RESEARCH ARTICLE

PREDATORS AS NATURAL AND APPLIED BIOCONTROL AGENTS OF SUGARCANE WOOLLY APHID *CERATOVACUNA LANIGERA* IN INDIA: AN APPRAISAL

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Abstract

In this study on sugarcane woolly aphid *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae), a pest native to north-eastern India that invaded tropical Indian states from 2002 onwards, we examined the role of predators as natural biocontrol agents and the possibility of using *Micromus igorotus* Banks (Neuroptera: Hemerobiidae) and *Dipha aphidivora* (Meyrick) (Lepidotpera: Pyralidae) as applied biocontrol agents. In three study years (2005-08) at Coimbatore, Tamil Nadu State, India, the aphid was active throughout the year with greater intensity during October-January. *Dipha aphidivora* was more abundant than *Micromus* sp. and syrphids (Diptera: Syrphidae), and its activity in general coincided with that of the aphid. In augmentative field trials, *M. igorotus* released at about 800 adults/ha failed to not only enhance its natural population but also displace the predominant *D. aphidivora* after 30 days. In a series of trials, *D. aphidivora* released at rates exceeding 1,000 cocoons/ha enhanced its natural population and reduced aphid intensity; *Micromus* sp. and unidentified syrphids displayed negligible activity. The study demonstrated the dominant status of *D. aphidivora* among *C. lanigera* predators and the usefulness of its' augmentative releases in reducing the aphid populations during the years of invasion at the study site.

Key words: Sugarcane, woolly aphid, *Ceratovacuna lanigera*, *Dipha aphidivora*, *Micromus igorotus*, seasonal fluctuations, augmentative biocontrol

Introduction

Woolly aphid *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae) had been a pest of sugarcane in India in the states of West Bengal (Basu and Banerjee 1958), Assam, Sikkim, Tripura and Uttar Pradesh (Ghosh 1974). The aphid invaded the tropical Indian states of Maharashtra and Karnataka in 2002 and later spread to Tamil Nadu, Andhra Pradesh, and parts of Kerala and Bihar States (Patil et al. 2004; Joshi and Viraktamath 2004; Thirumurugan 2004; Srikanth 2007). Based on initial surveys conducted in Maharashtra, the predator *Dipha aphidivora* Meyrick (Lepidoptera: Pyralidae), originally documented on the aphid in Nagaland State (Tripathi 1995), was identified as a potential biological control candidate (Rabindra et al. 2002); the brown lacewing *Micromus igorotus* Banks (Neuroptera: Hemerobiidae) was later reported from Karnataka (Lingappa et al. 2004). As the aphid spread in peninsular India, the dynamics of both the pest and its natural enemies, primarily predators, were examined in an apparent bid to understand the latter's role in natural control of the aphid. While the aphid was active during May-October, the predators *M. igorotus* and *D. aphidivora* were abundant during June-

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November with significant associations among them and correlations with weather parameters (Deshmukh et al. 2007; Sarma et al. 2007; Tripathi et al. 2008; Sharanabasappa et al. 2009).

As a precursor to augmentative evaluation, *D. aphidivora* was mass multiplied in situ in shade net enclosures (Patil et al. 2004; Ghorpade et al. 2007) and in the laboratory by providing woolly aphid infested leaf sections in galvanized iron trays (Mukunthan et al. 2006). A semi-synthetic diet (Venkatesan et al. 2008) and frozen aphids (Srikanth et al. 2009a) were found to support the development of the predator to different levels. *Micromus igorotus* was also bred in the laboratory using the natural host (Vidya et al. 2007). In augmentative trials with *D. aphidivora*, release rates of 500-1000/ac (Sannaveerappanavar et al. 2005; Patil et al. 2007) and more than 5000 cocoons/ha (Srikanth et al. 2009b) or inoculative releases of 1000 larvae or pupae (Pokharkar and Ghorpade 2009) effectively suppressed aphid populations. *Micromus igorotus* was also evaluated in field studies in Karnataka (Sannaveerappanavar et al. 2005; Vidya et al. 2010).

In the early days of the aphid outbreak, the aphid was perceived to be less active during summer but observations indicated that the aphid would thrive throughout the year under ideal crop conditions. Further, a very high rate of augmentative release of *D. aphidivora* suppressed aphid populations only late in the season but could not prevent the spread of the aphid in the target field (Srikanth et al. 2009b) and subsequent yield loss (Sivaraman et al. 2013). In the light of these observations and the need to manage the aphid that was spreading rapidly in Tamil Nadu, we examined the seasonal dynamics of the aphid and predators for three consecutive years to bring further clarity on their seasonal patterns and the role of predators in natural control of the aphid. We also examined the feasibility of predator-based biocontrol of the aphid through augmentative releases of *M. igorotus*, the predator whose first occurrence on the aphid (Lingappa et al. 2004) and encouraging results of augmentative trials were reported from Karnataka that experienced the aphid invasion ahead of Tamil Nadu; the predominant *D. aphidivora* was assessed in independent trials. In this paper, we present the results of these studies, conducted with the primary intent of containing the spread of the invasive pest, and discuss them in the light of similar work carried out in other aphid invaded states.

Materials and methods

Study site

The studies were conducted at Coimbatore, Tamil Nadu State, during 2004-2008, the period that witnessed the invasion, establishment and proliferation of the aphid. Seasonal dynamics of the aphid and natural enemies were monitored in growers' farms throughout the activity period of the aphid. The predators *M. igorotus* and *D. aphidivora* were evaluated in both growers' farms and experimental plots of ICAR-Sugarcane Breeding Institute (SBI), Coimbatore, in augmentative trials initiated in the invasive phase and continued through the established phase of the aphid. Wide temporal and spatial variation in aphid intensity during these phases, and fragmented plot size often influenced the magnitude of experimentation, particularly predator release rates which were altered deliberately in an attempt to determine the optimum dosage. Control plots, which could not be maintained in some trials for similar reasons, were located 200 - 2000 m away from the release plots. The study site featured one of the two popular cultivars, namely Co 86032 and Co 62175 and the crop remained free from pesticide use.

