

# Management of Sweet Potato Weevil, *Cylas formicarius*: A World Review

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## ABSTRACT

Sweet potato is infested by many insect pests. Sweet potato weevil (SPW) *Cylas formicarius* (Fab.) is the important insect pest throughout the world, wherever it is grown. The weevil is managed by a package of practices together called integrated pest management (IPM). In India, a few genotypes of sweet potato have shown durable resistance throughout 2006 to 2011. A new screening method of germplasm, volatile-assisted selection (VAS), was developed to identify resistance/susceptibility in sweet potato genotypes based on the volatile chemicals that are released. Transgenic sweet potato was not successful at the field level. Farmers in Asia practice intercropping of sweet potato with ginger, *bhendi*, taro and yams to reduce the incidence of pests as well as to conserve soil moisture. Entomopathogenic fungi and nematodes are used successfully to control *C. formicarius* in the West and Latin America. Female sex pheromone (*Z*)-3-dodecen-1-ol (*E*)-2-butenolate has changed the pest dynamics in the field and has become an important tool in *C. formicarius* IPM. It was used to monitor and trap male weevils, thus reducing the reproductive success of female weevils. A number of botanical pesticides are available and their use is limited in developed countries. A few insecticides that were used to control *C. formicarius* were banned in recent years in many countries and it is essential to identify new molecules with low or no persistence in tubers and soil with toxic effects on weevils. We reviewed the research done on SPW during the last five decades to assess the management practices of SPW and to identify new strategies required to control the pest effectively and economically.

**Keywords:** biological control, botanicals, cultural methods, host plant resistance, insecticides, integrated pest management, pheromones, sweet potato

**Abbreviations:** AVRDC, Asian Vegetable Research Centre; **Bt**, *Bacillus thuringiensis*; **Cry**, crystalline; **CTCRI**, Central Tuber Crops Research Institute; **EPF**, entomopathogenic fungi; **EPN**, entomopathogenic nematode; **GC-MS**, Gas Chromatography-Mass Spectrometry; **IAA**, indole-3-acetic acid; **IIE**, International Institute of Entomology; **kPa**, kilo Pascal; **NAA**,  $\alpha$ -naphthaleneacetic acid; **NMR**, Nuclear Magnetic Resonance; **SPW**, sweet potato weevil; **USDA-TARS**, United States Department of Agriculture-Tropical Agricultural Research Station

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## INTRODUCTION

Sweet potato is an important crop in many countries and east and south Asia, and its production is limited by insect pests. Sweet potato weevil (SPW) *Cylas formicarius* (Fab.), which is supposed to have originated from India about 80–100 million years ago (Wolfe 1991), has become widely dispersed mainly in tropical and subtropical regions of the world, and recently has been found in higher latitude areas as well (Nilakhe 1991; IIE 1993). The establishment of *C.*

*formicarius* in Muroto (Japan) shows that it can tolerate low temperature in winter to some extent, though it is a tropical and subtropical insect (Kandori *et al.* 2006). The weevil continues to infest sweet potato at higher altitudes of 1600 m above mean sea level in Meghalaya, India (Korada *et al.* 2010a). In an observatory trial, a change in dynamics of population of *C. formicarius* was noticed. Our experiments suggested that the number of *C. formicarius* were increased in relation to female population over a period of time in between 2005 to 2010. These changes in sex ratio would

have a tremendous impact on its damage potential and its management methods. Though several eradication programmes for this pest were taken in some countries, still *C. formicarius* continue to be one of the major insect pests in the sweet potato producing countries worldwide. An overview of research in sweet potato weevil management during the last twenty years was reviewed by Korada *et al.* (2010b). In the present chapter, the status of research done in SPW management worldwide during the last five decades is discussed to evolve suitable and viable management strategies.

## PLANT RESISTANCE

The shape of the tuber, tuber length, neck length and thickness plays an important role in preference by *C. formicarius* apart from the inherent nutritional quality of the sweet potato plant and tuber. Round tubers are preferred more than elongate and spindle shaped ones. Teli and Salunkhe (1996) reported that round and oval tubers of sweet potato were more infested in the field by *C. formicarius* than long stalked, spindle and elongate ones. Tuber damage in the field was negatively correlated with stalk length and positively with girth of tuber. Rind thickness and length of tuber were not correlated with weevil incidence. Pink and red coloured cultivars were generally less susceptible than white and brown coloured ones. Cultivars with thin foliage and lobed leaves with purple coloration at emergence were less susceptible. They also reported that the sweet potato variety CO3 was the most resistant to SPW feeding (Teli and Salunkhe 1995). Low yielding varieties with small roots were less infested than higher yielding entries (AVRDC 1990). Tuber damage by SPW in many varieties or cultivars differ only in relative terms.  $\beta$ -Carotene-rich accessions were found to be susceptible to SPW (AVRDC 1987a).

The interaction between drought stress and genotype was significant for adult feeding indicating a different response between the two genotypes 'Beauregard' and 'Excel' (Mao *et al.* 2004). Drought stress seemed to favour the feeding and oviposition of *C. formicarius* but it reduced larval survival rate. The magnitude of response of the two genotypes appeared to differ (Mao *et al.* 2004). Drought stress may increase the activity of oviposition stimulant present in the genotypes because weevils deposited more eggs on drought stressed plants (Mao *et al.* 2004). Some of the plant metabolites are produced and influenced by environmental stress which would have a bearing on resistance or tolerance. Jackson and Peterson (2000) reported sub-lethal effects of sweet potato resin glycosides on *Plutella xylostella*. Caffeic acid showed adverse effects on a generalist herbivore, *Helicoverpa zea* (Summers and Felton 1994) and sweet potato pathogenic fungi (Harrison *et al.* 2003a). Recent analyses showed that the levels of resin glycosides and caffeic acid vary between sweet potato genotypes and within genotypes among years or areas of production (Harrison *et al.* 2003a, 2003b) and showed insecticidal activities (Peterson and Harrison 1992; Jackson and Peterson 2000). This may indicate a relationship between the quantity of these two compounds and the antibiosis of sweet potato. It also may indicate that the production of these compounds is subject to environmental influence.

