Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J E Z S Journal of Entomology and Zoology Studies

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3 (4): 28-31

Received: 13-06-2015
Accepted: 15-07-2015

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Biology and population dynamics of new colour morph of *Chromaphis juglandicola* Kalt. (Hemiptera: Aphididae) in Kashmir, India.

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Abstract
White morph of *Chromaphis juglandicola* Kalt. has been reported for the first time by the authors during present study undertaken in Kashmir valley. Laboratory investigations comparing the two colour morphs did not show any difference in the developmental characteristics of white and yellow aphids, but a slight reproductive advantage for white morphs. The reproductive rate of white morph was greater over first two days of adult life with mean values of 6.5 and 11.1 nymphs, while that of yellow ones was 2.7 and 4.6 nymphs ($p < 0.05$) for 1st and 2nd days respectively. Of the walnut aphid population present in the orchards of the valley, white morph accounted for zero to 35% in the month of May, 2013 and 77.6% to 93% in the month of September, 2013. Percentage of aphids (both morphs) showed fluctuations during the years with a declining trend in numbers towards Oct-Nov. The percentage of white morph compared to yellow one increased during the same period.

Keywords: *Chromaphis juglandicola*, white morph, Kashmir, Walnut

1. Introduction
Walnut green aphid or *Chromaphis juglandicola* Kalt. occurs as a pest on the walnut tree [1, 2, 3, 4, 5, 6, 7, 8, 9]. It is yellowish in colour and is usually found feeding underside of leaves [1, 4, 6, 8]. In 2003, white morph of *C. juglandicola* was discovered in Yuba County, Scramento Valley, California [10]. The fundatrix hatches from the winter eggs during April and the first days of May, and feeds on the buds or the young foliage [3, 5, 7]. The first generation and its descendants throughout the spring and summer are all alate parthenogenetic viviparous females until the time for sexual reproduction comes (in all usually 8-11 generations). These alate females are able to spares infestations. The sexual forms occur from September to November. The eggs are laid on the bark of stems or the base of buds. The species overwinters as eggs [1]. Their feeding reduces tree vigour, nut size, yield, and quality. In addition to direct feeding damage, they excrete copious amounts of honey-dew that falls onto nuts, leaves and shoots. Honey-dew supports growth of the black sooty mould fungus. This fungus reduces light penetration to the leaf surface reducing its photosynthetic capacity. Being black, it also absorbs heat to predispose nuts to sunburn and subsequent kernel quality loss due to high temperatures. High populations of aphids may also cause leaf drop, exposing more nuts to sunburn. If heavy populations are allowed to develop (i.e. > 15 aphids per walnut leaflet) and remain for as little as 14 days uncontrolled, current season's nut quality is reduced along with a substantial reduction in the following season's crop [11]. The main aim of the present study was to observe the seasonal dynamics of the new morph of walnut green aphid. Laboratory studies were aimed to compare the biological characteristics of the new colour morph (white) with normal one (yellow) so that a plausible explanation can be made about the appearance of new morph.

2. Materials and Methods
For present study five sites (S1 to S5) were selected covering all major zones of the Kashmir valley during 2012-2013. Walnut orchards were randomly selected for survey. Monitoring was done at each site by selecting 20 trees randomly in an orchard. For each tree 5 leaves were sampled accounting a total of 100 leaves at one site. The level of infestation and number of white morphs was recorded. White morph was identified with naked eye on less infested leaves and with the help of magnifier on heavy infested leaves (>20 aphids/leaflet).

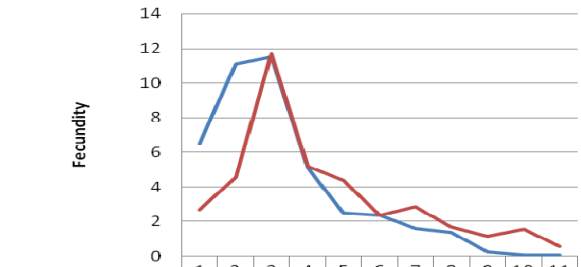
For investigating biological characteristics under laboratory conditions, both colour morphs were reared on walnut saplings grown in pots at Animal House, Department of Zoology, University of Kashmir. Both morphs were reared separately on walnut leaves using clip cages (10 Replicates). Following parameters were compared between white and yellow aphids viz. adult longevity, total progeny production (fecundity), or daily progeny production and reproductive rate [12, 13].

2.1. Statistical analysis
The data obtained from experimental groups was processed in MS-Excel 2007 for calculating Mean and SD. Later was then subjected to t-test using Primer software. Differences were considered to be statistically significant if $p < 0.05$.

