REVIEW ARTICLE

GLIMPSES OF RESEARCH ON BIOCONTROL OF SUGARCANE PESTS IN INDIA: RETROSPECT AND PROSPECTS

J. Srikanth*

Abstract

Among the biotic stresses that afflict sugarcane, insect pests occupy a good second position behind diseases in India. Yet, they constitute a major limiting factor attacking the crop from planting to almost harvest inflicting yield and sugar losses. The stable crop habitat and minimal pesticide usage accord a prominent role for biological control. Research on biocontrol in the early decades revolved around surveys for identification and studies on basic biology of natural enemies. The principles of conservation, re-distribution, and introduction and colonization of predominant parasitoids, followed since early times, continue to guide the course of biological control with remarkable success. Augmentative control has been assessed through development of mass multiplication techniques and standardization of field evaluation protocols for selected candidate biocontrol agents. *Trichogramma chilonis* Ishii serves as a typical example of the most exploited biocontrol agent on a commercial scale. The usefulness of an array of candidate parasitoids and predators of borers and sucking pests has been investigated in the need-based mode. The fungus *Beauveria brongniartii* (Saccardo) Petch attained commercial production against the white grub *Holotrichia serrata* F. following its successful evaluation in elaborate trials. *Bacillus thuringiensis* Berliner isolates occurring in sugarcane soil examined recently revealed a couple of scarabaeid specific holotype *cry* genes from them. These isolates hold promise against white grub when delivered in the form of formulations, besides the long-term possibility of deploying transgenics with *cry* genes. Kairomonal principles derived from borers and sugarcane as attractants to the larval parasitoid *Cotesia flavipes* (Cameron) were examined. Production of biocontrol agents and dissemination of technologies are promoted by sugar industry and/or commercial insectaries. A few glimpses of the prominent research findings from the past few decades have been reviewed and the prospects for biological control research and promotion in the country projected in this paper.

Key words: Sugarcane, biological control, research progress, sugar industry, transfer of technology, research prospects, overview

Introduction

Insect pests, despite constituting the second most important biotic stress in sugarcane after diseases, cause considerable yield and quality losses. Pest profiles in subtropical and tropical India display both distinctiveness and overlap (Table 1). While the extreme climatic conditions in subtropical India support moderate crop growth but high pest abundance, the moderate tropical climate favours good crop growth yet low pest levels. Borers, sucking pests and subterranean pests are the broad groups of insect pests that attack sugarcane in different crop stages displaying diverse distribution. Sugarcane entomology work in the country (David et al. 1986) and pest problems in subtropical India with notes on their management (Varma 1993) were described. A few newer pests were recorded subsequently (Mukunthan and Nirmala 2002), including the exotic fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) (Srikanth et al. 2018).

The unique features of sugarcane crop-pest system, such as long duration of the crop, staggered planting, multitude of pests that support natural

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J. Srikanth

ICAR-Sugarcane Breeding Institute, Coimbatore-641 007, Tamil Nadu, India.

*Corresponding author: srikanth_jsk@yahoo.co.in

Table 1. Major pests of sugarcane in India[@]

Pest	Order: Family	Scientific name	Geographical distribution [#]
I. Borers			
Early shoot borer	Lepidoptera: Crambidae	*Chilo infuscatellus* Snellen	T, ST
Internode borer	Lepidoptera: Crambidae	*Chilo sacchariphagus indicus* (Kapur)	T
Top borer	Lepidoptera: Crambidae	*Scirpophaga excerptalis* (Walker)	T, ST
Stalk borer	Lepidoptera: Crambidae	*Chilo auricilius* Dudgeon	ST
Root borer	Lepidoptera: Pyralidae	*Polyocha depressella* (Swinhoe)	T, ST
Gurdaspur borer	Lepidoptera: Crambidae	*Acigona steniellus* (Hampson)	ST
Plassey borer	Lepidoptera: Crambidae	*Chilo tumidicostalis* Hampson	ST
II. Sucking pests			
a. Foliage feeders			
Pyrilla	Hemiptera: Lophopidae	*Pyrilla perpusilla* Walker	T, ST
Woolly aphid	Hemiptera: Aphididae	*Ceratovacuna lanigera* Zehntner	T
Whiteflies	Hemiptera: Aleyrodidae	*Aleurolobus barodensis* Maskell	T, ST
		Neomaskellia bergii (Signoret)	T, ST
b. Cane colonizers			
Scale insect	Hemiptera: Diaspididae	*Melanaspis glomerata* (Green)	T, ST
Pink mealybug	Hemiptera: Pseudococcidae	*Saccharicoccus sacchari* (Cockerell)	T, ST
III. Subterranean pests			
Termite	Isoptera: Termitidae	*Odontotermes obesus* (Rambur)	T, ST
		Microtermes obesi Holmgr.	T, ST
White grub	Coleoptera: Scarabaeidae	*Holotrichia serrata* (Fabricius)	T
		Holotrichia consanguinea Blanchard	ST

[@] David et al. (1986); Varma (1993)

[#] T - tropical; ST - subtropical

enemy continuum, higher economic thresholds and low pesticide usage make the crop a semi-perennial habitat conducive for both natural and applied biological control. Active participation of governmental agencies and sugar industry make biological control an implementable reality in sugarcane (Srikanth et al. 2016). Several significant findings and achievements marked the progress of biocontrol research in the country over the past few decades. Observations on parasitoids of the leafhopper *P. perpusilla* and whiteflies *A. barodensis* and *N. bergii* (Misra 1920) are some of the earliest research attempts in biological control of sugarcane pests. The next decade witnessed the initiation of mass multiplication of the egg parasitoid *Trichogramma chilonis* Ishii