The search for sweet potato cultivars resistant to SPW has been conducted for decades, but little success has been achieved, partly because of the inconsistent expression of the resistance (Collins *et al.* 1991). Identification of environmental factors that affect the expression of resistance and the knowledge of the magnitude of these variations would assist in the development of cultivars with stable SPW resistance. In addition, secondary plant compounds are often associated with host plant resistance. Sweet potato resin glycosides and caffeic acid are two such compounds found in the sweet potato storage roots that have shown insecticidal activities (Peterson and Harrison 1992; Jackson and Peterson 2000). Any effect of environmental factors on the level of these two compounds may provide insights on

SPW resistance.

The sweet potato varieties 'H85-168' had the least SPW damage, while 'H85-70' and 'H85-16' were also better than the local check in Puri, Balasore, Mayurbhanj and Keonjhar districts, Orissa, India during 1988-1990 (Bhat and Naskar 1994). Cabrera *et al.* (1990) conducted two field experiments, one carried out in Isabela and one in Mayaguez, Puerto Rico and observed that the total yield of sweet potato cultivars was not significantly affected by the damage caused by *C. formicarius elegantulus*. In Isabela, cv. 'WRAS-3', 'WRAS-7', 'WRAS-31', 'WRAS-36', 'Mojave' and 'Gem' were highly susceptible to attacks by *C. f. formicarius* whereas cv. 'Regal', 'Sumor' and 'WRAS-27' were moderately susceptible. In Mayaguez, cv. 'WRAS-36', 'Mojave', 'Miguela' and 'Gem' were highly susceptible and cv. 'WRAS-3', 'WRAS-7', 'Resisto', 'Sumor', 'WRAS-25' and 'WRAS-35' were moderately susceptible. 'Regal', 'WRAS-37' and 'WRAS-40' were the most resistant varieties in Mayaguez. Three sweet potato breeding lines, 'MS-501', 'MS-503', and 'MS-510' are released by the Mississippi Agricultural and Forestry Experiment Station. They have moderate levels of resistance to the *C. f. elegantulus* and to the wire worm (*Conoderus* sp.), *Diabrotica* sp. (cucumber beetle), *Systema* sp. (flea beetle) complex (Thompson *et al.* 2001).

In Bihar, India, among 13 short duration sweet potato cultivars evaluated, one cv 'X24' had the fewest SPW damage (0.42 t/ha) but produced the lowest marketable tuber yield (4.35 t/ha) (Sarkar *et al.* 1992). Studies carried out in Kerala, India with 40 sweet potato hybrids showed that the cv. Selopia is the most resistant to *C. formicarius* followed by cv. 'H 268' and cv. 'Maruthipoovan' (Palaniswami and Mohandas 1992). In Coimbatore, Tamil Nadu, India, out of 6 sweet potato clones, 'CO-1X47' and 'OP76/217' were found to be resistant to *C. formicarius* (Kakaty *et al.* 1992). Lowest oviposition rates and fecundity, and maximum developmental time were recorded for cv. 'Deoria local' in Kanpur, India (Gupta and Pandey 1993). Crossman *et al.* (1998) evaluated sweet potato germplasm and studied the contribution of cultural practices including mulching and irrigation on yield and the reduction of *C. f. elegantulus* infestation of sweet potato in the United States Virgin Islands (USVI). The germplasm evaluation trials were conducted using cultivars obtained from the USA, Puerto Rico (USDA-TARS), St. Kitts, and the USVI. Results indicated a high % SPW infestation on storage roots of high yielding cultivars, but local cultivars produced lower yields than introduced cultivars. Most cultivars from Puerto Rico were very well adapted for production in the USVI. Mulching generally increased the yield of cultivar Sunny, with the plastic mulch producing the highest yield of medium sized roots while grass mulch reduced SPW infestation of storage roots. Irrigation significantly increased marketable yields of storage roots compared to the rainfed treatment. Additionally, irrigation maintained at 40 kPa produced the highest yield of medium sized storage roots which was significantly higher than the 20 kPa and rainfed treatments. Among 100 US sweet potato plant introductions (PIs) SPW injury was less severe in 'PI508523', 'PI531116', and 'PI564107' which have potential use for increasing insect resistance improvement programme (Thompson *et al.* 1999).

Sweet potato varieties differ in infestation based on kairomonal compounds present in the plants and tubers. A triterpenol acetate was identified in the root surface of a SPW susceptible cultivar 'Centennial', but not in more resistant cultivars (Nottingham *et al.* 1989). Wilson *et al.* (1988) demonstrated that a methylene chloride surface extract of the periderm of storage roots of the susceptible line Centennial stimulated oviposition of *C. f. elegantulus*. It has been established that the major component (compound I) of the surface extract was an oviposition stimulant of female weevils (Wilson *et al.* 1989). The mass spectrum of compound I displayed a molecular ion at  $m/z$  468 with a molecular formula of  $C_{32}H_{52}O_2$ . The fragmentation pattern was

indicative of a pentacyclic triterpene with isopropyl and acetate. Based on  $^{13}\text{C}$ -NMR and GC-MS data and physical evidence, the structure of compound I was established and tentatively named boehmeryl acetate (Son *et al.* 1990a). Boehmeryl acetate extracted from the surface of sweet potato storage roots appears to act as an ovipositional stimulant for the SPW (Son *et al.* 1990b). Later, in India, Palaniswami *et al.* (2000) reported that among four sweet potato varieties viz., 'Kanhagad', 'RS-III-2', 'Sree Bhadra' and '56-2', weevils attracted more to periderm extracts of variety RS-III-2. The ratio of male and female weevils attracted ranged from 2 to 7:1. Duration of biological activity of the crude extract and pure compound was in the range of 18-22 days. For the first time, the two triterpenoids, boehmerone and boehmerol isolated from the bark of *Boehmeria excelsa* (Bert. ex Steud) Wedd (Oyarzun 1987) commonly known as 'manzano' is an endemic shrub growing on Robinson Crusoe Island, Juan Fernandez Archipelago, Chile.

