RESEARCH ARTICLE

Status of woolly aphid *Ceratovacuna lanigera* and establishment of the parasitoid *Encarsia flavoscutellum* in sugarcane germplasm

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Abstract

Woolly aphid *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae), a native of north-eastern India, appeared in the world sugarcane germplasm maintained at the ICAR-Sugarcane Breeding Institute Research Center (ICAR-SBIRC), Kannur, Kerala State, India, first in 2004. The aphid colonized Indian hybrids and accessions of *Saccharum officinarum, Saccharum sinense, Saccharum robustum* and *Saccharum barberi* in the first year of occurrence and every year thereafter. Early detection and spot application of insecticide were adopted to manage the aphid and protect germplasm. Soap solution was applied in the later years to conserve the predators *Dipha aphidivora* Metrics (Lepidoptera:Pyralidae) and *Micromus* sp. (Neuroptera: Hemerobiidae) that occurred intermittently. The parasitoid *Encarsia flavoscutellum* Zehntner (Hymenoptera: Aphelenidae) maintained at Coimbatore, Tamil Nadu State, India, was released in the germplasm thrice, i.e. in January 2009, November 2014 and June 2015. Post-release, *E. flavoscutellum* parasitism was detected first during April-June 2015 at low levels (2.0-3.5%). Clear-cut evidence of establishment was witnessed during September-October 2021 when aphids showed a high 27.0% parasitism. Since *E. flavoscutellum* was found to regulate populations of woolly aphid wherever it established, it is expected to reach similar equilibrium with the aphid in the germplasm, *D. aphidivora* and *Micromus* sp. playing a complementary role at high aphid densities. However, continuous monitoring of aphid and natural enemies, and use of safer emergency control measures would go a long way in maintaining the germplasm free from woolly aphid.

Keywords: Sugarcane; Germplasm; Woolly aphid; Ceratovacuna lanigera; Status; Parasitoid; Encarsia flavoscutellum; Colonization; Establishment

Introduction

The woolly aphid *Ceratovacuna lanigera* Zehntner (Hemiptera: Aphididae), primarily a pest of sugarcane in north-eastern India, entered peninsular India, beginning with Maharashtra and Karnataka States in 2002 and spreading to Andhra Pradesh (2003) and Tamil Nadu (2004) States (Srikanth 2007). It remained a serious pest of sugarcane for about five years until biological control through augmentative deployment of predators (Srikanth et al. 2015) and introduction and colonization of the parasitoid *Encarsia flavoscutellum* Zehntner (Hymenoptera: Aphelenidae) (Srikanth et al. 2012) brought stability to the aphid populations. The world collection of sugarcane germplasm being maintained at the Research Center of the ICAR-Sugarcane Breeding Institute (ICAR-SBIRC), Kannur, Kerala State, India, constitutes a crop island since the location is devoid of sugarcane cultivation and, hence, affords natural protection against immigrant pests and diseases. Yet, the germplasm is subject to periodical colonization by resident and migrant pests (Mahesh et al. 2020).

Coinciding with its occurrence in different parts of Tamil Nadu (Srikanth 2007), woolly aphid made its first appearance in sugarcane germplasm in 2004 (Anonymous 2005a) and continued to occur in the subsequent years. Insecticide or soap solution was used in all the years as soon as the attack was detected in accordance with the zero-tolerance norm of pest or disease occurrence in the germplasm. In this paper, we chronicle the occurrence of the aphid in the germplasm over the years since its first occurrence in 2004, record the establishment of the parasitoid *E. flavoscutellum* following its inoculative releases and discuss the prospects of natural regulation of the aphid in the unique crop island system.

Materials and Methods

As soon as woolly aphid was detected in the germplasm collection at ICAR-SBIRC, Kannur, in April 2004 in routine pest monitoring activity (Mahesh et al. 2020), details of accessions infested and natural enemies observed were recorded. The first occurrence of the aphid and its status monitored in subsequent years were documented in Annual Reports of ICAR-SBI either by the Entomologist at the Center or those from the main Institute visiting the Center. Chronological progression of woolly aphid over the past decade-and-half was compiled and enumerated in this study.