(=*evanescens minutum* Riley) on the laboratory host *Corcyra cephalonica* (Stainton) for use against shoot borer *C. infuscatellus* (Kunhikannan 1931). While the subsequent commercialisation of this egg parasitoid exemplified augmentative use of a natural enemy, several instances of successful introduction and colonization within the country were recorded in sugarcane: establishment of *Isotima javensis* Rohw. in peninsular India against top borer *S. excerptalis* (Kalra and David 1964); *Epiricania melanoleuca* Fletcher deployment against pyrilla in subtropical India (Misra and Pawar 1984); *Encarsia flavoscutellum* Zehntner from north-eastern India against woolly aphid *C. lanigera* invading tropical India (Anonymous 2005) leading to its establishment (Sharanabasappa et al. 2008) and prevention of yield and quality losses (Srikanth et al. 2012). The present review recapitulates salient past research findings and attempts to prioritise future research and development needs in biological control of sugarcane pests in India.

Retrospective research record

Early developments

Borer pests

Early studies included the identification of parasitoids and predators occurring in different parts of the country, such as the egg parasitoid *Telenomus dignoides* Nixon (Krishnamurti and Usman, 1954) and the larval parasitoid *Cotesia flavipes* (Cameron) (Gupta 1954). The entomopathogenic nematode (EPN) *Neotylenchus* sp. was reported on larvae of top borer (David 1962).

Mass multiplication attempts began in 1930 with *T. chilonis* using *C. cephalonica* as a factitious host against shoot borer (Kunhikannan 1931) and details of the parasitoid rearing were described (Subramaniam and Rao 1940). Breeding of cultures through several generations at progressively increasing temperature and decreasing humidity indicated the possibility of producing tolerant strains (Abraham 1970).

Mass releases of *Trichogramma* enhanced parasitism rates of shoot borer and internode borer eggs, and increased cane yield in Mandya, Karnataka (Subramaniam 1941). Superparasitism in mass rearing leading to no or weak progeny, poor ecological adaptability of the parasitoid, differential laboratory rearing and target field temperatures, and lack of host abundance in the field were cited as reasons for its failure in the field (Narayanan and Mukherjee 1953). Despite such debate, other studies of inundative releases with variable dosage and frequency indicated the parasitoid's usefulness against internode borer (Sithanantham et al. 1973).

The tachinid *Sturmiopsis inferens* Tns. parasitized shoot borer throughout the year at Coimbatore (David et al. 1980) but hibernated in larvae of *C. auricilius* under north Indian conditions (Chandra and Avasthy 1988). A rearing technique was developed for the parasitoid (Jai Rao and Baliga 1968) and in laboratory tests shoot borer granulosis virus (GV) displayed superiority over the parasitoid (Easwaramoorthy and Jayaraj 1989). Several exotic species of tachinids were evaluated against sugarcane borers in the laboratory and field with partial or no success (David et al. 1986).

In studies on braconids, a rearing technique was described for *C. flavipes* (Butani 1972). In field trials, the exotic *Allorhogas pyralophagus* Marsh could be recovered when released against borers in subtropical India (Maninder et al. 1990).

Tetrastichus howardi (Olliff) (=*Tetrastichus ayyari* Rohwer), a pupal parasitoid of borers including *C. infuscatellus* and *C. sacchariphagus indicus*, was multiplied on pupae of a number of hosts (Cherian and Subramaniam 1940). Rearing techniques for *Isotima javensis* Rohw. were described (Raja Rao

1964a) and its inoculative releases against top borer in Tamil Nadu (Gupta 1960) and Karnataka (Puttarudriah and Usman 1961) enhanced parasitism rates and arrested borer proliferation.

Sucking pests

Dispensation of parasitized egg masses of pyrilla placed in wooden cages fitted with wire gauge was recommended against pyrilla (Muliyil and Lakshmanan 1942). Conservation and augmentative releases of *Epiricania melanoleuca* Fletcher in Haryana, Uttar Pradesh, Gujarat and Maharashtra were beneficial (Misra and Pawar 1984). The parasitoid was established successfully on pyrilla in Karnataka (Ansari et al. 1989) and Orissa (Patnaik et al. 1990).

Epizootics of the entomopathogenic fungus (EPF) *Metarhizium anisopliae* Metch led to 60-75% adult mortality and 71-75% nymphal mortality of pyrilla during September-October (Kulshreshtha and Gursahani 1961).

Population of *Adelencyrtus mayurai* Subba Rao fluctuated more or less synchronously with that of its host *M. glomerata* (Dutta and Devaiah 1988). The exotic predator *Chilocorus cacti* L. was multiplied in the laboratory and its releases were effective in controlling the scale (Misra et al. 1984).

Other pests

The milky disease bacterium *Paenibacillus popilliae* (Dutky) (=*Bacillus popilliae* Dutky) (David et al. 1973) and various EPF and EPN were evaluated against *H. serrata* (David and Easwaramoorthy 1990). The EPF *Beauveria brongniartii* (Saccardo) Petch exhibited potential against the white grub (Jayaramaiah and Veeresh 1984).