Host plant nutritional parameters have an influence on the infestation of SPW. The relationship between potash and silica in sweet potato stems is negatively correlated with *C. formicarius* infestation (Singh *et al.* 1993). Nutrients like nitrogen (N) and potassium (K) seems to influence storage root surface chemistry of sweet potato varieties. In a study on qualitative and quantitative effects of N and K nutrition on storage root surface chemistry of three sweet potato cultivars, two cultivars displayed moderate resistance ('Jewel' and 'Regal') and one was susceptible ('Centennial') to SPW. Boehmerol and boehmeryl acetate were found on the surface of Centennial but not on Jewel or Regal roots. Another compound, previously identified as peak 4, was found on Regal with a retention time very close to that of boehmeryl acetate. In the field, boehmeryl increased quadratically with increase in K rate (0, 112 or 224 kg ha<sup>-1</sup>), the same was true for boehmeryl acetate, but at 112 kg N ha<sup>-1</sup> only. Peak 4 increased linearly (pot experiment) or quadratically (field experiment) with increases in the K rate, while N did not have a significant effect (Marti *et al.* 1993). Methanol extracts of external and internal tissue of sweet potato cultivars 'Centennial', 'Jewel', 'Regal' and 'Resisto' were reported to have differences in quantities of carbohydrates, organic acids and chlorogenic acid (3-O-caffeoylquinic acid). With the exception of malic acid, the concentration of carbohydrates, organic acids and phenolics did not correlate with cultivar susceptibility to the SPW (Son *et al.* 1990b, 1991c). Methodology for the rapid qualitative and quantitative screening of sweet potato germplasm for concentration of the oviposition stimulant, boehmeryl acetate and its alcohol, boehmerol was developed (Son *et al.* 1991a).

The volatile chemistry of some clones of sweet potato was studied in relation to the olfactory behaviour of *C. f. elegantulus* (Wang and Kays 2002). They indicated that volatiles from storage roots and aerial parts are attractive to female weevils, the former being substantially greater. Three oxygenated monoterpenes (nerol, Z-citral and methyl geranate) found in storage roots but not in aerial parts, were identified as attractants. Differences in relative attraction among four sweet potato cultivars to female weevil were inversely correlated with the composite concentration of headspace sesquiterpenes (Wang and Kays 2002). Selection of clones with decreased volatile attractants and/or increased deterrents using an analytical means of quantification may significantly facilitate developing resistance to SPW. Recently, Korada *et al.* (2010c) coined a new term 'volatile assisted selection (VAS)' for identifying and selecting sweet potato genotypes resistant to *C. formicarius* based on differential volatile emission from sweet potato plants. The infestation pattern of SPW on four sweet potato genotypes, viz. 'Goutam', 'Sourin', 'Gouri' and 'CIP-6' showed that 'CIP-6' was the most susceptible. Electroantennogram studies with *C. formicarius* revealed that the electrophysiological response of female antenna to the volatile extracts of aerial plant parts and roots was higher than the male antenna. In olfactometer studies, the headspace

volatiles of genotype 'CIP-6' attracted more number of female *C. formicarius* weevils than volatiles of 'Gouri', 'Goutam' and 'Sourin'. Variation in the preference of SPW to sweet potato genotypes is attributed to differential emission of volatiles from the aerial parts and roots (Korada *et al.* 2010c). Further, Rajasekhara Rao *et al.* (2010) identified eight sweet potato genotypes, namely, 'S-643', 'Howrah', '90/235', 'SB 72/7', 'BP-2', 'BX 102', '90/693' and '1197', with high degree of *C. formicarius* resistance. They also found a few compounds from flower extracts of 'Howrah' and '90/235' that have shown electrophysiological and behavioural activity in their studies with gas chromatography coupled electroantennogram detector (unpublished). Identification of these compounds is under progress. Identification of biologically active compounds and other host plant volatiles that modulate insect behaviour represents an important step in developing an analytical approach to breeding for resistance to specific insect pests.

## TRANSGENICS

The expression of foreign genes in plants to confer pest resistance is an appropriate alternative to reduce crop losses. Most of the references focus on the efficacy of *Bacillus thuringiensis* (*Bt*) Berliner against different pest insects (Keller and Lagenburch 1993). Moran *et al.* (1999) evaluated clones of sweet potato (*Ipomoea batatas* cv. 'Jewel') transgenic for the cryIIIA gene from *Bacillus thuringiensis* subsp. *tenebrionis* to SPW. The cryIIIA delta-endotoxin gene was cloned under the regulation of the 35S CaMV promoter and the TMV Omega fragment. Biological activity experiments to evaluate the insecticidal capacity of transgenic plants were performed against SPW under both controlled and field conditions. Garcia *et al.* (2000) carried out transformation of leaves and stems of sweet potato with *Agrobacterium tumefaciens* strain C58C1-pGV2260 carrying the *Bt* endotoxin gene and nptII. Transformants were cultured on media containing various combinations of IAA, NAA, zeatin riboside, kinetin, benzyladenine and paclobutrazol. Resistance trials under controlled conditions indicated that weevil damage on tubers was up to 5 times lower in transgenic compared with control plants. Isakova *et al.* (2007) identified, cry1A, cry1B and cry1C genes encoding the toxic proteins in 13 *Bt* strains isolated in Ukraine and then determined the toxicity of these strains against lepidopteran and coleopteran pests from the south eastern United States. The cry1 toxins from three *Bt* strains were solubilized and trypsinized for bioassay against the boll weevil (*Anthonomus grandis* Boheman) and *C. formicarius*. Toxicity of all three strains was higher or comparable to that of the commercial *Bt* product FoilReg. (ECOGEN Inc., Langhorne, PA) containing cry3 toxin specific for coleopterans. Two of the Ukrainian *Bt* strains containing cry1B toxins were toxic to both lepidopteran and coleopteran pests. This study thus revealed new *Bt* strains toxic to lepidopteran and coleopteran pests from the southeastern U.S. indicating specific target pests for a broad spectrum of cry1 toxins, including natural and trypsin activated forms of cry1B proteins.

