	18	19	-	-	13	13	32
Totals	119	154	1280	1552	132	101	591	824	2371

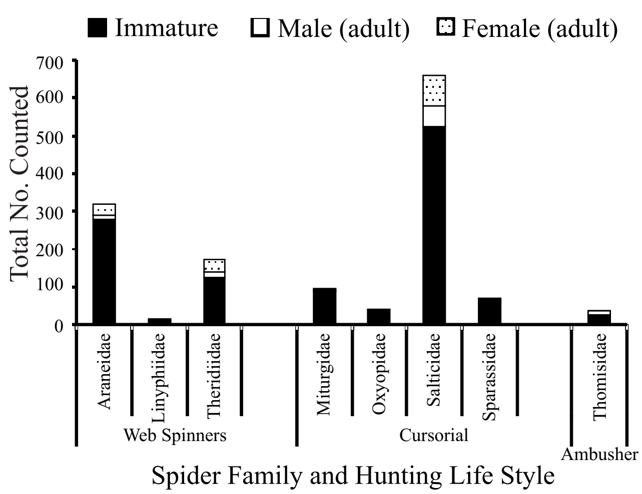

Fig. 1
The number of immature, adult male and female spiders of the most common families collected from tap sampling of citrus at Square 9 and PARS, University of Agriculture, Faisalabad.

substantially lower in citrus in 2012 when compared to 2011. Immature stages of *C. insulanum* were abundant on citrus foliage Feb-April 2012 and July-Sept 2012 (Fig. 3A), time periods when ACP eggs, nymphs, and adults would have been available as prey (Fig. 4).

The spider fauna of Pakistan is poorly known. Citrus is an economically important commodity in Punjab that can benefit from improved knowledge of spiders that may attack various pest species thereby providing important biological control services. This study generated an inventory of spider species sampled in two unsprayed citrus orchards in Faisalabad Punjab, Pakistan, over the period March 2011 to June 2013, and it provides an important foundation for future research on spider biodiversity in citrus. Two different survey methods were used to sample spiders, tap sampling of foliage, and passive interception with a Malaise trap. It is important to recognize that the two different collecting techniques used in this study resulted in disparate spider fauna compositions which reflected the method of sampling. Consequently, the choice of sampling approach for measuring spider abundance significantly influences conclusions on the quantification and biodiversity in crops and the significance of possible agro-ecosystem services that they provide.

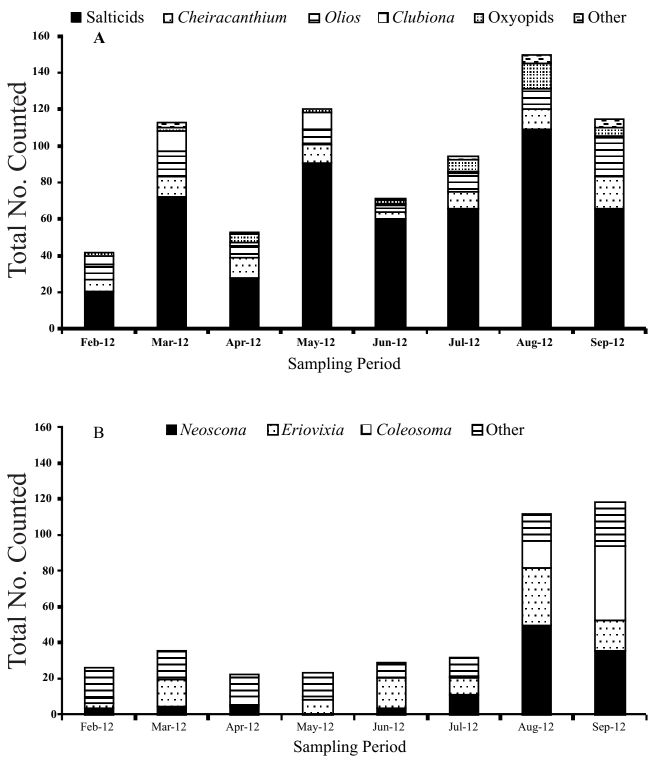

Fig. 2
Tap sample collections showing the immatures of the most common genera or family by hunting guild (A) cursorial spiders and (B) web spinners collected from February to September 2012 at Square 9 and PARS, University of Agriculture, Faisalabad Pakistan.

Tap sampling (as opposed to Malaise trap sampling) likely gave the best assessment of spider species, their relative temporal abundance, and likelihood of being important natural enemies of tree-inhabiting citrus pests. Tap sampling results suggest that jumping spiders (salticids) and cursorial hunters, are potentially important predators of citrus pests. These spider species, especially immature stages (i.e., salticid spiderlings), may be important predators of ACP eggs and nymphs, and possibly adults too. ACP are small prey and hunting methods employed by salticids, patrolling vegetation and using either vision or vibrations to alert them to the presence of prey, may enable them to find and attack this pest. In comparison, webspinners are passive predators, collecting and filtering aerially-dispersed arthropods that drift into webs, an interception method directly analogous to the Malaise trap used in this study.