Recent trends

Borer pests

In continuing studies on *T. chilonis*, searching range and parasitization ability of a heat tolerant strain were found to be superior to those of the native strain (Singh and Maninder 2008). Among three sugarcane-adapted populations, internode borer population showed superior biological attributes (Baitha et al. 2017). Three weeks of storage at low temperature affected several biological parameters of the parasitoid (Geetha 2010a). Olfactometer studies with hexane washings of internode borer eggs, scales and adult body indicated that *T. chilonis* reared on *C. cephalonica* was able to recognize and respond to the native host cues (Geetha 2010b).

In field studies, *C. cephalonica* eggs parasitized by *T. chilonis* were placed in gelatin capsules and the adults were released by opening the capsules in the field (Maninder et al. 1998). The parasitoid was effective against stalk borer when combined with *C. flavipes* (Anonymous 2000a). Role of release frequency and dosage, and crop age at first release in the field efficacy of *T. chilonis* against internode borer was investigated (Geetha 2009). The number of release points for ensuring uniform dispersal was determined based on dispersal pattern (Geetha and Balakrishnan 2010). Compatibility studies of sex pheromones and *T. chilonis* indicated superiority of the parasitoid (Geetha 2010c). Use of 5% tomato extracts or soybean intercrop enhanced *T. chilonis* efficiency against internode borer (Thirumurugan et al. 2006).

Mass multiplication methods of *C. flavipes* were standardized (Srikanth et al. 2000a; Srikanth and Salin 2003a) and advantages of group-rearing and individual-exposure methods compared (Srikanth et al. 2003). Response of the parasitoid to volatiles from host frass and infested plants (Srikanth et al. 2000b; Salin et al. 2000) and chemical profile of larval regurgitants and headspace volatiles vis-a-vis parasitoid attraction were examined (Salin et al. 2017).

In field studies, *C. flavipes* was most active on *Chilo partellus* Swinhoe (Lepidoptera:

Crambidae) in sorghum followed by internode borer and shoot borer of sugarcane in tropical India (Srikanth et al. 1999). A native population of *C. flavipes* was more effective than an Indonesian population against borers (Maninder and Brar 1996a). Parasitoid releases reduced the progress of internode borer infestation with some variation (Easwaramoorthy et al. 1998). At 2000 females/ha/month split into four doses of 500 each from July to October, the parasitoid was effective against *C. auricilius* (Tanwar and Varma 2001). Sequential releases of *T. chilonis*, *C. flavipes* and *T. howardi* reduced incidence of borers (Thirumalai and Selvanarayanan 2009). A field-release station was devised to liberate ready-to-emerge cocoons of *C. flavipes* whose dosage was fixed based on cocoon mass weight (Srikanth et al. 2017).

In studies on top borer in subtropics, the importance of time of release of *I. javensis* was established (Maninder and Brar 1996b). The borer was able to withstand summer variations in the field better than the parasitoid *T. howardi* (Baitha 2007).

Population dynamics of *St. inferens* in shoot borer indicated an overall increase in parasitism rates in the habitat over time (Easwaramoorthy et al. 1991a; Srikanth et al. 2009a). The parasitoid was multiplied on diet-reared larvae of pink borer *Sesamia inferens* (Walker) (Lepidoptera: Noctuidae) (Easwaramoorthy et al. 1991b). Augmentative releases at low dosage enhanced parasitism levels in *C. auricilius* (Rai et al. 1999) but higher release rates reduced *C. infuscatellus* incidence inconsistently (Srikanth et al. 2009a).

Habitat management practices like manual weeding, earthing-up, detrashing, furrow irrigation and post-harvest trash-burning reduced spider abundance (Srikanth et al. 1997). However, pulse intercrops did not enhance spider and coccinelld abundance significantly (Srikanth et al. 2000c).

A granulovirus was recorded on sugarcane top borer for the first time with infection levels of 1.6-14.4% (Singaravelu et al. 1999). Shoot borer GV acted in a density dependent manner showing significant correlation with borer incidence (Srikanth et al. 2000d). The virus was the most predominant natural enemy of shoot borer followed by *St. inferens* whereas *C. flavipes* was least active with no influence of weather factors (Srikanth et al. 2001a).

Shoot borer GV was safe to albino rats (Easwaramoorthy and Jayaraj 1990) and very specific to the borer (Easwaramoorthy 1992). A wettable powder formulation was viable up to 12 months of storage (Easwaramoorthy and Santhalakshmi 1999).

Crude suspension and purified preparation of shoot borer GV were equally effective against the borer in the field (Easwaramoorthy and Jayaraj 1991). While application of GV and *B. thuringiensis* reduced shoot borer incidence most, *Beauveria bassiana* (Balsamo-Criv.) Vuill. reduced it least (Mala and Solayappan 2001). Application of GV at 10^5 and 10^9 IBs/ml, either alone or with endosulfan, reduced shoot borer infestation significantly (Rao and Babu 2005).

Bacillus thuringiensis (*Bt*) formulations differed in toxicity to shoot borer and internode borer (Easwaramoorthy et al. 2000a). *Hirsutella nodulosa* Petch was recorded for the first time from India on *C. sacchariphagus indicus* (Easwaramoorthy et al. 1997); the fungus was active throughout the year except during summer (Easwaramoorthy et al. 1996). Conventional stem-culture technique and PCR-based approach using specific SCAR primers established endophytic colonization of *B. bassiana* isolates some of which were found to be pathogenic to internode borer in the laboratory (Ramasubramanian et al. 2014).