Thompson *et al.* (1994) studied genetic variance component and heritability estimates of freedom from *C. f. elegantulus* injury to sweet potato and yield of sweet potato by parent offspring regression and variance component analysis. Heritability estimates by variance component analysis based on half-sib families for percent and number of uninjured roots were 0.25 and 0.83, respectively. Individual plant heritability estimates for uninjured root percent and number were 0.03 and 0.13, respectively. Heritability estimates by parent offspring regression for uninjured root percent and number were 0.35 and 0.52, respectively. Genetic variance was mostly additive for all the traits except the stem diameter. Genetic correlations between total root number, uninjured root number and percent uninjured roots ranged from 0.66 to 0.87, indicating that selection for uninjured root number should most effectively increase un-

injured root number and percent, as well as total root numbers. Predicted gains in uninjured root percent and number were 8.8 and 0.87%, respectively, in the progeny derived from inter-mating the highest four out of 19 families for uninjured root number. The 0.87 gain in uninjured root number equals a 24% increase in one breeding cycle.

## CULTURAL PRACTICES

Cultural practices include those methods which prevent the build up or survival of the insect pests by including advancing planting time, mulching, trap crops, crop rotations, mixed or intercropping, sanitation, birth perches etc. Central Tuber Crops Research Institute (CTCRI), Kerala, India reported on the use of different cultural practices effectively for suppression of SPW. Several sweet potato mixed cropping systems are practiced by farmers in North Eastern Region of India. The component crops include ginger, bhendi, maize, taro and yam. Low incidence of *C. formicarius* was noticed in these systems and a detailed description of interaction of intercrops and multifunctional cropping systems are described by Rajasekhara Rao (2005), Rajasekhara Rao *et al.* (2006) and Korada *et al.* (2010a).

Removal of alternate host plants (*Calystegia soldanella*, *C. hederacea* and *Ipomoea indica*) reduced the SPW infestation in Japan (Komi 2000). Teli and Salunkhe (1994a) reported that sweet potato planted during June-August yielded higher yields (9.41 to 10.32 t ha<sup>-1</sup>) and with least tuber damage (41%) than the crops planted in January-April (above 52%). The orientation of planting of cuttings also influences the tuber yield and the damage by *C. formicarius* (Levett 1993). In a study conducted in dry lowlands of Papua New Guinea, planting of 4 nodes vertically in the soil produced the lowest percentage of non marketable tubers in cv. 'L22', while 6 nodes placed horizontally was the most effective for 'L44'. Increasing cutting number at a planting site from 1 (36700 cuttings/ha) to 2 decreased tuber size in cv. 'L44' but not in cv. 'L22', and increased the proportion of marketable tubers which had internal damage from the *C. formicarius*. In another experiment by Levett (1993), in which *C. formicarius* weevil damage was generally severe, vertical planting of 2 cuttings with 2 nodes, or vertical or horizontal planting of 1 cutting with 4 nodes resulted in minimum damage. Delaying harvest date from 108 to 133 days after planting increased total yield in both trials by 7-8% but increased internal damage of marketable tubers and the percentage of small and non marketable tubers. In both trials, 'L44' produced higher yield, with fewer but larger tubers and a lower percentage of non marketable tubers than 'L22'. Deep planting into the hill and planting into the furrow and earthing up over 6 weeks, offers protection against weevils (Macfarlane 1987). Re-ridging the crop during tuber initiation and formation stage prevent the weevil from laying the eggs as well as its entry into the tuber. The efficacy of re-ridging sweet potato crop, as a cultural practice for reducing *C. formicarius* incidence was investigated over two seasons at Vellayani, Kerala, India (Palaniswami and Mohandas 1994). Treatments consisted of up to seven re-ridgings at 10 day intervals between 30 and 90 days after planting (DAP). Re-ridging significantly reduced the weevil damage to the tubers; the damage was lowest with 7 re-ridgings, which was not significantly different from 5 re-ridgings between 50 and 90 DAP.

Mulching with rice straw or black plastic reduced the infestation by SPW in the root zone (AVRDC 1987b). The availability of mulch is a problem especially in dry land areas coupled with higher temperatures. A ten time reduction in SPW is noticed in sweet potato intercropped with rice, cowpea or *Colocasia* (24.0-57.7 weevils/5 kg tubers) as compared with 105.3 weevils for sweet potato followed by flooding and 217.5 weevils for sweet potatoes as a monocrop sequence (Pillai *et al.* 1987). In low land rice fields in India, the cropping sequences such as rice-sweet potato-cowpea; rice-rice-sweet potato and rice-sweet potato-rice were effective in controlling the weevil infesta-

tion (Pillai *et al.* 1996). Tuber damage in the effective crop rotations ranged from 7-9%, compared with 52% in the sole crop of sweet potato. Sweet potato intercropped with maize plots had a lower percentage of weevil, *C. formicarius* and a chrysomelid *Typophorus nigritus* damage, with 14.37 and 18.89% less tubers being affected in 1992 and 1993, respectively than in plots with sweet potato alone in Cuba (Suris *et al.* 1995).

## BIOLOGICAL CONTROL

Biological control is encouraging or using beneficial organisms for controlling insects and is most suitable for all types of cropping systems. Castineiras *et al.* (1985) calculated the economic indices for the biological and chemical control of the principal sweet potato pest in Cuba, the *C. formicarius elegantulus*. Biological control of the pest was calculated to reduce production costs to 13.93% as compared to chemical control. Net profit and yield were also higher in the case of biological control.