Extensive biological control research efforts with the predator *Dipha aphidivora* Meyrick (Lepidoptera: Pyralidae), necessitated by proliferation of the aphid in sugarcane tracts of tropical India (Srikanth 2007), at ICAR-Sugarcane Breeding Institute (ICAR-SBI), Coimbatore, Tamil Nadu State, India, produced promising results but with delayed effect of predatory activity (Srikanth et al. 2009 & 2015). Subsequent introduction and colonization of *E. flavoscutellum* from Assam (Anonymous 2005b) led to its establishment and natural regulation of the aphid (Srikanth et al. 2013), and prevention of yield loss in sugarcane (Srikanth et al. 2012).

Following successful in situ maintenance of *E. fla-voscutellum* in shade-net house at ICAR-SBI (Srikanth et al. 2007), consignments of the parasitoid in the form of parasitized aphid colonies on leaf

bits held in aerated plastic boxes were transported to Kannur and dispensed in the field by inserting the leaf bits in leaf axils within 24 h of excision. Parasitoid adults that emerged during transit were released by opening and tapping the boxes in the canopy of colonized plants. To assess the establishment of the parasitoid, mature aphids collected from the field were dewaxed with ethyl alcohol and digested in phenol in the laboratory as per the procedure standardized earlier (J. Srikanth et al. unpubl. data). The cleared specimens were examined under the microscope to locate adult stages of the parasitoid inside them. Parasitism levels were assessed from a variable number (200-300) of grown-up stages known to host the parasitoid (Hazelhoff 1929), ignoring the young ones. As it is difficult to locate immature stages of the parasitoid inside even the cleared aphid specimens, percent parasitism was based only on individuals with visible adults.

Results and Discussion

Woolly aphid status

The arrival of the aphid in the germplasm during April-June 2004 was marked by colonization (Fig. 1a) of Indian hybrids and accessions of four Saccharum spp., namely S. officinarum, S. sinense, S. robustum and S. barberi. By March 2005, the aphid spread to 14 foreign hybrids and 83 Indian hybrids (Table 1). To protect germplasm from the explosive aphid, systemic insecticide was applied as spot treatment as and when colonies appeared or showed signs of expansion. The aphid continued to occur year after year (2005-20) at varying abundance levels attacking the germplasm randomly (Table 2) but predominantly Indian hybrids and accessions of four Saccharum spp. However, accessions of Saccharum spontaneum and Erianthus spp. were not found to be attacked by the aphid in any of the years which suggested that these could be good sources of resistance. In the early years,

Month/ year of attack	Germplasm attacked	Accessions infested	Natural enemy status	Remarks
April-June 2004	i. Indian hybrids	Co 716, Co 717, Co 718, Co 818, Co 819, Co 820, Co 822, Co 823 and Co 825	Micromus sp. observed	First occurrence during April- June 2004; managed with spot application of systemic insecticide
	ii. Saccharum officinarum	Warni Bola, Rasdali, Rayada, Old Jamaica, Lakhapur, Rat gros ven- tre, 57 NG 174, 57 NG 175, IM 76-235, IK 76-112 and NG 77-44		
	iii. Saccharum sinense	Uba Seedling, Uba White and Cavengerie		
	iv. Saccharum robustum	Duruca vicoria, MOL 4861 and MOL 5698		
	v. Saccharum barberi	Mungo 254 and Nargori		
March 2005	i. Foreign hybrids	14 nos.		
	ii. Indian hybrids	83 nos.		