Although tap sampling probably generated a more comprehensive list of spider species acting as biological control agents, it still may not be sufficient to accurately inventory tree inhabiting species. For example, the cursorial yellow sac spider, *Cheiracanthium insulanum*, was very common in Malaise trap samples but not in tap samples. These spiders create protective silk sacs inside leaves whose edges they curl over and fasten with silk. It is possible that yellow sac spiders were prevalent in tap sampled citrus trees but they were not dislodged from their protective bivouacs when branches were tapped. Consequently, in addition to tap sampling, placement of sticky traps inside the citrus canopy

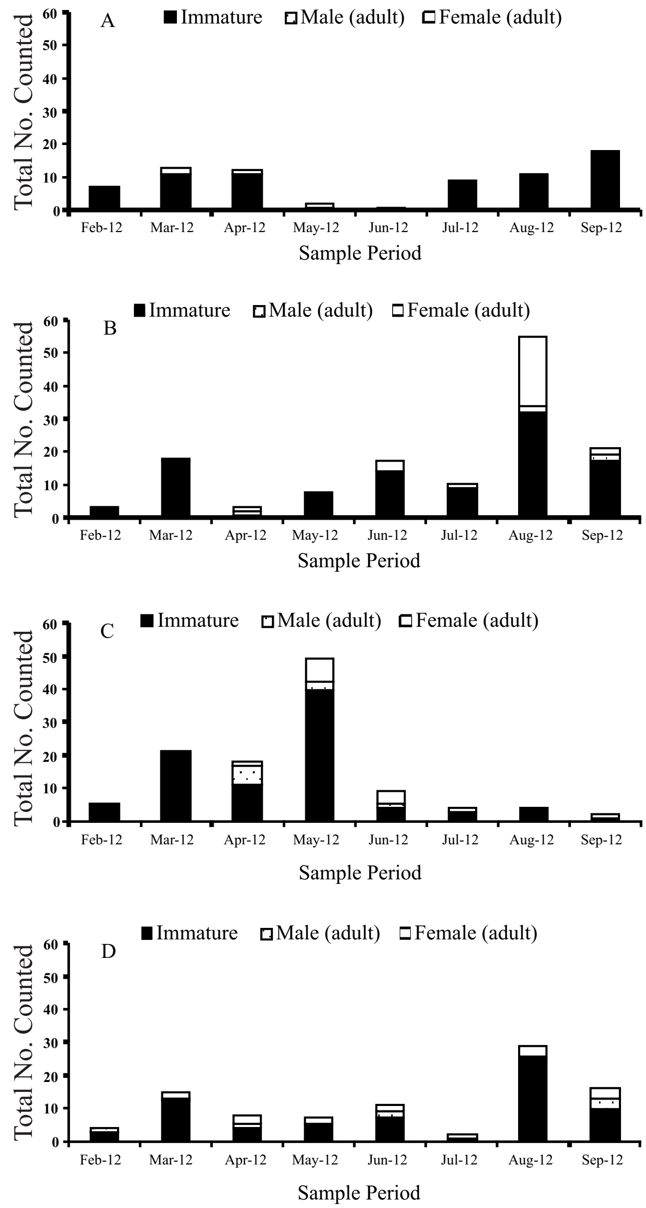

Fig. 3
Seasonal captures from tap samples in citrus at Square 9 and PARS, University of Agriculture Faisalabad, from February to September 2012 for four of the most common spider species: (A) *Cheiracanthium insulanum* (Miturgidae) (cursorial predator), (B) *Eriovixia excelsa* (Araneidae) (web spinner), (C) *Phintella* sp. #1, and (D) *Thyene imperialis* (latter two are both Salticidae; cursorial predators). Y-axis scales are the same for all graphs allowing easier comparisons among the four species.

might result in a better assessment of yellow sac spider (and other spider species) abundance in citrus trees. It is possible that *C. insulanum* could be important predators of ACP.