## Parasites

A solitary, idiobiont, ectoparasitoid *Bracon yasudai* Maeto et Uesato sp. nov. from the south west islands of Japan was reported on the larva of the West Indian sweet potato weevil (WISPW), *Eusepes postfasciatus*, and the SPW, *C. formicarius*, both feeding on *I. batatas* (Maeto and Uesato 2007). The percentage parasitism of the braconid on *E. postfasciatus* in the vines of *I. batatas* was 19.9-40.7% in the field. Another larval parasitoid *Bracon cylasovor* (Rohwer) was also reared from *C. formicarius* in the Philippines. In India, Palaniswami and Rajamma (1986) described natural enemies of the SPW. The braconids, *Rhaconotus* sp. and *Bracon* sp. and another unidentified hymenopterous parasitoid were found in larvae. The empidid fly *Drapetis* sp. and an unidentified mite preyed on larvae and adults respectively. The parasitism occurred in July-December, reaching a peak of 32.5% in October. The duration of the egg, larval, prepupal and pupal stages of *Rhaconotus* sp. averaged 2, 9.6, 1 and 7.2 days, respectively; the adult life-span averaged 12.8 days in males and 22.5 days in females. Application of insecticides like endosulfan, fenthion or fenitrothion at 0.05% a.i. each as soil drenching at 50 and 80 DAP was effective against *C. formicarius* and did not have significant effects on actinomycetes, bacteria and fungi in sweet potato fields (Palaniswami *et al.* 2002). Jansson and Lecrone (1991) reported that *Euderus purpureas*, an eulophid, was found parasitizing *C. formicarius* in southern Florida. The success of these parasitoids at field level is doubtful since they were always found in very few numbers.

## Entomo-pathogenic nematodes

The entomo-pathogenic nematodes (EPNs) seem to be promising for the control of SPW. Among different species, *Heterorhabditis* is found to be most effective, infective and pathogenic than *Steinernematids*. Jansson *et al.* (1989) evaluated *Heterorhabditis bacteriophora* ('HP88' strain) and *Steinernema carpocapsae* (All and G-13 strains) against *C. formicarius elegantulus*. The LD<sub>50</sub> to *C. f. elegantulus* larvae were 2.6, 2.9 and 3.4 infective juveniles/insect for 'All', 'G-13' and 'HP88', respectively. The LD<sub>50</sub> to pupae of these nematodes were 4.9, 5.9 and 4.8 infective juveniles/insect, respectively. The 'G-13' strain was the most effective in killing the larvae within infested sweet potato roots, followed in decreasing order by 'HP88' and 'All' nematodes. Efficacy in killing pupae within roots did not differ among the three nematodes or between life stages for each nematode. Field applications of 'HP88' and 'All' nematodes were more effective than chemical insecticides in reducing weevil densities on plants. 'HP88' nematodes were more effective than 'All' nematodes and chemical insecticides in reducing weevil damage to fleshy roots. Figueroa *et al.*

(1991) used various strains of *Heterorhabditis* spp. for the control of SPW and WISPW. Jansson *et al.* (1991) indicated that *H. bacteriophora* ('HP88' strain), was more infective than *S. carpocapsae* ('All' strain). Subsequent field tests showed that one application of 'HP88' was as effective in protecting sweet potato storage roots from weevil damage as three applications of the same nematode.

Mannion and Jansson (1992, 1993a, 1993b) also reported that *Heterorhabditid* nematodes (unidentified *Heterorhabditis* sp. FL2122 strain) were the most virulent and pathogenic than many of the *Steinernematids*. *Heterorhabditid* nematodes were more pathogenic to pupae than *Steinernematid* nematodes. Weevil adults were the least susceptible stage to nematode infection. Larval mortality from nematode infection increased significantly over a 4 day period for all nematodes with the exception of *S. feltiae*, which caused high mortality within 24 h. *Heterorhabditid* nematodes produced significantly more infective juveniles per cadaver than did *Steinernematids*. *Heterorhabditis* favoured the sand arena while *S. carpocapsae* preferred the loam soil. Jansson *et al.* (1991) reported that the number of applications of *H. bacteriophora* did not significantly reduce number of *C. formicarius* but did consistently reduce damage to sweet potato storage roots. Nematode application rate had no effect on densities of *C. formicarius* or damage caused; suggesting that a single application early in the growing season was adequate. Damage to plants treated with insecticides was intermediate to that on nematode treated and untreated plants.

Mannion and Jansson (1992b) described the distribution and dispersal of EPNs within the soil rhizosphere. They reported that EPNs could locate and infect the SPW upto a distance of 15 cm within the soil. Among the 5 species of EPNs, *H. bacteriophora* strain HP88 and *Heterorhabditis* sp. strain FL2122 (isolated in Florida) causing more mortality of bait insects (*Galleria mellonella*) than *S. carpocapsae*, *S. feltiae* strain N-27 and *S. glaseri* within the storage roots of sweet potato. All the nematode species were able to move at least 15 cm horizontally from the *C. formicarius* cadavers within the roots in soil in the laboratory. The heterorhabditids and, to a lesser extent, *S. feltiae* and *S. glaseri* (but not *S. carpocapsae*), were able to locate and infect apionids in roots 15 cm from their original source roots. All these species and *S. carpocapsae* Mexican strain were recovered 60 cm horizontally from the point of application (as inoculum) in the field, with the heterorhabditids and *S. glaseri* causing most mortality in the bait insects.