 Table 1. First occurrence of woolly aphid Ceratovacuna lanigera in germplasm at ICAR-SBIRC, Kannur (2004-05)

regular monitoring, early detection and spot application of insecticide were adopted as this approach was established to be useful in managing the aphid effectively (Srikanth et al. 2009). However, when *D. aphidivora* started appearing consistently and *E. flavoscutellum* began establishing during 2018-21, soap solution was applied in view of its effectiveness against low intensity aphid attack and relative safety to *D. aphidivora* compared to commonly used insecticide (Mukunthan et al. 2008). Low levels of occurrence and early control methods adopted to protect germplasm in all the study years precluded detailed assessment of aphid intensity.

Natural enemy status

Despite rigid quarantine procedures and highly regulated movement of genetic material in and out of ICAR-SBIRC, pests enter germplasm from alternative crops (Mahesh et al. 2019a) or weeds in the habitat and through long-range natSource: Anonymous (2005a) & authors' observations

ural dispersal. Woolly aphid may have reached the germplasm through dispersal of alates from distant sugarcane belts. The predator *Micromus* sp. (Neuroptera: Hemerobiidae) was observed in 2004 along with the first occurrence of the aphid (Table 1); webbings of the predator *D. aphidivora* were observed in the colonies in July 2005, the second year of occurrence of the aphid; syrphid larvae were active in the colonies in 2021 alone (Table 2).

In earlier studies, *D. aphidivora* was found to be more predominant than *Micromus* sp. or syrphids in Tamil Nadu whereas *Micromus igorotus* Banks was the dominant predator in Karnataka (Srikanth et al. 2015). In the present habitat, the general predator *Micromus* sp. may have dispersed from alternative host-host plant systems within the habitat whereas the woolly aphid-specific *D. aphidivora* may have originated from sugarcane and reached the germplasm. Early detection and destruction of aphid colonies by clipping of infested leaves and application of soap solution or insecticide may have precluded buildup of the predators and led to their intermittent detection in the subsequent years.

Establishment of E. flavoscutellum

The delayed appearance of the aphid in 2009-10 season, following the first release of *E. flavoscutellum* in the previous year (2008-09) (Table 2), was assumed to be due to the parasitoid released in the previous year (Anonymous 2010) though no visible signs of the parasitoid activity were recorded. Aphid sample processed and examined in November 2014, when the second consignment of the parasitoid was released, did not indicate parasitoid activity (Table 3), a probable offshoot of insecticide application. However, initial signs of establishment were indicated by 3.5% parasitism in April 2015 and 2.0% parasitism in June 2015. Despite the absence of parasitism in a sample processed in January 2019, clear-cut evidence of *E. flavoscutellum* establishment was witnessed in

Table 2. Chronological progression of woolly aphid Ceratovacuna lanigera in germplasm at ICAR-
SBIRC, Kannur, since first occurrence

Year	Aphid status	Germplasm infested	Natural enemy status	Remarks	Source
2005-06	Present	Co 783, Co 784, Co 785, Co 851, Co 853 and Co 854	Dipha aphidivora webbings observed in July 2005	Aphid occurred in August 2005; systemic insecticide applied to contain spread	Anonymous (2006)
2006-07	Present	-	-	Low incidence	Anonymous (2007)
2007-08	Present	Co 62055, Co62057, Co 62058, Co 62059, Co 62063; a few <i>Saccharum robus-</i> <i>tum</i> accessions	-	-	Anonymous (2008)
2008-09	Present	Indian and foreign hybrids; accessions Creoula and Creoula Rayada of- <i>Saccharum</i> officinarum; a few accessions of <i>Saccharum robustum</i>	First consignment of <i>Encarsia flavoscute-llum</i> adults and parasitized aphids from Coimbatore released in January 2009	Spread of the aphid was contained by insecticide	Anonymous (2009)
2009-10	Present	Co 1116, Co 1117 and Co 62175	-	Late occurrence assumed to be due to <i>Encarsia</i> released during 2008-09; managed with insec- ticide	Anonymous (2010)
2010-11	Present	Co 62079, Co 62080, Co 62081 and Co 62082	-	Insecticide applied to prevent spread	Anonymous (2011)
2011-12	Present	Indian hybrids during September-December	<i>Micromus</i> sp. observed up to December	Effectively managed using insecticide	Anonymous (2012)

Cont'd...