Seasonal dynamics

In 2004, i.e. the first year of occurrence of the aphid at Coimbatore and, in general, Tamil Nadu (Srikanth 2007), the activity of the aphid and its predators was monitored at monthly intervals. Each month, observations were recorded in a different plot of 0.2 ha or larger area harboring uniform 5 to 6-month old crop in order to eliminate the effect of crop age on aphid and predator abundance. In the target field, 5-20 plants were located randomly depending on the spatial spread of attack and in each plant five leaves, beginning from the top most leaf with visible dewlap and moving downwards, were selected. Aphid intensity on individual leaves was recorded on a 0-5 rating based on the extent of colonization assessed visually as 0: 0%, 1: 0-20%, 2: 20-40%, 3: 40-60%, 4: 60-80% and 5: above 80% of leaf area covered by colonies (Anonymous 2005a). The mean rating per leaf from the five leaves constituted the aphid intensity for the plant selected and the mean rating per leaf computed from the 5-20 plants sampled represented the aphid intensity in the field. Predator activity was recorded as percentage of colonized leaves in the first five months (November 2004 - March 2005) but as actual predator numbers per leaf subsequently. In each selected leaf, the number of larvae of the agile *Micromus* spp. (Fig. 1(a)) was recorded first with least disturbance to the leaf. The webs built by *D. aphidivora* were teased open with a needle to expose the concealed larvae and their number was recorded; the number of cocoons formed on the leaf surface was also recorded. Mean number per leaf computed from the five leaves represented the activity of the two major predators and unidentified syrphids (Diptera: Syrphidae) in individual plants and the mean number per leaf computed from the 5-20 sample plants indicated their overall activity in the field.

Field evaluation of *M. igorotus*

Predator culture

Consignments of the predator were obtained from the University of Agricultural Sciences, Dharwad

Fig. 1. Life stages of *Micromus igorotus*, a predator of sugarcane woolly aphid *Ceratovacuna lanigera*: (a) larva (b) cocoon (c) adult

(UAS(D)), Karnataka State, India, as cocoons (Fig. 1(b)) formed on corrugated brown paper in laboratory cultures. The cocoons were held in well ventilated plastic jars until the first adults (Fig. 1(c)) emerged.

Augmentative trials

Trial-1 with *M. igorotus* was conducted during 2004-2005 in a grower's farm where a 6-month old 0.6 ha crop (cv Co 62175) showed only 100 m^2 infested area in small patches in the initial period of aphid invasion. Adults (20) and cocoons (132) of *M. igorotus* were released uniformly in the infested patches in November 2004. The delicate adults were released by tapping the plastic container and the cocoon bearing brown paper pieces were stapled to leaves. Pre- and post-release observations (15 days later) of aphid intensity and predator abundance were recorded in 6-10 randomly selected canes according to the protocol followed for population dynamics studies described above. In trial-2 conducted in another grower's farm the same year, 178 adults of *M. igorotus* that emerged from the second consignment were released in December 2004 in a 7-month old 0.4 ha crop (cv Co 86032) that showed about 600 m^2 total attacked area in three closely located patches. Observations of aphid intensity and predator abundance were recorded in 15-20 canes before the release of the predator and 30 days after release.

In trial-3 conducted on the campus of ICAR-SBI during 2005-2006, 787 adults were released in December 2005 in about 0.1 ha infested patch in the middle of a 1.0 ha plot of 9-month old (cv Co 86032) crop that had established *D. aphidivora* colonies and slight syrphid activity but no *Micromus* sp. presence. Pre-and post-release observations (30 days later) of aphid activity and predator abundance were recorded in 11-13 canes.

Field evaluation of *D. aphidivora*

Predator culture and field deployment

Predators required for the field trials were multiplied in the laboratory following a tray-rearing method developed earlier (Mukunthan et al. 2006). Since it was difficult to handle the agile and concealed larvae (Fig. 2(a)) or delicate moths (Fig. 2(c)), cocoons (Fig. 2(b)), formed on 20 cm leaf sections at the end of the laboratory rearing cycle, were dispensed by inserting the cocoon-laden leaf sections in leaf axils of canes (Fig. 3) at about 10 different points in the field depending on the size of the plot and rate

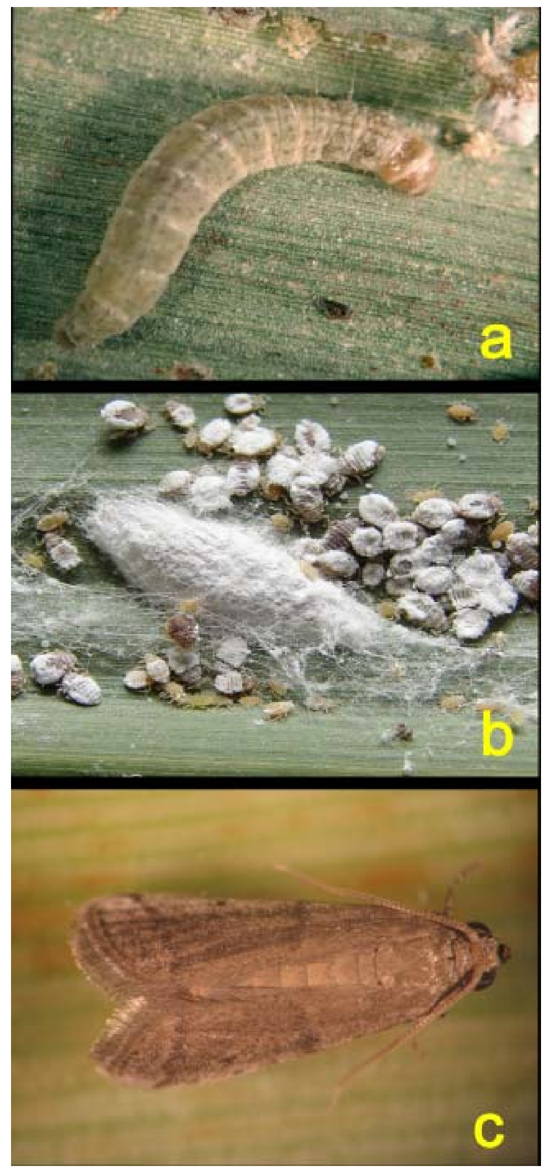

Fig. 2. Life stages of *Dipha aphidivora*, a predator of sugarcane woolly aphid *Ceratovacuna lanigera*: (a) larva (b) cocoon (c) adult

Fig. 3. *Dipha aphidivora* cocoon-laden leaf sections from laboratory cultures inserted in leaf axils for field delivery against sugarcane woolly aphid *Ceratovacuna lanigera*

of release. Prior to the commencement of trials, sample cocoon-laden leaf sections from laboratory tray rearings were inserted in leaf axils, collected 24 h later and held in the laboratory to assess whether or not cocoons were subject to the risk of ant predation. About 81.1 - 94.5% adult emergence from these sample cocoons confirmed the feasibility of the method.