Regarding the dose of the EPNs required, Jansson and Lecrone (1994) mentioned that single application of aqueous suspensions of *H. bacteriophora* HP88 nematodes (4.9 billion infective juveniles/ha) was as effective as application of *G. mellonella* cadavers (83,700/ha) infected with HP88 nematodes in reducing the damage caused by *C. formicarius*. Jansson and Lecrone (1997) observed beneficial interactions between sweet potato varieties and entomopathogenic nematode population within the roots. Studies were conducted on two cultivars, 'Jewel' and 'Picadito' differing in susceptibility to the insect pest. The effectiveness of *S. carpocapsae* 'All' strain and *H. bacteriophora* 'HP88' strain, was compared with that of two chemical insecticides in combination. Number of live and nematode infected weevils was higher on 'Jewel' than on 'Picadito'. Weevil counts were lowest on plants treated with 'All' followed in increasing order by those treated with chemical insecticides, 'HP88' and non-treated plants. Root damage and percent marketable tuber did not differ between cultivars, however, lower damage scores and higher percent of root marketability tended to be found on plants treated with 'HP88' nematodes and chemical insecticides than those treated with 'All' nematodes and non-treated plants. Chemical insecticides sometimes showed fewer efficacies than the entomopathogenic nematodes. Kinoshita and Yamanaka (1998) reported that *S. carpocapsae* registered for turf pest control in Japan was very effective against larvae of *C. formicarius* and was found to be superior to chemical insecti-

cides. Ekanayake *et al.* (2001) reported that *H. megidis* caused significantly higher mortality in larvae, pupae and adults of *C. formicarius* (80-90%) compared to *S. feltiae* or carbofuran in Sri Lanka. Although carbofuran application resulted in considerable reduction in crop damage by the weevil, the degree of control was significantly lower than those of the two nematode species. Banu and Rajendran (2002) reported that *C. formicarius* was infected by EPN *H. indica* in Tamil Nadu. Subramanian (2003) mentioned that *C. formicarius* larva was also an effective source for multiplication of EPNs *in vivo*. The larvae of *C. formicarius* inoculated with 10 infective juveniles per larva yielded higher number of infective juveniles of *H. indica* and *S. glaseri* similar to other hosts *Corcyra cephalonica*, *Dichrocrocis punctiferalis* and *Chilo sacchariphagus indicus*. Yamaguchi *et al.* (2006) suggested that *S. carpocapsae* ('All' strain) would be an appropriate and effective bio-control agent for suppressing *C. formicarius* population. The pathogenicity of *S. carpocapsae* ('All' strain), *S. feltiae*, *S. riobrave* and *S. glaseri* ('326', '328', 'Mungyeong' and 'Dongrae' strains) was tested against the SPW under laboratory conditions. *S. carpocapsae* induced highest mortality in both sexes of the adult SPW (>90%). About 30-60% of pupae and larvae in the root of sweet potatoes were killed by *S. riobrave*, *S. glaseri* ('Mungyeong' strain) and *S. glaseri* ('Dongrae' strain). The efficacy of *S. carpocapsae* ('All' strain) on the suppression of the weevil population was then tested in a sweet potato field. Application of *S. carpocapsae* ('All' strain) successfully reduced the root damage rate and root damage index by approximately 60 and 40% respectively, as compared to root damage in the control fields.

## Entomo-pathogenic fungi

Most entomo-pathogenic fungi (EPF) belonging to Hyphomycetes, Zygomycetes, order Entomophthorales and Ascomycetes attach to a wide variety of insect pests of different crops. These pathogens enter the body of the insect (both larvae and adults) through natural body openings or by direct penetration through the cuticle. Most promising entomo-pathogen infecting SPW is *Beauveria bassiana* (Bals.) Vuill which is used in the form of sprays or in combination with pheromone traps for its successful infection and dispersal of weevils. Su (1989) described the infection process of *B. bassiana* and disease development in SPW. The time taken to form hyphal bodies of *B. bassiana* grown on potato dextrose agar (PDA) media decreased with increasing temperature. The mortality of *C. formicarius* was 85% when treated with  $1.06 \times 10^4$  conidia ml<sup>-1</sup>, and 100% at  $1.59 \times 10^4$  conidia ml<sup>-1</sup> or higher concentration. An internal examination of dead *C. formicarius* after varying inoculation periods showed that the muscle, fat body, tracheal matrix cells and digestive tract were infected. The fat body cells showed disruption 24-48 h after inoculation and were filled with hyphae, 72 h or more after inoculation; 120 h after inoculation, the body cavity was filled with hyphae and conidia. The pathological changes to muscle, tracheal matrix and digestive tract were similar to those observed in the fat body cells. *B. bassiana* found in some of the soil samples in Mt. Seven Stars, Taiwan was found infective to SPW (Su *et al.* 1988).

Burdeos and Villacarlos (1989) evaluated several isolates of other EPF, *Metarhizium anisopliae*, *Paecilomyces lilacinus* including *B. bassiana*. The BPI Tiaong isolate of *M. anisopliae* gave the lowest LC<sub>50</sub> ( $8.42 \times 10^5$  spores ml<sup>-1</sup>). *B. bassiana* was moderately virulent ( $1.54 \times 10^7$ ), while *P. lilacinus* caused very low weevil mortality even at high spore concentration (LC<sub>50</sub>  $3.0 \times 10^8$  spores ml<sup>-1</sup>). *M. anisopliae* and *B. bassiana* caused 50% mortality at 3 and 4 days, respectively. A positive correlation was obtained between soil moisture and mortality of *Metarhizium* treated adults. Soil pH, unlike soil sterilization, showed no significant effect on the pathogenicity of the fungus. Villacarlos and Granados Polo (1989) evaluated the efficiency of *M. anisopliae* for the control of SPW in screen house and field trials

in 1986-88 in Leyte, Philippines. Screen house tests showed that potted plants treated with the fungus produced a higher percentage of uninfested tuberous roots compared with untreated plants, which was comparable with potted plants growing in soil treated with carbofuran. The highest percentage of infected weevils was recorded 3 weeks after application of the fungus. Field application of *M. anisopliae* either as conidial suspension at 4 weeks after planting or as conidia and mycelia on palay substrate at 3 WAP during the rainy season reduced infested roots by 9%. The number of adult weevils was reduced by 48% in treated plots compared with untreated plots. Though *B. bassiana* was the most pathogenic to SPW in the laboratory, other pathogens were also found to be pathogenic viz. *M. anisopliae* var. *anisopliae*, *M. flavoviride* var. *minus*, *Verticillium lecanii*, *P. lilacinus* and *P. fumosoroseus* (Khan *et al.* 1990).