Susceptibility of nine lepidopteran insects to the EPNs *Steinernema glaseri* Steiner, *Steinernema feltiae* (Filipjev) and *Heterorhabditis indicus*

(Poinar, Karunakar and David) was examined (Karunakar et al. 1999). EPNs developed and reproduced in GV infected shoot borer and internode borer larvae (Karunakar et al. 2002). Three selected isolates of EPN caused 100% mortality of *C. infuscatellus* (Sankaranarayanan et al. 2003). Five species of *Steinernema* and *Heterorhabditis* showed variable penetration and pathogenicity in late instar larvae of internode borer (Sankaranarayanan et al. 2008).

Sucking pests

Conservation and augmentative releases of *E. melanoleuca* against pyrilla were beneficial in several states (Pawar et al. 2002). Deployment of 7,000-10,000 cocoons or 1.0 million eggs/ha provided equally high levels of parasitism at a fifth of chemical control cost (Rajak et al. 2009). Infection levels of the EPF *Hirsutella citriformis* Speare (Singaravelu et al. 2003), and phenology and natural enemies of pyrilla (Mahesh et al. 2019) were assessed in a unique sugarcane crop island system containing sugarcane germplasm.

The beginning of this century witnessed the invasion of the tropical Indian states, viz. Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh by woolly aphid *C. lanigera*, a pest of sugarcane in north-east India (Joshi and Viraktamath 2004; Srikanth 2004). *Dipha aphidivora* Meyrick (Lepidoptera: Pyralidae) (Rabindra et al. 2002) and *Micromus igorotus* Banks (Neuroptera: Hemerobiidae) (Lingappa et al. 2004) emerged as potential candidates in Maharashtra and Karnataka, respectively. The fungus *Acremonium zeylanicum* (Petch) W. Gams and H.C. Evans was reported as a first record on woolly aphid in Sankeshwar, Karnataka (Tippannavar et al. 2006). Molecular diagnostic kits for correct identification of *D. aphidivora* and *M. igorotus* were developed by cloning and characterizing the Folmer region of *mtCOI* (Ramasubramanian et al. 2017).

Population dynamics studies indicated positive relationship between woolly aphid and its predators (Deshmukh et al. 2007; Sarma et al. 2007; Tripathi et al. 2008). The aphid was active throughout the year with peaks during October-January in tropical India; *D. aphidivora* was more predominant than *M. igorotus* (Srikanth et al. 2015a).

Dipha aphidivora was mass multiplied on the host in shadenet (Patil et al. 2004) and galvanized iron trays in the laboratory (Mukunthan et al. 2006). Frozen host supported late instars of the predator indicating its use as transit food for the predator (Srikanth et al. 2009b). A simple technique for multiplying *Micromus igorotus* was also developed (Vidya et al. 2007).

Field releases of *D. aphidivora* enhanced predator numbers and decimated aphid populations (Patil et al. 2007; Srikanth et al. 2015a) but did not prevent the spread of the aphid in the field (Srikanth et al. 2009c) and the subsequent yield loss (Sivaraman et al. 2013). *Micromus igorotus* was evaluated successfully in field studies in Karnataka (Vidya et al. 2010) whereas it failed to establish in release areas at Coimbatore (Srikanth et al. 2015a).

The parasitoid *E. flavoscutellum* established in southern India about a year after it was introduced from Assam (Anonymous 2005). Thereafter, the parasitoid usually appeared along with the first appearance of the aphid (Srikanth et al. 2007) and restricted the spatial and temporal spread of the pest thereby preventing economic loss to the crop (Srikanth et al. 2012; Sivaraman et al. 2013). A protocol for its detection, conservation and distribution for effective control of the aphid has been outlined (Srikanth et al. 2008). The parasitoid continues to trail the aphid causing significant levels of parasitism whenever the aphid occurs sporadically in the crop system (Srikanth et al. 2013).

EPF evaluated against woolly aphid in the field either produced variable results (Nirmala et al.

2007) or failed to suppress host population despite showing concentration-dependent mortality in the laboratory (Ramegowda et al. 2007). Other studies with *M. anisopliae* and *B. bassiana* showed encouraging results (Pokharkar and Ghorpade 2009).

Amitus minervae Silv. and *Encarsia ochai* Viggiani were observed parasitizing the whitefly *A. barodensis*, with the former showing high natural parasitism and multiplication rate in the laboratory (Ananthanarayana et al. 1994). *Camponotus compressus* F. was found to interfere with the activity of the predatory coccinellid *Cryptolaemus montrouzieri* Mulsant against pink mealybug *S. sacchari* by physically removing larvae of the predator (Srikanth et al. 2001b).

Other pests

A mass culture method using molasses-based media and formulation with press-mud as carrier were developed for *B. brongniartii*. A laboratory was set up in a sugar factory in Tamil Nadu to mass multiply and supply the fungus to its growers (Easwaramoorthy et al. 2002). Removal of expensive components economized the mass culture method (Tamizharasi et al. 2005) and addition of supplements enhanced spore production (Balakrishnan et al. 2011; Srikanth and Santhalakshmi 2012). The viability and virulence of selected formulations were evaluated against *H. serrata* (Srikanth et al. 2006). Compatibility of pesticides with three species of EPF was examined (Prabhu et al. 2007). An improved bioassay method for evaluating EPF by maintaining treated larvae under starvation was standardized (Srikanth et al. 2011a).