Year	Aphid status	Germplasm infested	Natural enemy status	Remarks	Source
2012-13	Present	-		Low incidence	Authors' obser- vations
2013-14	Present	Few accessions attacked	-	Controlled with insecticide	Anonymous (2014)
2014-15	Present	Co 416, Co 629, Co 896, Co 62032, Co 62213, Co 62214, CoK 28 and Maneria IMP-1648; a few accessions of <i>Saccharum robustum</i> and seedling plot in September; aphids surviving in a small patch of seedling plot in November	Second consign- ment of <i>Encarsia</i> <i>flavoscutellum</i> adults and para- sitized aphids from Coimbatore released in seedling plot harboring aphid in November 2014; third consignment released in June 2015	Aphid sample collected and pro- cessed in Novem- ber 2014 did not show parasitoid, possibly due to insecticide appli- cation	Anonymous (2015)
2015-16	Present	-	-	Low incidence	Anonymous (2016)
2016-17	Present	-	-	Low incidence	Anonymous (2017)
2017-18	Present	-	-	Low incidence	Anonymous (2018)
2018-19	Present	Accession NG 77-84 of red fleshed <i>Saccharum</i> <i>robustum</i>	Dipha aphidivora and <i>Micromus</i> <i>igorotus</i> observed	Incidence in isolated pockets; controlled using soap solution and insecticide	Anonymous (2019)
2020	Present	Accessions 28 NG 34, 28 NG 35, 28 NG 36 and 28 NG 37 of <i>Saccha-</i> <i>rum officinarum</i> in December	-	Clipping and de- struction of infest- ed leaves, followed by application of soap solution	Anonymous (2020)
2021	Present	Indian hybrids SEL 76/59, Co 201, Co 96011, Co 96017, Co 96018, Co 98007, Co 99006 and IC 233 in September 2021	<i>Encarsia</i> adults located on colonized leaves, parasitoid emerged from aphids in captiv- ity and processed aphids showed 24.0% parasitism during Septem- ber-October; syrphid predator was active	Soap solution used in border rows to prevent spread; small patches of infestation being monitored for spread	Authors' observations

Table 2. Chronological progression of woolly aphid *Ceratovacuna lanigera in* germplasm at ICAR-SBIRC, Kannur, since first occurrence

Date of release	Date of assessment	% parasitism@	Remarks
January 2009	-	-	-
November 2014	November 2014	0.0	General sample
June 2015	23 April 2015	3.5	Indian hybrids
	19 June 2015	2.0	Indian hybrids
	06 January 2019	0.0	Indian hybrids
	28 September 2021	24.0	Indian hybrids
	08 October 2021	6.0	Co 201
		27.0	IC 233
		20.0	Co 96017

Table 3. Pattern of Encarsia flavoscutellum parasitism in woolly aphid Ceratovacuna lanigera follow-
ing inoculative releases at ICAR-SBIRC, Kannur

@Phenol processed sample; n=200-300 mature or grown-up aphids

September 2021: aphids exhibited pin-hole emergence passage ways of adult parasitoids on wax coating (Fig. 1b & c); processed aphids showed developing adults inside (Fig. 1d) with 24.0% parasitism (Table 3); adults emerging from parasitized aphids congregated on colonized leaves (Fig. 1e & f). Another sample collected from three spots of germplasm 10 days later, i.e. in October 2021 indicated high parasitism levels (20.0-27.0%) in two accessions, namely Co 96017 and IC 233. However, parasitism was low (6.0%) in the third accession (Co 201) planted in the border, probably due to the soap solution applied to prevent inward spread of the aphid.