Spraying *B. bassiana* (isolated from *C. formicarius*) at  $1.6 \times 10^4$  conidia ml<sup>-1</sup> at planting and rootstock formation, and broadcasting soybeans containing *B. bassiana* into the rows at planting controlled *C. formicarius* effectively (Su 1991a). Application of *B. bassiana* isolated from honey bees at  $1 \times 10^6$  conidia ml<sup>-1</sup> at planting and rootstock formation gave the best results. The type of soil also influences the success of infection of *B. bassiana* on SPW (Su 1991b). Thirteen out of 80 different soils collected from central and southern Taiwan were pernicious to SPW when they were added at planting and the mortality caused to the SPW by *B. bassiana* was over 80%. Spraying of the fungal pathogen at  $1.6 \times 10^4$  conidia/ml before planting and at the time of storage root formation, broadcasting soybean seeds containing the pathogen and broadcasting of pernicious soils collected in Guan-Miaw into the burrow of the row at planting were effective in controlling the weevil in the field. Hou (1997) and Lagnaoui *et al.* (2000) also reported that *B. bassiana* was lethal to *C. formicarius*. Environmental factors also govern the successful infection of SPW by entomo-pathogens. Yasuda *et al.* (1997) reported that less than 43% relative humidity and temperature below 15°C are not conducive for *B. bassiana* infection to adults of SPW.

Yasuda (1999) developed an auto infection system consisting of a modified sex pheromone trap and a bottle with exit holes containing conidia of *B. bassiana* ( $9.3 \times 10^{10}$  g<sup>-1</sup> medium) was designed and tested to control *C. formicarius* in Japan. Male weevils were attracted to the system by the pheromone and exited the bottle after infection. In the sweet potato field where the systems were placed, the highest mortality of males by the disease was 96.2% on 21 days after treatment and that of females was 33.0% on 49 days. Rates of fungus carrier were 57.9% in males and 31.6% in females. In the treated field, the sex ratio of the SPW population was greatly skewed to females. Females seemed to be infected through mating with fungus carrier males. The advantages of this system are: (i) Attracted males are efficiently auto infected with the fungus, (ii) only SPW is infected by this system, (3) the fungus is easy to mass produce, (4) the cost of this system is low as compared to conventional field sprays and (5) once attracted and infected, males can be expected to return to their habitat which accelerates dispersal of the fungus. Corn oil is used to enhance the infectivity of EPF. Yasuda *et al.* (2000) reported that formulations of *B. bassiana* conidia in a 10% corn oil mixture showed more superior infectivity in both sexes of *C. formicarius* than the formulation of conidia only in laboratory assays.

Low cost and effective technology of production of *B. bassiana* as a cottage level industry to control SPW in Cuba was successfully established and adopted by many farmers (Lagnaoui *et al.* 2000). Use of the fungus is particularly attractive because it relieves farmers of the high cost of chemical pesticides. The use of ants against weevils is another component of the IPM strategy adopted for the SPW in Cuba. Two species of predatory ants, *Pheidole megacephala* and *Tetramorium guineense* (*Pheidole guineensis*) are common inhabitants of banana plantations. Rolled banana leaves were used as 'temporary nests' to transport the ants

from their natural reservoir to sweet potato fields, where they prey upon weevils and other insects. Setting up colonies in the field 30 days after planting with 60-110 nests ha<sup>-1</sup> has kept weevil infestations at low levels (3-5%) (Lagnaoui *et al.* 2000).

The entomo-pathogens are also used for conversion of certain metabolites. *B. bassiana* ATCC 7159, the fungus which is responsible for the muscaridine disease in insects, has been used to affect the bioconversion of many substrates including alkaloids, steroids and terpenes. This strain, formerly known as *B. sulfurescens* or *Sporotrichum sulfurescens*, has also been used to effect the reduction of various carbonyl compounds (Davies *et al.* 1989).  $\alpha,\beta$ -unsaturated xenobiotics e.g. ( $\pm$ )-carvone, have been reduced to their corresponding saturated ketones or alcohols depending on the length of the incubation period (Kergomard *et al.* 1982). *B. bassiana* is also known to selectively hydroxylate non-activated carbon atoms (Goswami *et al.* 1987; Hammoumi *et al.* 1991; Lamare *et al.* 1991; Holland 1992). Incubation of cadina-4,10(15)-dien-3-one with *B. bassiana* ATCC 7159 resulted in the production of nine novel sesquiterpenes which were effective against *C. f. elegantulus* (Buchanan *et al.* 2000). These metabolites were identified as (4*S*)-cadin-10(15)-en-3-one, (4*S*)-3 $\alpha$ -hydroxycadin-10(15)-ene, (4*R*)-3  $\alpha$ -hydroxycadin-10(15)-ene, (4*S*)-3  $\beta$ -hydroxycadin-10(15)-ene, (4*S*)-3  $\beta$ -hydroxycadina-10(15),12(14)-diene, (4*S*)-13-hydroxycadin-10(15)-en-3-one, (4*S*)-12-hydroxycadin-10(15)-en-3-one, (4*R*)-3  $\beta$ ,14-dihydroxycadin-10(15)-ene and 3 $\alpha$ -hydroxycadina-4,10(15)-diene. The allylic alcohol 3  $\alpha$ -hydroxycadina-4,10(15)-diene was also biotransformed to afford cadina-4,10(15)-dien-3-one, (4*S*)-cadin-10(15)-en-3-one and (4*S*)-12-hydroxycadin-10(15)-en-3-one. (4*S*)-cadin-10(15)-en-3-one showed enhanced activity to SPW when compared with the naturally occurring compound cadina-4,10(15)-dien-3-one. An increase in activity was also noted for compounds (4*S*)-3 $\alpha$ -hydroxycadin-10(15)-ene and (4*R*)-3 $\beta$ ,14-dihydroxycadin-10(15)-ene at higher concentrations. The insecticidal activities of the metabolites were compared with that of farnesyl methyl ether. The latter is a potent growth regulating agent at low doses, but is also capable of inflicting high mortality on adult *C. formicarius* at slightly higher concentrations (Mansingh and Steele 1975; Mansingh and Rawlins 1977).