Beauveria brongniartii was found effective against different instars of *H. serrata* in laboratory, pot-culture and field experiments (Easwaramoorthy et al. 2004). At a single field dosage of 2.5 x 10^{12}/ha, the fungus survived in the soil for more than five years as evidenced from infection levels in field-collected grubs (Srikanth et al. 2010). *Metarrizium anisopliae* application at 1×10^{13} spores/ha was next best to chlorpyriphos in reducing grub numbers and enhancing crop yield and returns (Rachappa et al. 2004). The fungus reduced grub populations and increased yield significantly at lower (Manisegaran et al. 2011) and higher (Kesarasing et al. 2010) dosages and as a formulation too (Chelvi et al. 2011).

The first scarabaeid specific holotype *Bt* gene (*cry8Sa1*) from India was obtained from an isolate of *Bacillus thuringiensis* (*Bt*) cultured from a white grub endemic soil sample (Singaravelu et al. 2012 & 2013). Further screening of native *Bt* strains revealed the presence of a nematode-active *cry6* gene and sequence from one isolate showed homology to nematode-active *cry6A* gene (Singaravelu et al. 2014). A *Bt* isolate harbouring six *cry* genes was identified and partial sequencing of these genes indicated that *cry1D* and *cry1E* could be holotypes at the tertiary level; the *Bt* isolate showed promise against shoot borer in preliminary bioassays (Singaravelu et al. 2017).

A new EPN *Heterorhabditis indicus* (Poinar, Karunakar and David) was isolated from *H. serrata* (Poinar et al. 1992). EPN belonging to *Heterorhabditis* spp. and *Steinernema* spp. isolated from subtropical India were identified and their pathogenicity tested against *G. mellonella* and first instar *H. serrata* (Sankaranarayanan et al. 2017a). Molecular identification, diversity and bioassays of EPN against *H. serrata* were investigated in tropical sugarcane crop system (Sankaranarayanan et al. 2019).

Mass production potential of the EPN-symbiotic bacterium complex, i.e. *H. indicus*-*Photorhabditis luminescens* was ascertained successfully (Ehlers et al. 1998). The symbiotic bacteria *Photorhabdus* spp. and *Xenorhabdus* spp., associated with the

EPN *Heterorhabditis* spp. and *Steinernema* spp., respectively, were isolated, identity established and insecticidal property assessed against *H. serrata* (Sankaranarayanan et al. 2017b).

Steinernema glaseri and *H. indicus* showed promise in white grub control (Karunakar et al. 2000). Biocontrol potential of two species each of *Heterorhabditis* and *Steinernema* against pupae and adults of *H. serrata* was examined (Sankaranarayanan et al. 2006); a combination of EPN and EPF produced higher levels of mortality than individual treatments (Sankaranarayanan and Singaravelu 2012). In field efficacy studies, an isolate of *Heterorhabditis indica* caused up to 78% reduction in white grub population (Sankaranarayanan et al. 2019).

An apparently new braconid, two *Pediobius* spp. (Hymenoptera: Eulophidae) and one *Eurytoma* sp. (Hymenoptera: Eurytomidae) were recovered from the leaf miner *Asamangulia cuspidata* Maulik (Coleoptera: Chrysomelidae: Cassidinae: Hispini), which was observed in sugarcane as a first record at Coimbatore. While the unidentified braconid contributed 70% to the overall parasitism rate of 39.3%, the remaining parasitoids accounted for 30% with likely hyperparasitism among them (Srikanth et al. 2015b).

The progress hitherto

Research retrospective

Fundamental work

Early studies in biological control revolved around surveys and seasonal dynamics of natural enemies and these continued to remain an integral part in the subsequent decades (Easwaramoorthy et al. 1983; Easwaramoorthy et al. 1996; Srikanth et al. 1999 & 2009a). Such fundamental studies provided insights into new associations, region-specific dynamics, role of density-independent weather factors (David and Ananthanarayana 1991; Srikanth et al. 2000d), predominance of specific natural enemies and density dependent natural control (Srikanth et al. 2000d).

Basic studies revealed the causes for under-performance of biological control agents. Variability in the performance of *Trichogramma* spp. was related to loss of vitality, reduced longevity and fecundity, preponderance of males and malformation in individuals due to superparasitization (Chacko 1969). The role of semiochemicals and their possible use to enhance field parasitization of *C. flavipes* were indicated by the attraction of parasitoid adults to cues from non-target hosts (Salin et al. 2000; Srikanth et al. 2000b). Competitive interaction between *St. inferens* and GV and the superiority of the latter in shoot borer (Easwaramoorthy and Jayaraj 1989) explained the negative association between them in field populations of the borer (Srikanth et al. 2000d). Often, understanding of life history traits provided clues to the efficacy of natural enemies as in the case of maggot distribution behaviour of *St. inferens* adults (David et al. 1988), and dispersal and host searching ability of *I. javensis* (David et al. 1986). Mating and host finding behaviour of *C. flavipes* adults after eclosion could be inferred from their group dynamics in the laboratory (Srikanth and Salin 2003a).

Conventional studies

In very early studies, egg parasitoids of pyrilla were conserved and enhanced by holding the egg masses in cages with wire mesh (Muliyil and Lakshmanan 1942). Toxicity of insecticides to parasitoids (Sithanantham and Paul 1977), predators (Mukunthan et al. 2008) and EPF (Prabhu et al. 2007) was examined to harmonize their use despite the low pesticide usage (Anonymous 2013) in the crop. The impact of cultural practices on natural enemies was investigated (Srikanth et al. 1997) to enable their selective adoption.

Enhancement of natural enemies in intercropping and selective weed regimes highlighted the importance of habitat diversity and manipulation (Srikanth 2010).