Augmentative trials

The series of trials began with small scale field colonization (trial-1) in November 2004, the initial period of aphid invasion in the study site. About 150 cocoons of the predator were released in a 0.8 ha area of 8-month old ratoon (cv Co 86032) that had about 100 m^2 aphid infested patch but no predator activity. Observations of aphid intensity and predator abundance were recorded before predator release and 15 and 30 days later from 6-15 randomly sampled canes; the activity of *D. aphidivora* was expressed as percentage of leaves colonized.

Trials-2 & 3 were conducted during November 2005 - January 2006 on the campus of ICAR-SBI, the first in a 0.4 ha plot and the second in a 0.1 ha plot of 8-month old crop (cv Co 86032). The predator was released at rate equivalents of 1000 and 5000 cocoons/ha, and observations on aphid rating and predator numbers were recorded from 18-20 randomly selected canes.

In trial-4 on ICAR-SBI campus, *D. aphidivora* was evaluated in a 0.5 ha seed multiplication plot comprising primarily the cultivar Co 86032 planted in September 2005. Woolly aphid attack that began in January 2006, when the crop was about 5 months old, continued through the summer up to June 2006. The predator was released in a staggered manner at a rate of 1500 cocoons/ha twice at 6 and 8-9 months age of the crop. Observations on aphid rating and predator numbers were recorded from 16-24 randomly selected canes at 30 day intervals when the crop was 7-10 months old. An aphid attacked crop (9 months age) in a different location was maintained as a control plot in which similar aphid and predator data were recorded from 20 canes for comparison. In trials-5 & 6 conducted in the experimental plots of ICAR-SBI, the predator was released in November 2006 at the rates of 1250 and 2500 cocoons/ha in 8 month old crop occupying 0.32 and 0.10 ha respectively; an isolated plot of 0.33 ha was maintained as control. Observations of aphid and predator were recorded from 10-12 randomly selected canes.

Data analysis

The monthly data of mean aphid rating and predator numbers from seasonal studies were correlated with weather parameters namely mean monthly maximum and minimum temperatures, mean monthly forenoon and afternoon relative humidity (RH), and total monthly rainfall using Pearson's product moment correlation. For the comparison of pre- and post-release aphid and predator data within predator release and control plots in augmentation studies,

Table 1. Woolly aphid *Ceratovacuna lanigera* and predator activity levels in sugarcane at Coimbatore, Tamil Nadu, India (2004-05)

Month and year	Aphid rating per leaf	Percent of leaves colonized by predators		
		Dipha aphidivora	*Micromus* sp.	Syrphids
November 2004	2.08	0.0	0.0	0.0
December 2004	2.15	90.2	0.0	8.2
January 2005	3.47	57.3	0.0	6.7
February 2005	1.69	25.6	0.0	0.0
March 2005	1.49	27.1	2.4	0.0

non-parametric tests for dependent samples were employed using individual sample plants as replications or blocks and time of observation as treatments. Wilcoxon matched pairs rank test was used to compare two observations and Friedman ANOVA by ranks & Wilcoxon matched pair rank tests with a Bonferroni correction were used to compare three or more observations. The analyses were performed using StatSoft Inc (2004).

Results

Seasonal dynamics

When the aphid appeared first in a grower's sugarcane farm at Coimbatore in June 2004, *D. aphidivora* was found associated with it in the initial surveys; *Micromus* sp. and an unidentified syrphid appeared later. By January 2005, when the aphid spread to neighboring farms, *D. aphidivora* was the most predominant predator with occasional occurrence of the other two predatory groups. Shortly afterwards, the aphid was recorded on the campus of ICAR-SBI in various experimental plots.

In the first year of its occurrence (2004-05), mean aphid rating/leaf was similar in November and December 2004, peaked in January 2005 and declined thereafter reaching a low in March 2005 (Table 1). *Dipha aphidivora* activity, assessed as percentage of colonized leaves, was the highest in December 2004 but declined until March 2005; *Micromus* sp. and syrphids were less regular and abundant. Aphids collected from the field and maintained in the laboratory during this period did not yield any parasitoids.

Woolly aphid and predator dynamics monitored for the next three years (April 2005 - March 2008) indicated that the aphid was prevalent in almost all months (Fig. 4). Its' activity peaked during August-October, more prominently during 2005-06 and 2006-07, and declined in summer months. *Dipha aphidivora* displayed similar round-the-year activity with peaks during the same period as the aphid but with a few more months of inactivity. *Micromus* sp. was inactive in most months with far fewer numbers than those of *D. aphidivora*. The mean monthly activity levels (Table 2) and overall seasonal fluctuations (Fig. 4) not only indicated the gradual decline in aphid intensity and predator numbers over the three year study period but also the clear-cut differences between populations of the two predators. Over the three study years, the highest numbers of *D. aphidivora* larvae and cocoon per leaf (November-January) decreased from 11 (2005-

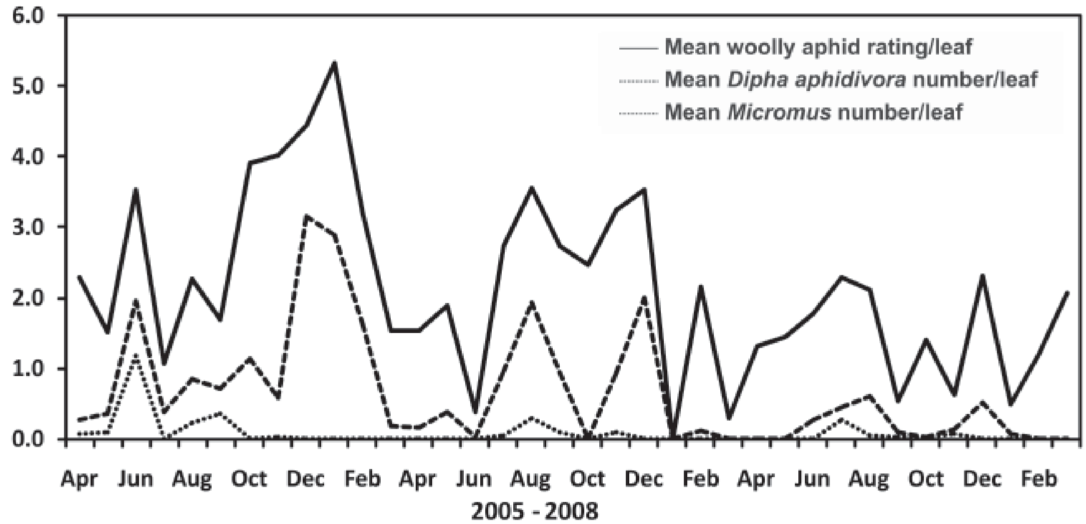

Fig. 4. Seasonal dynamics of sugarcane woolly aphid *Ceratovacuna lanigera* and its predators at Coimbatore, Tamil Nadu, India, during three study years (April 2005 – March 2008)