3. Results
C. juglandicola is a yellow coloured aphid feeding on underside of walnut leaves. White morph of *C. juglandicola* (Fig. 2a & 2b) has been reported for the first time in Kashmir valley by the authors at Danwethpora Kokernag in August, 2012. The white morphs were seen feeding randomly with yellow ones on both less and heavily infested leaves (Fig. 3). The percentage of white morph varied from site to site as well as during different months of the year, 2013 (Table 1). However percentage of white morph comparative to yellow morph showed increasing trend during the year (Fig. 4), although overall number of aphids decreased towards last 5 months of the year. During January-April, 2013, no infestation was recorded. Aphids started appearing after 20th of May, 2013. The percentage of white aphids was small or negligible during May at most sites reaching highest percentage (24%) at S2. During June highest percentage was recorded at S3 (54.8%). During July, highest percentage of white morph was found at at S3 (61.2%) and in August at S5 (81.1%) and in September at S1 (93%). During October onwards aphids started disappearing from trees. At S1, percentage of white morphs during May was zero, in June it was 20% and increased to 93% during September. At S2, the percentage of white morph was found to be 24% during May and increased to 77.6 % during September. At S3 it was 23.4% in May and increased to 90% during September. At S3 aphid was seen during October in very small numbers and white one's percentage were found to be 81.2%. At S4, it was 35.8 % during May and 82.5% during September. At S5 it was zero during May and increased to 85.8% during September.

Laboratory studies revealed that white morph and yellow morph do not have any difference in the developmental characteristics, but a slight advantage for white ones in reproductive characteristics. Although white morphs did not show greater longevity, total progeny production, or daily progeny production compared to yellow morphs, the reproductive rate of white aphids was greater over the first two days of adult life (Fig. 1). During the first two days of adult life white morph gave birth to higher number of nymphs with mean values 6.5 and 11.1, while that of yellow ones was 2.7 and 4.6 ($p < 0.05$) (Table 2). The fecundity for remaining days of adult life was more or less comparable.

4. Discussion
Phenotypic polymorphism (i.e. differences in body characters, such as colour and size of individuals of the same species) is widespread in animal taxa [14]. Many species of aphids are characterized by the production of a range of colour morphs that may differ in some biological properties such as growth rate, host range and susceptibility to natural enemies [15, 16, 17]. Several hypotheses have been proposed to justify the occurrence and maintenance of polymorphism in natural populations of aphids [18]. According to most of these hypotheses, differential pressures of environmental conditions, especially natural enemies, on different colour morphs maintain colour morphism in aphid populations [19, 16]. Appearance of a new colour morph of walnut aphid and its increasing percentage compared to yellow morphs during the year clearly hints towards greater fitness or advantage which the former has over the later [20]. Appearance of a new colour form gives clear inference that directional selection is going on [21]. Though adult longevity and total progeny production is same for both morphs but reproductive advantage of white ones during early days of adult life might give them added advantage to leave more progeny. As the longevity of adult aphids under field conditions is likely to be much shorter than in the lab, this small advantage early in the adult life of the white morph can translate to more rapid population growth than for the yellow morph. There might be some forces operating which are favouring the white ones over yellow morphs. White morphs may have advantage in getting protection from natural enemies like its pasasitoid *Trioxys pallidus* or else. These inferences have not been proven yet and await further research on the subject. Our future aim will be to compare host instar preference of different natural enemies for each colour morph and also to compare the susceptibility of white and yellow walnut aphids to walnut pest management products using simple laboratory bioassays.



	1	2	3	4	5	6	7	8	9	10	11
White morph	6.5	11.	11.	5.1	2.5	2.4	1.6	1.4	0.3	0.1	0.1
Yellow morph	2.7	4.6	11.	5.2	4.4	2.4	2.9	1.7	1.2	1.6	0.6

Fig. 1: Fecundity of white and yellow morphs of *C. juglandicola* during 11 days of adult life

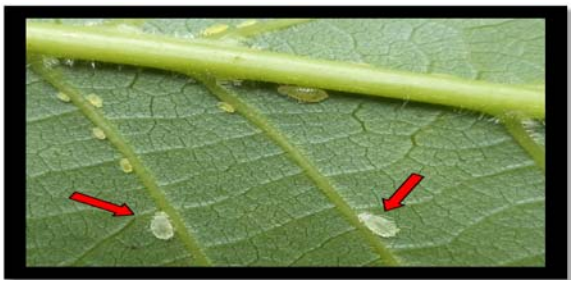


Fig. 2a: White morph of *C. juglandicola*

Fig. 2b: White morph and yellow morph enlarged

Fig. 3: Heavily infested leaf showing random distribution of white morphs with yellow ones