Some outstanding examples in the introduction and colonization mode within the country include the successful establishment in Tamil Nadu (Raja Rao 1964b) and Karnataka (Puttarudriah and Usman 1961) of top borer parasitoid *I. javensis*is transported from Uttar Pradesh and establishment in tropical India of *E. flavoscutellum* from Assam against woolly aphid (Anonymous 2005; Srikanth et al. 2012). Colonization and establishment of *St. inferens* in Tamil Nadu (David et al. 1986) exemplified inoculative release within a geographical area. A combination of inoculative, inundative and supplementary release strategies were used in the effective utilization of *E. melanoleuca* against pyrilla in subtropical India (Chaudhary and Sharma 1991).

Artificial diets were developed for *C. sacchariphagus indicus* (Mehta and David 1978) and *C. infuscatellus* (Anonymous 2002) to facilitate the production of parasitoids and entomopathogens. Mass culture techniques were developed for several homopteran host insects (David et al. 1986) for need-based utilization.

For large-scale augmentative deployment, mass culture methods were developed for several parasitoids, such as *T. chilonis* (Manjunath 1991), *St. inferens* (David and Kurup 1991), *C. flavipes* (Srikanth et al. 2003), *I. javensis* (Raja Rao 1964a) and *E. melanoleuca* (Chaudhary and Sharma 1991). Similarly, methods were developed for the predators *D. aphidivora* (Mukunthan et al. 2006) and *M. igorotus* (Vidya et al. 2007) in a short span of time.

Shoot borer GV was formulated as a wettable powder (Easwaramoorthy and Santhalakshmi 1999) and an artificial diet was developed for *C. infuscatellus* (Anonymous 2002) to facilitate mass production of the virus on diet-reared larvae. Mass culture and formulation techniques were developed for *B. brongniartii* using sugar industry by-products (Easwaramoorthy et al. 2002; Tamizharasi et al. 2005) and viability of the formulations was evaluated (Srikanth et al. 2006). Addition of media supplements improved the methods further (Balakrishnan et al. 2011; Srikanth and Santhalakshmi 2012).

In intensive field studies over the past eight decades on *T. chilonis*, dosage, frequency and release technique were investigated in the inundative mode against subtropical and tropical borers (Manjunath 1991). Despite differences in viewpoints on its effectiveness (Mukunthan 2006), the parasitoid was evaluated to revalidate its field efficacy (Geetha 2009). Variable results with *C. flavipes* against subtropical (Maninder and Brar 1996a) and tropical (Easwaramoorthy et al. 1998) borers, and differential parasitism rates of *St. inferens* in *C. auricilius* (Rai et al. 1999) and *C. infuscatellus* (Srikanth et al. 2009a) in augmentative trials suggested the involvement of tri-trophic factors (Salin et al. 2000; Srikanth et al. 2000b), besides the role of target host, parasitoid strains and dosage, and climatic conditions. Selective establishment of woolly aphid predators (Srikanth et al. 2015a) in augmentative trials indicated the role of differential habitat suitability.

Commercial formulations of *B. thuringiensis* did not become popular possibly due to the internal feeding habits of the borers, dosage and volume of sprays required, and canopy constraints in the grand growth phase, despite the positive results against some subtropical borers (Kalra and Kumar 1963). The year-to-year variation in field virulence of *B. brongniartii* against *H. serrata* (Srikanth et al. 2010) indicated the possible role of crop management practices. However, laboratory observations on microbial ecology (Geetha et al.

2011 & 2012) need to be extrapolated to field situations with caution. Field evaluation of EPF against shoot borer with encouraging results (Mala and Solayappan 2001) and woolly aphid with variable results (Nirmala et al. 2007) suggested factors, such as hostile canopy, feeding habits of pests, specific climatic requirements of the EPF and lack of host specific isolates.

Novel approaches

Unconventional areas, such as improved natural enemies and semiochemicals were explored in sugarcane biological control. Adaptability to variable temperature and humidity, besides insecticide tolerance, were the subject of investigation with positive results (Anonymous 2000a). Six geographical isolates of shoot borer and internode borer GVs compared for DNA homology and biological activity did not show significant differences (Easwaramoorthy et al. 1999). ELISA and SDS-PAGE techniques showed no differences in nucleocapsids and granulin of the two GVs, except for polypeptide composition (Easwaramoorthy et al. 2000b). Attractants identified in shoot borer frass (Salin et al. 2012) and larval regurgitant-induced headspace volatiles (Salin et al. 2017), and positive response of *C. flavipes* to volatiles from borer damaged sugarcane plant (Salin et al. 2000) are likely to be the forerunner for further studies on semiochemicals to enhance parasitoid attraction. PCR-based technique standardized to establish endophytic colonization of *B. bassiana* in sugarcane (Ramasubramanian et al. 2014) and molecular diagnostic kits used for correct identification of natural enemies, such as *D. aphidivora* and *M. igorotus* (Ramasubramanian et al. 2017) should serve as useful tools in further studies on biocontrol of sugarcane pests.

Industry participation

Sugar industry extends active support to the promotion of biological control in sugarcane. Several cooperative and private sugar factories in different states, including Tamil Nadu (Anonymous 2000b), established biological control laboratories to multiply natural enemies of region-specific pests. With pan-India spread, *T. chilonis* tops the list of biocontrol agents taken up for large-scale production. In Tamil Nadu, some sugar factories produce *T. chilonis* through a rural entrepreneur model to distribute to their growers (Anonymous 2015). A private factory produces a cost-effective formulation of *B. brongniartii* for white grub management in the laboratory set up after elaborate studies established effectiveness of the fungus (Easwaramoorthy et al. 2002 & 2004; Srikanth et al. 2010). Some sugar mills in subtropical India produce *E. melanoleuca* for pyrilla management (Chaudhary and Sharma 1991).