06) through seven (2006-07) to five (2007-08). The peak numbers of *Micromus* sp. per leaf (June-August) declined from 16 in 2005-06 to five in 2006-07 and 2007-08.

Weather factors vs. aphid and predator abundance

Among the weather factors, total monthly rainfall influenced the monthly aphid intensity positively over the three study years (Table 3). While mean monthly maximum and minimum temperatures affected *D. aphidivora* activity negatively, mean monthly forenoon RH showed a similar negative effect on *Micromus* sp. numbers. Among the three individual study years, 2005-06 witnessed maximum number of significant correlations: forenoon RH positively influenced aphid rating (r = 0.632; P = 0.028); minimum temperature negatively impacted *D. aphidivora* numbers (r = -0.737; P = 0.006); forenoon RH negatively affected *Micromus* sp. (r = -0.648; P = 0.023). In the remaining two years, the positive relationship of afternoon RH with *Micromus* sp. (r = 0.688; P = 0.013) was significant in 2007-08 alone. Correlations among aphid intensity, *D. aphidivora* and *Micromus* sp. were not

Table 2. Mean monthly woolly aphid *Ceratovacuna lanigera* and predator activity levels in sugarcane at Coimbatore, Tamil Nadu, India (April 2005 - March 2008)

Year	Aphid rating per leaf		*Dipha aphidivora* (No. per leaf)		*Micromus* sp. (No. per leaf)	
	Overall mean	Range	Overall mean	Range	Overall mean	Range
2005-06	1.72	0.69-3.43	1.01	0.18-3.16	0.16	0.00-1.18
2006-07	1.42	0.00-2.46	0.58	0.00-2.00	0.04	0.00-0.28
2007-08	1.28	0.41-2.07	0.15	0.00-0.57	0.03	0.00-0.26

Table 3. Correlations of monthly woolly aphid *Ceratovacuna lanigera* and predator abundance in sugarcane vs. mean monthly weather parameters at Coimbatore, Tamil Nadu, India (2005-08)

Weather parameter	Aphid rating per leaf	*Dipha aphidivora* (No. per leaf)	*Micromus* sp. (No. per leaf)
Maximum temperature (°C)	-0.287 ^{ns}	-0.391*	0.002 ^{ns}
Minimum temperature (°C)	0.031 ^{ns}	-0.402*	0.293 ^{ns}
Forenoon RH (%)	0.298 ^{ns}	0.152 ^{ns}	-0.420*
Afternoon RH (%)	0.317 ^{ns}	0.105 ^{ns}	0.128 ^{ns}
Total rainfall (mm)	0.493**	-0.073 ^{ns}	-0.128 ^{ns}

* $P<0.05$; ** $P<0.01$; ^{ns} $P>0.05$

significant either for the individual years or data pooled for all three years.

Predators in augmentative control

Predator dosages, always expressed as equivalent numbers per ha, were designated as 'absolute' to indicate the numbers released in the total area of the target plot and 'actual' to denote the numbers available for the infested area of the plot. At low aphid intensity levels in the invasive phase, actual dosages, computed on the basis of percentage of infested area assessed visually, were considerably higher than absolute dosages. However, in the established phase when the distribution of the aphid in trial plots became nearly uniform, absolute and actual dosages became more or less equal which were termed as 'field' dosage.

Field evaluation of *M. igorotus*

At 250/ha absolute or 1500/ha actual dosage in a plot with no predator activity (trial-1), *D. aphidivora*, *Micromus* sp. and syrphids appeared 15 days later in the same order of magnitude (Table 4); aphid intensity decreased significantly during the same period. The crop was harvested 15 days later as seed material obviating further observations. The pest spread to an adjacent ready-to-harvest plot with a lower infestation rating; *D. aphidivora* and *Micromus* sp. colonized these leaves in the same order of magnitude.

In slight contrast, at 450/ha absolute or 3000/ha actual dosage in a plot with abundant *D. aphidivora* and low syrphid populations but no *Micromus* sp. activity (trial-2), *M. igorotus* showed no establishment 30 days later (Table 4). *Dipha aphidivora* colonization rate showed a slight significant decline but the predator was present in all sampled plants just as in pre-release observation; the activity of syrphid too decreased. Aphid infestation rate showed a marginal, yet non-significant, increase during the same period but it showed a gradual decline in the subsequent weeks probably due to maturity of the crop.

In trial-3 conducted in the following year (2005-06) under similar conditions of established *D. aphidivora* and syrphid populations but no *Micromus* sp. activity (Table 4), *M. igorotus* release at 787/ha absolute or 7870/ha actual rate led to its minimal presence 30 days later. The predominant *D. aphidivora* decreased non-significantly and the previously small syrphid numbers maintained status

Table 4. Evaluation of the predator *Micromus igorotus* against woolly aphid *Ceratovacuna lanigera* in sugarcane plots at Coimbatore, Tamil Nadu, India

Observation	Aphid rating perleaf	Per cent of predator-harboring leaves[#]		
		Micromus igorotus	***Dipha aphidivora***	**Syrphids**
Trial-1: Grower's plot (November - December 2004)				
Pre-release	2.77	0.00	0.00	0.00
Post-release[a] (15 days)	1.73	31.80 (0.69)	70.83	16.00
Z-value[@]	2.293[*]	-	-	-
Adjacent plot (30 days)	0.95	45.70 (0.85)	57.30	0.00
Trial-2: Grower's plot (December 2004 - January 2005)				
Pre-release	2.14	0.00	90.20	8.17
Post-release[b] (30 days)	2.37	0.00	80.99	1.33
Z-value	1.661 [ns]	-	2.260[*]	-
Trial-3: Experimental plot (December 2005 - January 2006)				
Pre-release	2.29	0.00	3.19	0.07
Post-release [c] (30 days)	2.24	0.09	2.79	0.06
Z-value	0.178 [ns]	-	0.769 [ns]	-

[#] Predator figures in parentheses and trial-3 are numbers per leaf
[a] Absolute dosage of ≈250/ha and actual dosage of 1500/ha
[b] Absolute dosage of ≈450/ha and actual dosage of 3000/ha
[c] Absolute dosage of ≈787/ha and actual dosage of 7870/ha
[@] Wilcoxon matched pairs test; [*] $P<0.05$; [ns] $P>0.05$

quo. Aphid activity decreased marginally yet non-significantly in the same 30 day period. A second observation 45 days after release did not reveal any *Micromus* sp. activity indicating its failure to establish in the release site.