The phenology of ACP life stages infesting citrus flush show distinct increases and decreases which was most pronounced for adult ACP (Fig. 4C). Tap sampling indicated that immature salticids and *C. insulanum* are present in citrus when ACP is abundant (Fig. 4A, B, C). These predators may have the ability to prey on ACP, especially vulnerable eggs

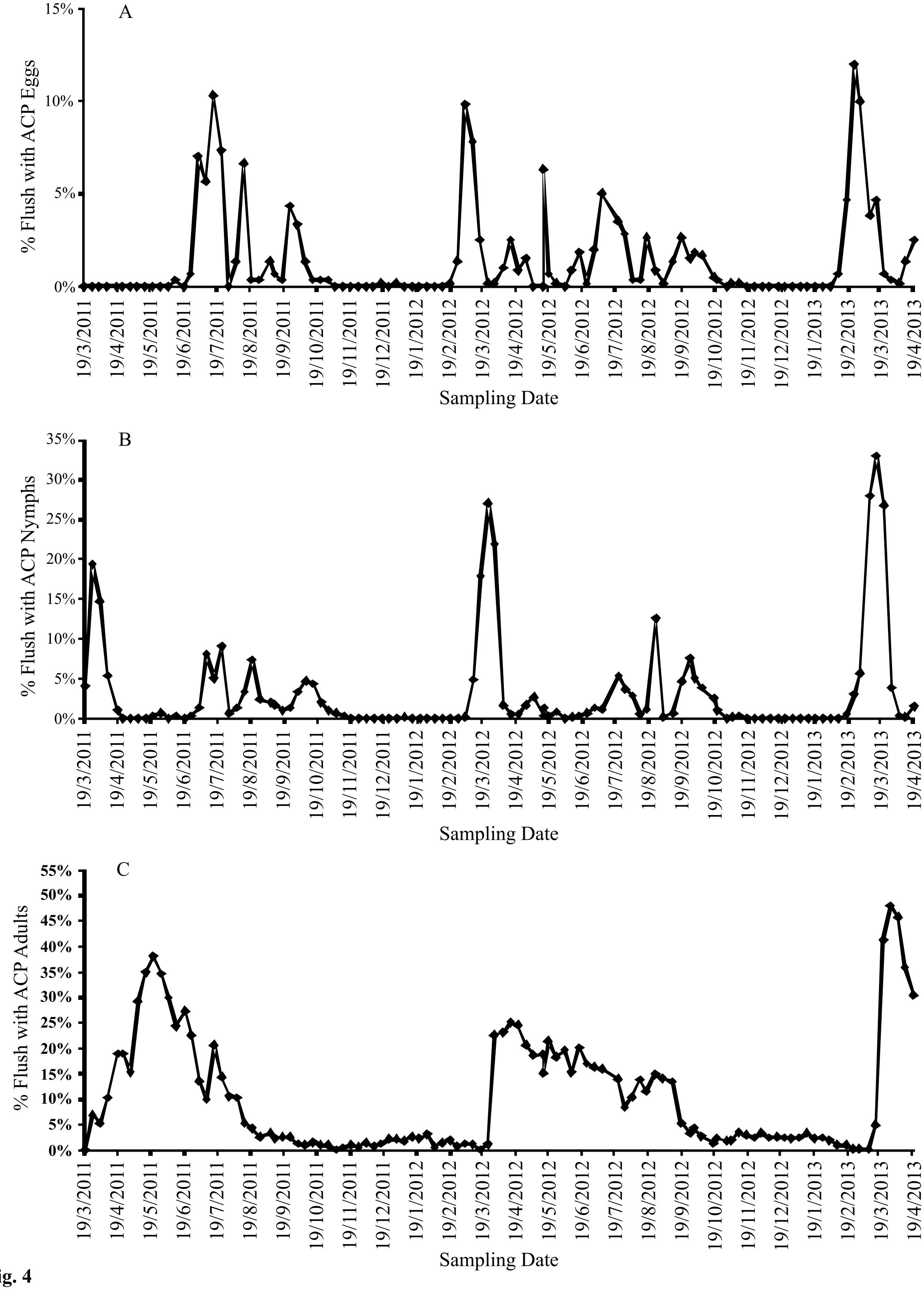

Fig. 4

Percentage citrus leaf flush infested with Asian citrus psyllid (ACP) (A) eggs, (B) nymphs, and (C) adults averaged across Square 9 and PARS, University of Agriculture, Faisalabad over March 2011 to April 2013.