The way forward

Research priorities

Identification of potential natural enemies should be a continuous process as this would help determine candidate biocontrol agents for introduction and colonization against introduced and augmentative control of resident pests. In this context, deployment of parasitoids of top borer, pyrilla and woolly aphid represents the classical mode in a restricted sense. Established yet less explored associations deserve emphasis while identifying potential biocontrol agents. The parasitoid complex of the leaf miner *A. cuspidata* identified in recent surveys at Coimbatore, where the pest was observed for the first time, reiterated the importance of such surveys as the natural enemies so identified could be part of a backup plan to be utilized if the pest assumed serious proportions (Srikanth et al. 2015b). For biocontrol-based management of invasive exotic pests like the fall armyworm, though not as serious on sugarcane as it is in maize presently, native biocontrol agents

(CABI 2018; Shylesha et al. 2018) and new associations, either in sugarcane or maize, may be given as much importance as natural enemies from its native home or other geographical areas to be used in the classical mode. The spectrum of natural enemies documented from different parts of the world, including India (CABI 2018), provides a wide choice to select candidate biocontrol agents for classical or augmentative mode of control. Molecular diagnostic kits (Ramasubramanian et al. 2017) need to be developed for natural enemies in sugarcane crop system to be used as a complementary taxonomic tool for their correct identification.

Lack of mass production technologies for host insects and biocontrol agents often limits the large-scale utilization of potential candidates in the augmentative mode. The obligate shoot borer GV suffered long from such a constraint but subsequent standardization of artificial diet for the borer (Anonymous 2002) opened up the avenue for its in situ production on diet-reared larvae. Development of the still elusive artificial diet for top borer will enable mass production of *I. javensis* for large-scale and early deployment in subtropical India where the pest occurs in distinct broods. While it would be easier to multiply predators like *D. aphidivora* on semi-synthetic diet (Venkatesan et al. 2008), the tachinid *St. inferens* is a good candidate among parasitoids for the development of the more difficult in vitro mass production technique in the light of its viviparous mode of reproduction. Low-cost mass culture media developed hitherto for *B. brongniartii* (Easwaramoorthy et al. 2002; Srikanth et al. 2006) should lead to the development of simpler economic formulations for potential EPF.

Selection of inferior populations with low genetic vigour and searching ability could be a major concern associated with mass production of biocontrol agents on factitious hosts. For example, by maintaining parasitoid populations on natural and laboratory hosts for several generations, Bertin et al. (2017) provide evidence to confirm the prediction that the use of factitious hosts can lower fitness of *Trichogramma galloi* Zucchi on the target pest. Although such misgivings can be extended to *T. chilonis* produced on *C. cephalonica* in insectaries, recent studies revealed that the parasitoid has retained its ability to recognize and respond to native host volatiles (Geetha 2010c) allaying at least some apprehensions. Despite the availability of theoretical measures to circumvent the issues of fitness (Bertin et al. 2017), which may need verification through elaborate research, it is prudent to consider alternatives and avoid dependence on a single candidate agent (Srikanth 2012) such as *T. chilonis* with debatable record (David et al. 1986). Within the egg parasitoid guild, there is a need to turn our attention to potential candidates such as *Telenomus* spp. that exhibited greater levels of association with the host (Easwaramoorthy et al. 1983; Rajendran 1999; Baitha 2012; J. Srikanth et al. unpubl. data) while continuing work with the time-tested *T. chilonis* on futuristic lines outlined by Sithanantham et al. (2013).

Improved field liberation techniques are needed to release parasitoids and predators at the right stage, dosage and field points taking in to account the ease of handling, dispersal range of the agent and field predation. A novel method of release of *T. chilonis* adults was attempted (Maninder et al. 1998) and based on the dispersal pattern of *T. chilonis*, the number release points was fixed as at least 13 per acre (Geetha and Balakrishnan 2010). Woolly aphid predator *D. aphidivora* was dispensed in the field as cocoons formed on leaf bits in laboratory rearings, with prior tests to ensure lack of predation and high adult emergence from cocoons (Srikanth et al. 2015a). In this context, the field-release station designed and protocol developed for dispensing cocoons of *C. flavipes* can be extended to other smaller or larger parasitoids and predators with suitable modifications (Srikanth et al. 2017).

Enhancement of natural enemy adaptability through conventional selection methods, attempted to develop heat tolerant strains of *T. chilonis* (Kumar et al. 2008), needs to be extended to other parasitoids like *St. inferens* for use against borers during summer. Insecticide tolerant strains of *T. chilonis* (Anonymous 2000a) or other parasitoids may appear less relevant in sugarcane under the current minimal pesticide usage (Anonymous 2013) scenario, particularly in tropical India, but would be a necessity in the pesticide-intensive subtropical sugarcane. Introduction of populations from harsher subtropical to milder tropical habitats is likely to enhance parasitoid performance due to the inherent plasticity of the natural enemy in the former environment (Srikanth and Salin 2003b). Parasitoids multiplied on non-target hosts from within their host range are known to learn host cues when exposed to the target host or its cues in the laboratory prior to field release (Lewis et al. 1991). The oligophagous *C. flavipes*, multiplied on its natural hosts (Srikanth et al. 2000a), may be a more suitable candidate than the polyphagous *T. chilonis*, mass-produced on an unnatural host (Manjunath 1991), for verification and adoption of this principle of associative learning.