No separate control could be maintained for all three trials due to non-availability of identical plots. However, seasonal dynamics data for the corresponding period (Table 2 and Fig. 4) indicated no *Micromus* sp. activity in the study site in the absence of predator releases.

Field evaluation of *D. aphidivora*

In trial-1 carried out in the initial stages of aphid invasion, the infested patch in the 8-month old crop had 100% leaf colonization with moderate aphid rating but no *D. aphidivora* activity prior to predator release (Table 5). However, following the release of the predator at 190/ha absolute or 15,000/ha actual dosage, colonization of leaves by the predator increased significantly in the first 15 days but non-significantly in the next 15 days (Friedman ANOVA by ranks: χ^2 = 16.59; df=2; N=15; P=0.003;

Table 5. Evaluation of the predator *Dipha aphidivora* against woolly aphid *Ceratovacuna lanigera* in a grower's sugarcane plot at Coimbatore, Tamil Nadu, India (November-December 2004) (trial-1)

Observation	Aphid colonization on leaves		Percent of leaves with *Dipha aphidivora*
	Rating per leaf	Percent of infested leaves	
Pre-release	2.33 a[1]	100.00 a	0.00 a
Post-release[@](15 days)	2.18 a	91.45 a	47.92 b
Post-release (30 days)	1.18 b	68.70 b	53.57 b

[@]Absolute dosage of ≈190/ha and actual dosage of 15,000/ha

[1]Means followed by the same letter in a column are not significantly different by Friedman ANOVA by ranks and Wilcoxon matched pairs rank test significant with a Bonferroni correction (P<0.017)

coefficient of concordance = 0.553). The percentage of aphid colonized leaves (Friedman ANOVA by ranks: χ^2 = 17.15; df=2; N=15; P=0.0002; coefficient of concordance=0.572) and mean aphid rating (Friedman ANOVA by ranks: χ^2 = 18.41; df=2; N=15; P=0.0001; coefficient of concordance = 0.614) decreased non-significantly in the first 15 days but significantly in the next 15 days with no further spread of the aphid. Occasional *Micromus* sp. and syrphid activity was observed during the trial period. Seasonal dynamics data (Table 1), which served as control, indicated that predator colonization decreased in the absence of augmentation but the aphid rating increased progressively in the corresponding period.

In the two concurrent on-campus trials-2 & 3 during November 2005 - January 2006, the experimental plots with initial predator activity showed more or less uniform spatial infestation by the aphid which equated the absolute and actual dosages into a single field dosage. In the two trials carried out with field dosages of 1000 and 5000 cocoons/ha, mean *D. aphidivora* number per leaf increased significantly by nearly 19.6 and 3.0-fold with the highest number of cocoons per sampled leaf rising from 5 to 22 and from 4 to 7 in the two trials, respectively. During the same period, aphid rating decreased significantly by 25.2 and 44.0% in 40 and 30 days, respectively (Table 6). Leaf colonization rates of *D. aphidivora* and aphid showed similar trends. The far fewer *Micromus* sp. and syrphid numbers per leaf remained more or less same during the observation period. Further 30 days later, in trial-3, *D. aphidivora* colonization of leaves and mean number dropped down to 9% and 0.21 per leaf, and aphid colonization of leaves and mean rating decreased to 11% and 0.30 per leaf, respectively; the rest of the leaves harbored only old silken walkways (Fig. 5). In trial-3 of *M. igorotus* augmentation (Table 4), carried out during the same period (December 2005 - January 2006) with no establishment of the predator and which served as some sort of control for the present two *D. aphidivora* trials (2 and 3), mean aphid rating and *D. aphidivora* number per leaf remained constant over a 30 day period.

In the on-campus trial-4 conducted during January-June 2006 in a September planted seed plot, the aphid appeared in January and persisted through summer in a moderate form contradicting the earlier perception that the aphid displays reduced activity in summer. When *D. aphidivora* was released at

Table 6. Evaluation of the predator *Dipha aphidivora* against woolly aphid *Ceratovacuna lanigera* in experimental sugarcane plots at Coimbatore, Tamil Nadu, India

Observation	Aphid rating per leaf	*Dipha aphidivora* (No. per leaf)	Percent of colonized leaves		*Micromus* sp. (No. per leaf)	Syrphids (No. per leaf)
			Aphid	*Dipha aphidivora*		
Trial-2: November 2005 - January 2006						
Pre-release	3.29	0.34 (0-5)[$]	98.3	17.0	0.05	0.07
Post-release[a] (40 days)	2.46	6.68 (0-22)	51.7	92.0	0.10	0.24
Level of change	-25.2%	+19.6x	-	-	-	-
Z-value[#]	2.46[*]	3.920[***]	-	-	-	-
Trial-3: November - December 2005						
Pre-release	3.27	0.62 (0-4)[$]	100.0	38.8	0.16	0.37
Post-release[b] (30 days)	1.83	1.87 (0-7)	50.0	79.0	0.06	0.10
Level of change	-44.0%	+3.0x	-	-	-	-
Z-value	3.82[***]	3.05[**]	-	-	-	-

[$] Range of cocoon numbers on sampled leaves
[a] Field dosage of ≈1000 cocoons/ha
[b] Field dosage of ≈5000 cocoons/ha
[#] Wilcoxon matched pairs test; [*]$P<0.05$; [**]$P<0.01$; [***]$P<0.001$; [ns]$P>0.05$

Fig. 5. Silken walkways and cocoons of *Dipha aphidivora* on sugarcane leaves cleared of woolly aphid *Ceratovacuna lanigera* following predator augmentation