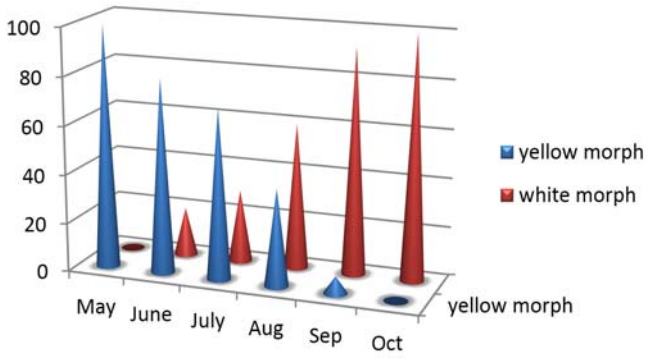


Fig. 4: Dynamics of colour morphs of the walnut green aphid in Kashmir during 2013.

Table 1: Seasonal dynamics of white morph of *C. juglandicola* during different months of the year 2013 in Kashmir

S. No.	Study site		JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
1	S1	Total no. of aphids/ leaflet (Mean)	-	-	-	-	12	25	24.3	6.6	3	-	-	-
		No. of white morph (mean)	-	-	-	-	-	5.00	7.3	4.00	2.8	2	-	-
		%age of white morph	-	-	-	-	0	20	30	60	93	100	-	-
2	S2	Total no. of aphids/ leaflet (Mean)	-	-	-	-	8.2	12.25	18	2.8	1.03	-	-	-
		No. of white morph (mean)	-	-	-	-	2	2.88	6.20	2.2	0.8	-	-	-
		%age of white morph	-	-	-	-	24	23.5	34.4	78	77.6	-	-	-
3	S3	Total no. of aphids/ leaflet (Mean)	-	-	-	-	14.18	25.68	27.53	4.02	4	1.33	-	-
		No. of white morph (mean)	-	-	-	-	3.33	14.09	16.86	2.75	3.60	1.08	-	-
		%age of white morph	-	-	-	-	23.4	54.8	61.2	68.75	90	81.2	-	-
4	S4	Total no. of aphids/ leaflet (Mean)	-	-	-	-	21.5	48	32.66	20	12.02	-	-	-
		No. of white morph (mean)	-	-	-	-	7.70	7.6	10.04	8.55	9.9	-	-	-
		%age of white morph	-	-	-	-	35.8	58	30.7	42.75	82.5	-	-	-
5	S5	Total no. of aphids/ leaflet (Mean)	-	-	-	-	6.82	22.4	26.08	7.15	1.56	-	-	-
		No. of white morph (mean)	-	-	-	-	-	6.11	12.71	5.80	1.34	-	-	-
		%age of white morph	-	-	-	-	0	27.2	48.8	81.1	85.89	-	-	-

Table 2: Fecundity of two colour morphs of *C. juglandicola* during 11 days of adult life (10 Replicates)

Days of Adult life	Fecundity of White morph (Mean±SD)	Fecundity of Yellow morph (Mean±SD)
Ist	6.5± 2.01^{a}	2.7± 1.41 ^{b}
2nd	11.1± 1.91^{a}	4.6± 0.69^{b}
3rd	11.5± 2.06^{a}	11.7± 2.11^{a}
4th	5.1± 2.5^{a}	5.2± 1.93^{a}
5th	2.5± 0.84^{a}	4.4± 2.17^{b}
6th	2.4± 1.26^{a}	2.4 ± 1.42^{a}
7th	1.6± 1.71^{a}	2.9 ± 0.99^{a}
8th	1.4± 0.51^{a}	1.7± 0.67^{a}
9th	0.3± 0.48^{a}	1.2± 1.22 ^{b}
10th	0.1± 0.31^{a}	1.6± 1.57^{b}
11th	0.1± 0.31^{a}	0.6 ± 0.84^{a}
Average	3.87± 1.24^{a}	3.54± 1.37^{a}

Values within each row that do not share the same superscript are significantly different ($^{a\text{-}b}P<0.05$). The data was evaluated by t-test to detect differential fecundity of two colour morphs. Differences were considered to be statistically significant if $p < 0.05$.

5. Acknowledgments

We thank Council of Scientific and Industrial Research (CSIR) for providing financial help *(Vide Sanction no. 09/251(0048)/2012-EMR-I)*. We also thank Head, Department of Zoology, University of Kashmir, Srinagar for providing the necessary laboratory facility. The authors have no conflict of interest.

6. Conclusion

Reporting of new colour morph of walnut green aphid, its increasing percentage vis-a-vis normal yellow morph during different months of the years in the walnut orchards and higher reproduction rate during early life is a matter of concern.

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