In a crop wherein inundative releases often do not lead to expected results (Easwaramoorthy et al. 1998), the use of semiochemicals may enhance their effectiveness. Parasitoids like *C. flavipes* which show preference to hosts in crops other than sugarcane (Srikanth et al. 1999) and *St. inferens* with its preference to *Se. inferens* (David and Kurup 1991) could be ideal candidates. Semiochemicals from either the preferred host plant or host insect or its frass may increase the retention time and enhance parasitoid efficiency. Chemicals identified from shoot borer frass and their behavioural bioassay in the laboratory (Salin et al. 2012) hold promise for further studies. The possibility of sex pheromones mimicking allelochemicals for parasitoids also needs investigation. Habitat manipulation through intercropping (Srikanth 2010) and habitat augmentation with lac cultivation (Roy et al. 2019) to enhance natural enemy populations and reduce pest abundance deserve promotion.

In the area of entomopathogens, *B. bassiana* or *M. anisopliae* need attention against the below-ground root borer *P. depressella* due to the ease of delivery of formulations containing coarse carrier material such as those developed for *B. brongniartii* (Srikanth et al. 2006) and the inaccessibility of subterranean larvae to parasitoids, despite the occurrence of a large number of them (Srikanth et al. 2014). Failure in preliminary field trials (Sardana 2000) with root borer EPF isolates (Easwaramoorthy and Santhalakshmi 1993) notwithstanding, studies need to be intensified for better isolates or species of EPF. Wide row spacing facilitates spray application of powder EPF formulations against borers and nanoparticle-based fungal formulations may improve their efficacy further. Identification of endophytic strains of *B. bassiana* (Ramasubramanian et al. 2014) should lead to their utilization against borers, more so the subterranean root borer, as in the case of banana weevil (Akello et al. 2009). Micro-irrigation system would enable better delivery of EPF and EPN formulations to target soil dwelling white grub or termites and EPF against tissue borers through endophytic action but needs standardization taking into consideration soil moisture thresholds.

Isolates of entomopathogens with greater virulence and tolerance to environmental adversities need to be identified and in this process molecular techniques can help establish genetic variability.

Identification of virulence determinants and their possible enhancement by biotechnological tools should form the long-term objective in genetic manipulation studies of EPF. Intensive and extensive work on *Bt* strains from diverse agro-ecosystems could yield isolates to be developed as formulations for application as biopesticides. Such studies would reveal more *cry* toxin genes effective against a wide range of soil and aerial pests (Singaravelu et al. 2012; 2013; & 2017) to be exploited through transgenic approach. When transgenics reach commercial cultivation in the near or distant future, which does not seem to be on the horizon presently due to socio-political and policy reasons, their impact on sugarcane natural enemy complex may need attention (Srikanth et al. 2011b). Evaluation of pathogenicity of EPN isolates from different geographical locations (Sankaranarayanan et al. 2017a) should pave the way for introduction and colonization of superior isolates in target areas. Promising EPNs need large-scale field validation against white grubs, either in isolation or in combination with the EPF *B. brongniartii* or *M. anisopliae* as consortia. Determination of insecticidal activity of symbiotic bacteria such as *Xenorhabdus* and *Photorhabdus* (Sankaranarayanan et al. 2017b) could lead to identification of toxin genes with wider applications in many crops, besides sugarcane.

Technology dissemination

The success of biological control depends largely on the availability of mass produced biocontrol agents to the end-user. This can be ensured by the establishment of adequate number of insectaries in sugarcane tracts. Research laboratories that generate mass-production and field evaluation technologies may provide the technical expertise needed for their transfer as well as the subsequent quality maintenance. Besides facilitating knowledge dissemination, i.e. transfer of the latest biocontrol technologies to growers, sugar industry can play a more active role by either mass producing biocontrol agents in R & D laboratories or encouraging entrepreneurial growers to establish production and service centres, as is already being practiced by a few factories. Such concerted efforts by sugar industry would ensure an uninterrupted supply chain of biocontrol agents to their registered growers, despite the possibility of the industry itself emerging as a competitor to commercial insectaries. Nonetheless, when integrated with other suitable pest management tools and practiced under a mandatory mode in all the registered farms of any factory, biological control will become an important component of area-wide pest management concept and approach (Faust 2008) in sugarcane.

Epilogue

The stable sugarcane habitat supports both natural and applied biological control, more so in the tropical belt. Conservation of system stability is the first component in biological control of sugarcane pests. Past experience in tropical sugarcane indicates that sporadic pest outbreaks often follow a decline in natural enemy activity whereas occasional pest flare-ups are associated with profuse proliferation of the predominant natural enemy. Judicious use of insecticides in the first case and avoidance of insecticides together with re-distribution of the proliferating biocontrol agent in the second case usually restore balance by the beginning of the next season. While introduction and colonization of major native natural enemies led to successful control of introduced pests, augmentative control against endemic or resident pests needs to be adopted in an area-wide mode in the spatially and temporally contiguous crop habitat. In the climatically harsher subtropical belt where pests and natural enemies display diapause and/or brood patterns, early targeting of the pest through inoculative releases may hold the key in the augmentative approach. Excessive dependence on pesticides against endemic pests, such as top borer in the subtropics and white grub in the tropics, is likely to convert sugarcane pest management from the natural/applied biological control mode to insecticide mode and sugarcane from an insurance crop to a catastrophic crop (Srikanth et al. 2016).

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