When *E. flavoscutellum* was introduced from Assam and colonized in tropical Indian States (Anonymous 2005b), the parasitoid established in about a year after first releases (Srikanth et al. 2007). Continuous availability of abundant host in the semi-perennial sugarcane habitat (Srikanth 2019) and favorable tropical climatic conditions may have led to such rapid establishment of the parasitoid. However, at ICAR-SBIRC, Kannur, the parasitoid required longer time for establishment, despite similar tropical conditions, probably due to the low aphid abundance in the diverse germplasm and prompt control measures adopted to contain the aphid.

At ICAR-SBIRC, previous season crop of the entire germplasm is retained until June to ensure emergency seed material for the current season crop planted in January-February in the event of germination failure (Mahesh et al. 2019b). The first consignment of E. flavoscutellum released in January 2009 at crop maturity stage and the second consignment released in seedling plot harboring aphid in November 2014 might have led to initial establishment of the parasitoid on the waning aphid populations, probably aided by its functional and numerical responses. Although the third consignment released in June 2015 coincided with the harvest of previous season crop, the current season crop might have provided adequate aphid populations and ensured continuity of the parasitoid. Thus, the parasitoid may have perpetuated within the germplasm on the spatio-temporally continuous aphid populations made available

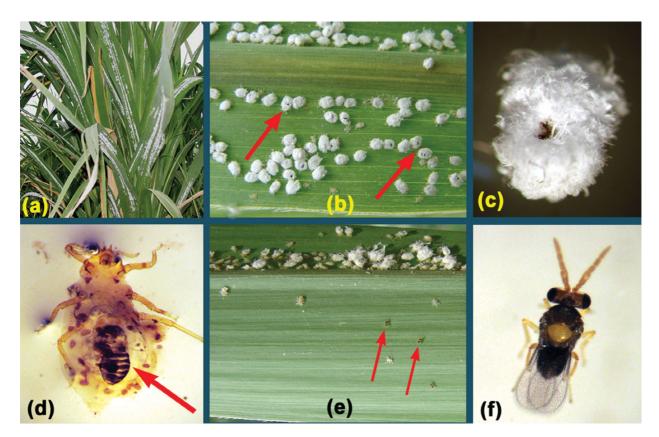


Figure 1. Woolly aphid *Ceratovacuna lanigera* and parasitoid *Encarsia flavoscutellum* in sugarcane germplasm at ICAR-SBIRC, Kannur: (a) aphid colonies in sugarcane (b) parasitized aphids showing pin-hole emergence passage ways of parasitoid in wax coating (red arrows) (c) enlarged view of a single aphid with parasitoid emergence passage (d) processed aphid showing developing parasitoid adult inside (red arrow) (e) parasitoids on aphid infested leaf (red arrows) (f) enlarged view of parasitoid adult

by the planting pattern of germplasm. The aphid is known to survive on other host plants (Joshi and Viraktamath 2004) but *E. flavoscutellum* does not seem to have many alternative hosts (Evans 1995). Thus, it is also possible that the parasitoid may have survived on woolly aphid colonizing alternative hosts in the habitat. The present observations demonstrated the successful establishment of *E. flavoscutellum* at ICAR-SBIRC, Kannur, following its releases, notwithstanding the possibility of its natural immigration from distant aphid infested sugarcane plantations.

Once established, *E. flavoscutellum* is known to follow and regulate populations of woolly aphid

in the habitat (Srikanth et al. 2012). It is likely to regulate the aphid in the germplasm in a similar manner, which, however, needs to be monitored for a couple of seasons. Besides, the previously colonized *D. aphidivora* and *Micromus* sp. may complement the action of *E. flavoscutellum*. Although the combined action of predators and parasitoid may preclude the need for insecticidal control, soap solution may be used as an emergency measure retaining some unsprayed spots as refugia for their survival. It would be useful to assess the toxicity of soap solution or other plant products to the parasitoid for ensuring its survival in the germplasm.

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