1500 cocoons/ha twice at 6 and 8-9 months age in a staggered manner, percentage of leaves harboring *D. aphidivora* and its' numbers per leaf (Friedman ANOVA: χ^2 = 66.50; df=4; N=20; *P*=0.0001; coefficient of concordance = 0.831), and percentage of leaves colonized by the aphid and its' rating (Friedman ANOVA: χ^2 = 65.27; df=4; N=20; *P*=0.0001; coefficient of concordance = 0.816) decreased gradually and significantly from 45 days to 135 days after first release (Table 7) until both disappeared. In the control plot, *D. aphidivora* numbers decreased and mean aphid rating increased significantly over a corresponding 45 day period. While the percentage of colonized leaves decreased substantially for the predator, it remained constant for the aphid during the same period. Maturity of

Table 7. Evaluation of the predator *Dipha aphidivora* against woolly aphid *Ceratovacuna lanigera* in a sugarcane seed multiplication plot at Coimbatore, Tamil Nadu, India (January - June 2006) (trial-4)

Observation	*Dipha* release plot				Control plot			
	Aphid rating per leaf	*Dipha aphidivora* (No. per leaf)	Percent of colonized leaves		Aphid rating per leaf	*Dipha aphidivora* (No. per leaf)	Percent of colonized leaves	
			Aphid	*Dipha aphidivora*			Aphid	*Dipha aphidivora*
Pre-release	2.37 a[1]	2.87 a (0-11)[@]	100.0	60.0	1.47	1.61 (0-8)	100.0	57.9
Post-release[#] (45 days)	1.65 b	0.91 b (0-5)	95.0	39.0	2.36	0.60 (0-7)	100.0	17.0
Post-release (75 days)	1.44 b	0.27 c (0-4)	86.7	17.5	0.00[$]	0.00[$]	0.0	0.0
Post-release (105 days)	0.27 c	0.08 c (0-3)	22.0	33.0	-	-	-	-
Post-release (135 days)	0.31 c	0.03 c (0-1)	22.5	1.3	-	-	-	-
Z-value[2]	-	-	-	-	3.82***	2.57*		

[#] Field dosage of ≈1500 cocoons / ha twice at 6 and 8-9 months age

[1] Means followed by the same letter in a column are not significantly different by Friedman ANOVA by ranks and Wilcoxon matched pairs rank test significant with a Bonferroni correction ($P<0.005$)

[2] Wilcoxon matched pairs test; $^{*}P<0.05$; $^{***}P<0.001$

[@] Range of cocoons on sampled leaves

[$] Aphid colonies disappeared from the plants apparently due to crop maturity the crop and the consequent disappearance of the aphid and predator populations precluded further observations.

In the on-campus trial-5 (November 2006 - January 2007) with a single release of the predator at a field dosage of 1250 cocoons per ha, *D. aphidivora* population increased significantly 20 days later but decreased significantly 45 days after predator release (Friedman ANOVA: $\chi^2 = 3.30$; df=2; N=12; *P*=0.192; coefficient of concordance=0.138) (Table 8). On the other hand, mean aphid rating remained unchanged initially but decreased significantly during the corresponding time intervals (Friedman ANOVA: $\chi^2 = 19.48$; df=2; N=12; *P*=0.0001; coefficient of concordance=0.812). In the on-campus trial-6 with a field dosage of 2500 cocoons/ha, *D. aphidivora* numbers did not change in the first 20 days but decreased significantly in the next 25 days (Friedman ANOVA: $\chi^2 = 16.17$; df=2; N=12; *P*=0.0003; coefficient of concordance=0.674). Aphid rating decreased significantly in the first 20 days and the pest disappeared in the next 25 days (Friedman ANOVA: $\chi^2 = 23.13$; df=2; N=12; *P*=0.0001; coefficient of concordance = 0.964). In the control plot, while *D. aphidivora* numbers

Table 8. Evaluation of the predator *Dipha aphidivora* against woolly aphid *Ceratovacuna lanigera* in experimental sugarcane plots at Coimbatore, Tamil Nadu, India (November 2006 - January 2007)

Observation	Aphid rating per leaf	Predator number per leaf		
		Dipha aphidivora	*Micromus* sp.	Syrphids
Trial-5				
Pre-release	2.32 a[#]	0.78 a	0.10	0.05
Post-release[@1] (20 days)	2.07 a	1.85 b	0.00	0.18
Post-release (45 days)	0.08 b	0.87 a	0.02	0.00
Trial-6				
Pre-release	2.12 a	2.46 a	0.02	0.00
Post-release[@2] (20 days)	0.33 b	3.25 a	0.02	0.00
Post-release (45 days)	0.00 c	0.32 b	0.00	0.00
Control				
First	2.57 a	1.03 a	0.00	0.00
Second (20 days)	1.53 b	1.97 b	0.00	0.15
Third (45 days)	0.00 c	1.15 ab	0.00	0.00

[@] Field dosage of ≈[1]1250 and [2]2500 cocoons/ha

[#] Means followed by the same letter in a column are not significantly different by Friedman ANOVA by ranks and Wilcoxon matched pairs rank test significant with a Bonferroni correction (*P*<0.017) increased and decreased significantly (Friedman ANOVA: χ^2 = 3.45; df=2; N=12; *P*=0.178; coefficient of concordance=0.144), aphid rating decreased progressively and significantly in the corresponding observation intervals (Friedman ANOVA: χ^2 = 23.53; df=2; N=12; *P*=0.0001; coefficient of concordance = 0.981).