and nymphs which have restricted mobility. In captivity, young spiderlings are susceptible to rapid starvation and are therefore likely to be aggressive hunters of small prey to satisfy their nutritional needs. Predation of ACP eggs and nymphs by spiderlings of spider species commonly collected during this survey could be evaluated through laboratory experimentation. Follow-up field experiments would be needed to conclusively demonstrate the role of spiders as important natural enemies of ACP. These experiments would need to control for many variables including prey size, prey and predator phenology, the influence of alternative prey and how it affects spider attack rates on ACP, and intraguild predation within and between spider taxa and other generalist predators inhabiting citrus. Direct observation of ACP eggs and nymphs over multiple 24-hr observation periods could capture diurnal and nocturnal predation events by spiders (Pfannenstiel, 2008) and could help verify the speculation that salticids and *C. insulanum* may be ACP predators, as well as identifying the predation potential of other species. Alternatively, molecular techniques could be used to determine prey consumption by field captured spiders (Welch *et al*., 2013) and possibly the identification of prey specificity and subsequent pest control by particular spider species (Chapman *et al*., 2013). Immunomarkers have high potential for marking prey in the field because of their low cost and ease of detection with ELISA testing. This technique could be used to detect ingestion of protein-marked prey to assess spider predation on ACP life stages (Kelly *et al*., 2012). In addition in North America, a yellow sac spider, *C. inclusum*, can exploit plant resources like pollen to survive which could promote the persistence of spider populations when prey are scarce (Pfannenstiel 2012). This could be important for spiders attacking ACP which exhibits periods during the year when population densities drop below detectable levels.

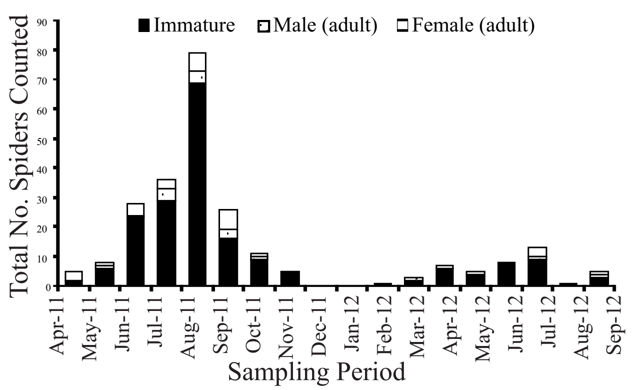

Fig. 5

Seasonal phenology of the yellow sac spider, *Cheiracanthium insulanum* (cursorial predator) from Malaise trap sampling at Square 9 University of Agriculture Faisalabad, over March 2011 to April 2013.

As generalist natural enemies, spiders have some desirable attributes; they are often ubiquitous throughout a crop and are voracious predators. On the other hand, spider development times may reduce their importance as predators in comparison to other predator species. Spiders typically require at least one season to grow and mature and therefore, most spider species produce one generation a year. Reproduction may also be highly synchronized, possibly with all the members of a particular species being in a cohort consisting of individuals of approximately the same size/age at the same time. Rigid cohort structures of this type may reduce the pest control efficacy of a particular spider species if the size of the pest of concern is outside the acceptable size range that the spider would attack. Additionally, many insect pests have multiple generations per growing season. If pest population phenologies are characterized by large outbreaks followed by prey scarcity (or prey change from a slow-moving, plant-restricted stage [e.g., larvae] to a highly motile adult stages [e.g., flying moths]), certain guilds of spider species may be unable to utilize these species and, therefore, might focus on alternative prey which would relax pressure on the pest of interest. The overall effect would be that the spider population, although relatively abundant in citrus for long periods, may only intersect the preferred life stage of the pest for attack during a short period. Therefore, spiders may not be biological control agents that are able to effectively reduce densities of a particular pest species.

An interesting result from this work involves the discovery of three theridiid spiders that were identified to species, all of which have geographically large distributions. Saaristo (2006) reports that *Phycosoma martinae* has a widespread Eastern hemisphere tropical distribution and is found from Aldabra to India, and in China, Korea, Ryukyu Islands, the Seychelles, and the Philippines. Levi (1967) states that *Coleosoma blandum* is often found in packages arriving from the tropics into the United States and that it may be native to the American tropics. It has been found in the United States (Florida), Africa (Ghana, Togo), India and the New Hebrides islands (now Vanuatu) with Saaristo (2006) documenting it on the Seychelles as well. *Chrysso pulcherrima* is a widespread pantropical species probably originating from the Eastern hemisphere and has been found in the Pacific and in West Africa (Levi 1967). It is unknown how many of these species are native to Punjab Pakistan or whether they are non-native species that have been accidentally introduced into the Indian subcontinent. If they are non-natives, it is remarkable that they were able to establish viable populations in the citrus ecosystem in Pakistan.

In conclusion, this study provides information in two areas where data were lacking; it contributes to an improved understanding of the Pakistani spider fauna by documenting the spider fauna in an agricultural crop (citrus) where spiders may be biological control agents of important pest species like ACP. Further, the information presented here provides the foundation for follow up laboratory and field studies that can investigate more thoroughly hypotheses put forward here, such as salticid or *C. insulanum* spiderlings potentially being important predators of ACP. Better understanding of the role spiders play as natural enemies will benefit the development of Integrated Pest Management programs for the Pakistani citrus industry.

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