Discussion

Population dynamics

General activity period of April-November observed in native north-eastern state of Assam (Phukan et al. 1988; Gupta and Goswami 1995; Sarma et al. 2007), and the recently invaded tropical Karnataka State (Deshmukh et al. 2007; Sharanabasappa et al. 2009) and the present study site in Tamil Nadu indicated that the aphid displayed more or less uniform seasonal pattern under diverse conditions in the country. However, observations made in a single crop planted in the main season in most of these studies would have reflected the influence of crop phenology to a greater extent than weather factors. In contrast, observations recorded in plots of uniform crop age every month in the present study, facilitated by the continuous availability of sugarcane planted by growers in a staggered manner for jaggery and fresh juice, eliminated the effect of crop growth factor. The present observations also indicated that 4 to 5-month old crop was optimum for first attack, apparently due to the availability of adequate foliage, and younger crop was attacked only when crop of optimum age was either under severe attack or not available in the habitat. Prevalence in almost all months and the lack of a pattern in the relationship with weather parameters indicated that the aphid could thrive round the year in the sugarcane agro-ecosystem of tropical India characterized by mild climate, and spatial and temporal continuity of crop of preferred age. The dissimilar nature and differential levels of relationship with weather factors observed in Assam (Phukan et al. 1988) could partly be due to the response of the aphid and predators to the specific climatic conditions of the study site, besides planting season and sampling methodology followed as pointed out above. Nevertheless, the greater activity of the aphid during October-December in the first two years of the present study could possibly be due to the following reasons: (i) crop planted during January-March provided optimum food resource for aphid colonization during October-December, despite the year round planting; (ii) the post-monsoon period provided ideal conditions for aphid multiplication which was supported by the positive correlations of rainfall and RH with aphid rating, as was also observed elsewhere (Tripathi et al. 2008); (iii) the lack of equilibrium between the stabilizing populations of aphid and predators which, incidentally, did not share a strong or significant relationship during the study period.

Among the predators, *D. aphidivora* displayed higher densities during October-December marked by low temperatures which apparently encouraged its rapid multiplication as indicated by its negative relationship with temperature. The predator displayed similar higher densities and negative relationship with temperature in Karnataka (Ravi et al. 2007). In the north-eastern Nagaland, the predator was active during July-November and it was suggested that after suppressing woolly aphid the predator may switch to pests of other crops (Tripathi 1995). However, in the present study site where sugarcane and woolly aphid were available throughout the year, the question of host switching for the predominant *D. aphidivora* apparently did not arise. Lack of a relationship between populations of the aphid and *D. aphidivora* in the present study, such as the one observed in Uttar Pradesh (Tripathi et al. 2008), and the decrease in aphid and predator populations from 2005-06 to 2007-08 suggested gradual attainment of equilibrium between them. The drastic decline in aphid numbers in 2007-08 was probably accelerated by the establishment, spread and early appearance (June 2007) of the parasitoid *Encarsia flavoscutellum* Zehntner (Hymenoptera: Aphelinidae), introduced from Assam and released during the previous season (Anonymous 2005b). The much reduced activity of *D. aphidivora* after April 2007 was apparently due to the dominance of the parasitoid which did not allow the aphid to multiply to levels sufficient to sustain the predator. In the years after its introduction and establishment, the parasitoid closely followed the aphid, competitively excluded *D. aphidivora*, stabilized aphid populations and prevented cane yield and quality losses (Srikanth et al. 2012 and 2013; J. Srikanth et al. unpubl. data).

Brown lacewings were associated with the aphid in smaller numbers than *D. aphidivora* in both seasonal dynamics and augmentative studies (discussed below) clearly establishing their secondary role. Despite the maximum number per leaf (16) in June 2005 being higher than that of *D. aphidivora*, apparently due to gregarious nature early in its life cycle and subsequent dispersal to alternative hosts in the habitat, *Micromus* sp. was not observed over several months. However, in some sugarcane habitats, the brown lacewing *M. igorotus* emerged as a major predator (Lingappa et al. 2004) with greater activity during June-December (Deshmukh et al. 2007; Sharanabasappa et al. 2009; Vidya et al. 2011). Syrphids remained irregular and far less abundant and hence were ignored in the seasonal dynamics studies. In contrast, *Eupeodes confrater* (Diptera: Syrphidae) and a few other predators, with significant positive influence on the aphid, seemed to have a role in its regulation in the north-eastern Assam State (Sarma et al. 2007).

Woolly aphid may have entered the southern tropical parts of the country from its native north-eastern states through the transport of infested leaves along with commercial seed or germplasm material. The occurrence of *D. aphidivora*, the major predator of the aphid in Nagaland (Tripathi 1995), ever since the aphid's first appearance in Maharashtra (Rabindra et al. 2002) indicated that the predator may have been introduced along with the aphid as larvae concealed in the silken walkways constructed by them on either side of the midrib or the sessile cocoons adhering to the leaf surface. It has established as the dominant predator on woolly aphid in several places (Tripathi et al. 2008), including the present study site, apparently due to its' oligophagous nature (Arakaki and Yoshiyashu 1988). With a sluggish larval stage, syrphids such as *E. confrater* and other hover flies native to the aphid's place of origin (Tripathi 1995) may have similarly entered the new areas, besides opportunistic local host switching by native populations of the polyphagous *E. confrater* or others. The same cannot be concluded about the agile *Micromus timidus* Hagen (Patil 2003), *M. igorotus* (Lingappa et al. 2004) and other unidentified brown lacewings which were not originally reported in the aphid's home (Tripathi 1995). It is possible that these polyphagous lacewings native to the invaded areas switched to the potential woolly aphid opportunistically. Although *Chrysopa* sp. (Neuroptera: Chrysopidae) was also recorded as another common predator of the aphid in Nagaland (Tripathi 1995), the differential dominance of various predators observed in different invaded areas was perhaps governed by the relative climatic suitability, and heterogeneity of host plants and hosts.

Field evaluation of *M. igorotus*

In the augmentative trials (2004-06) with *M. igorotus*, deployment of increased absolute dosages through trial-1 to 3, corresponding with the invasive to established phases of the aphid, ensured high actual dosages commensurate with proliferation of the aphid. The simultaneous appearance of all three groups of predators in the post-release observation of trial-1 indicated the short density-dependent time-lag in their buildup. On the other hand, the relative abundance of *D. aphidivora* indicated its dominance over the augmentatively released *M. igorotus* and naturally occurring syrphids, and a greater role in the significant reduction of aphid populations. Despite the lowest dosage of the predator used, the occurrence of *Micromus* sp. at considerable level in trial-1 that showed no initial predator activity suggested its ability to survive in the absence of competition, particularly from *D. aphidivora*. This is supported by the competitive exclusion of *Micromus* sp., notwithstanding the higher dosages adopted, in trials-2 and 3 which showed initial occurrence of *D. aphidivora*. The significant increase in aphid intensity in response to significant decrease in *D. aphidivora* colonization rate over the 30 day post-release period in trial-2 could be independent of the high release rates of *M. igorotus*. The non-significant changes in aphid and *D. aphidivora* in trial-3 over the observation period indicated the stable nature of the populations on the verge of decline in the late stage of the crop.

The poor survival of *M. igorotus* released in 6 and 7-month old crops in trials-1 and 2, respectively, and a 9-month old crop in trial-3 indicated that age of the crop apparently did not influence the establishment of the predator. More cocoons than adults were released in trial-1 but adults alone were released in the next two trials to ensure two aspects, namely high adult emergence and prevention of field predation of cocoons both of which, however, could not be ascertained in prior tests due to insufficient predator culture. Identical results in all three trials indicated that the stage of predator released had no role in its establishment. Further trials with this predator were discontinued as it failed to thrive through coexistence or competitive exclusion of *D. aphidivora* despite high release rates in the three trials.

In contrast to the present results, *M. igorotus* was more effective than *D. aphidivora* in field trials in Karnataka (Sharanabasappa et al. 2009; Vidya et al. 2011). At 2600 larvae/ha and as a part of a module with other control methods, including 1000 larvae/ha of *D. aphidivora*, *M. igorotus* reduced populations of the aphid 30 and 60 days after release (Mallapur et al. 2006). Further, enhanced release rates (500-1500 cocoons/ha) reduced the time taken to control the aphid from 90 to 30 days (Vidya et al. 2012). In Maharashtra too, *M. igorotus* at 2500 larvae/ha effectively reduced aphid populations and enhanced cane yield (Patil et al. 2007). Overall, these reports indicated that Karnataka and parts of Maharshtra provided the ideal climate and crop habitat for proliferation of the predator. It is possible that *M. igorotus* may have existed unidentified as a predator in other crop-aphid systems within these habitats and switched over to woolly aphid in sugarcane as it served as a new and abundant food resource. The failure of *M. igorotus* to establish even in the early phase of aphid invasion and under high release rates, and the absence of any other report from Tamil Nadu regarding its establishment or role in woolly aphid control suggested that the climatic conditions and predominantly monocrop sugarcane habitat in the state were not as favorable for the proliferation of *M. igorotus* as they were for *D. aphidivora*. Moreover, the lower abundance of unidentified *Micromus* sp. observed in seasonal dynamics studies also emphasized the poor suitability of sugarcane crop habitat for brown lacewings as a predatory group against woolly aphid.

Field evaluation of *D. aphidivora*

Under field augmentation, *D. aphidivora* enhanced its' natural population and reduced aphid intensity in 30-40 days, the period approximately equal to two generations of the predator (Mukunthan et al. 2006), when its initial populations were low as in trials-1 to 3, despite the differences in initial aphid intensity and predator release rates. Also, predator releases and observations recorded at 8 months age of the crop during November-January coincided with high activity of both the aphid and the predator as was observed in seasonal dynamics studies. Thus, the synchrony of the favorable post-monsoon period with suitable age of the crop seemed to further enhance the effectiveness of the released predator. However, gradual decline of both aphid and predator populations at high field dosages dispensed twice in a staggered manner in a late planted younger seed material crop (trial-4) suggested a few interesting points: (i) the high initial population of the predator apparently disallowed its' further enhancement despite augmentation; (ii) summer months (January-June) were less favorable for proliferation of both aphid and predator populations as indicated by seasonal dynamics observations, despite the general observation that 4 to 5-month old crop was suitable for aphid colony initiation and multiplication. In the last two trials (5 and 6), conducted once again during the favorable November-January period and in the suitable 8-month old crop, predator numbers increased non-significantly in trial-6 despite the higher dosage than in trial-5, probably due to the higher initial predator number. However, in both trials, aphid populations decreased in a predator dosage dependent manner over the 20-45 days observation period. This season witnessed the first signs of establishment of the introduced parasitoid *E. flavoscutellum* which could have been partly responsible for the unusual rapid decline of aphid intensity in both release and control plots of these two trials.

Despite the limitation of lack of control plots for some trials in the present study, *D. aphidivora* releases at single field dosages of 1000 - 1500 cocoons/ha enhanced its' numbers and reduced aphid intensity within 15-45 days but the extent of aphid control seemed to depend on the season and initial density of the predator. Similar positive results of aphid reduction with comparable release rates (Patil et al. 2007; Pokharkar and Ghorpade 2009) endorsed the range of release rates used in the present study. Also, reduction of aphid densities in 30-60 days when the predator was used in modules with *M. igorotus* and cultural practices (Mallapur et al. 2006) not only indicated the minimum duration required for the predator to overtake the host populations but also the need to deploy the predator in conjunction with cultural methods for higher level of control. Staggered releases of *D. aphidivora* at more than 5,000 cocoons/ha in our earlier study enhanced its' numbers and decimated aphid populations but only over a 4-month period and late in the season (Srikanth et al. 2009b). This was probably due to the delayed commencement of first releases, which was linked to the availability of abundant field populations of the aphid required for predator multiplication in the laboratory (Mukunthan et al. 2006). Such constraint emphasized the need to maintain continuous populations of woolly aphid for predator multiplication since frozen aphid material supported only late larval stages (Srikanth et al. 2009a) and semi-synthetic diet produced lower survival and fecundity (Venkatesan et al. 2008). The avoidance of entomopathogenic fungi due to their pathogenicity (Nirmala et al. 2007) and adoption of spot application of insecticides (Mukunthan et al. 2005) due to their toxicity (Mukunthan et al. 2008) to *D. aphidivora* perhaps played some role in the successful augmentative control of the aphid with this predator during the epidemic invasion of the aphid in tropical India. *Dipha aphidivora* augmentation in all these studies generally constituted supplementary or inundative approach since in most cases the predator was naturally present in the field, albeit at low levels. In this context, the usage of the term 'inoculative releases' by some authors (Pokharkar and Ghorpade 2009) is perhaps inappropriate, especially in habitats such as the present study site where both the aphid and predator were active throughout the year. However, the introduction and establishment of the parasitoid *E. flavoscutellum*, and the consequent natural control of the aphid in the study site towards the end of the present study period (J. Srikanth et al. unpubl. data) altered the scenario so much so that in the subsequent years the predator seldom built up to the levels observed during the present study period. Nevertheless, the stronger association of *D. aphidivora* than *E. flavoscutellum* with the aphid observed in Assam (Sarma et al. 2007), and the reduction of aphid populations in the present trials and other studies in tropical India (Mallapur et al. 2006; Patil et al. 2007; Pokharkar and Ghorpade 2009; Srikanth et al. 2009b) vindicated the role of *D. aphidivora* in woolly aphid regulation in the augmentative mode.

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