## PHEROMONES

The SPW infest mainly the tubers below the ground level and identification of foliar damage by this insect is difficult since many other chrysomelid beetles also cause similar type of punctures on the leaves. The cryptic feeding habits of the SPW larvae and the nocturnal activity of the adults limit the effectiveness of chemical insecticides used for its control. Though different parts of sweet potato attract weevils, the rate of emission of volatiles from them is very small and hence could not be used for monitoring. Soon after the identification of female sex pheromone (Heath *et al.* 1986), the course of weevil management changed in different parts of sweet potato growing countries around the world. The sex pheromone alone contributed to significant reduction in SPW population and tuber damage and resulted in increase in marketable tubers. When the number of weevils is too large in traps, they are often measured in weight or volume per unit measurement (Villacarlos *et al.* 1992). The potential of the sex pheromone (*Z*)-3-dodecen-1-ol (*E*)-2-butenolate, as a mating disruptant for the control of *C. formicarius* was studied in a sweet potato field (Mason and Jansson 1991; Yasuda *et al.* 1992; Yasuda 1995). Traps captured 65214 males of SPW during one and a half year collection in Japan (Yasuda 1995). The sex ratio of the population in such traps is largely female biased and the mating rate of females is reduced (Yasuda 1995). Sureda *et al.* (2006) found sex pheromone of feral SPW, *C. formicarius elegantulus* from Cuba, via solid phase microextraction analysis, to be identical to (*Z*)-3-dodecenyl (*E*)-2-bute-

noate, a previously reported compound. Females emitted 20 pg pheromone d<sup>-1</sup>. Palaniswami *et al.* (2000) reported that dichloromethane extract of sweet potato periderm contained a pentacyclic triterpenoid compound boehmeryl acetate which attracts both the sexes of SPW. They showed that with 100 mg of pure compound, the attraction was 60.88%. The number of weevils attracted towards the extracts from four sweet potato varieties was also different and it was maximum in the variety 'RS-III-2'. The attraction was high in this variety. The activity of the pure compound as well as the crude extracted lasted up to 18-22 days. The ratio of male and female weevils attracted ranged from 2 to 7:1.

Scanning electron microscopy (SEM) studies carried out on the male antenna of *C. f. elegantulus* (Sureda *et al.* 2006) showed several types of sensilla: sensilla trichoidea of type 1 (ST1) as long hairs (100-150 µm), sensilla trichoidea of type 2 as short hairs (50-60 µm), sensilla basiconica of type 1 as thick pegs (20-25 µm), sensilla basiconica of type 2 as curved pegs (10-15 µm), and sensilla basiconica of type 3 as thin and straight short pegs (15-20 µm). The same types were observed in female antennae but ST1 were far less abundant than in males. Sensilla chaetica were also found on the flagellum sub-segments in both sexes. In electrophysiological tests, the crotonate function in the pheromone structure was proved to be critical for activity since regular depolarizations (0.6-0.8 mV) were obtained with puffs on 1 µg of the attractant.

Weevils caught in the traps differ significantly during different parts of the day. Maximum weevil catch was recorded in the evening (Islam *et al.* 1991a; Yasuda *et al.* 1992; Chiranjeevi *et al.* 2002). Trap catches were greatest in the active tuber development stage of sweet potato, during February-March that coincides with the harvesting time. The high catch of weevil was reported during the late seasons of late February-March, when the potential for weevil damage of tubers was greatest (Mondal *et al.* 2006). Yasuda *et al.* (1992) reported that pheromone traps installed on the ground captured more males than those set above the ground. Most of the males were seen to approach the traps by walking. More males were captured when they were released downwind of the trap than when released from the upwind or other directions. The number of trapped males decreased gradually, thereafter and decreased rapidly after sunrise. Chiranjeevi *et al.* (2002) found that traps installed at canopy level were more effective than those erected at four feet above ground level.

Different types of traps are used for monitoring and mass trapping studies. When the sex pheromone is discovered and synthesized, various designs and types of traps are used for the weevil management viz. light trap, sticky trap, water trap, plastic funnel live traps, etc. (Lingren *et al.* 1980). Hwang *et al.* (1989) determined the efficacy of different formulations of sex pheromone and lure types in the field to determine the efficacy of 3 trap designs for catching the *C. f. elegantulus*. Single funnel, double funnel and netting funnel PET (polyethylene tetraphthalate) bottle traps baited with 1 mg synthetic sex pheromone were more effective at catching adult males of the pest. The males recaptured at varying distances from funnel PET traps baited with 1 mg synthetic sex pheromone were greatest at 5 m (69.1%). At 10, 20 and 40 m, these figures were 10.7, 1.5 and 0.4%, respectively. The success in capturing males was affected by temperature. A synthetic pheromone of SPW was tested in the field in Bangladesh using water pan trap in sweet potato (Islam *et al.* 1991a). A pheromone with 10 µg septum<sup>-1</sup> was very effective in trapping the males within 15 min. Plastic funnel trap and the universal moth trap (Uni-trap) were more effective in collecting *C. formicarius* males than a screen-cone boll weevil trap in southern USA (Jansson *et al.* 1992b). The plastic funnel trap was more efficient (72.8%) than the Uni-trap (58.0%) in capturing marked male weevils released at the base of the traps. These traps, however, were equally effective in preventing adults from escaping (2% escape) and were superior to the screen cone boll weevil trap (43% escape). The distance upto which the

sex pheromone acts depends mainly on the concentration and the number of traps used in unit area. Trap counts increased (although not consistently significantly) with an increase in pheromone dose between 1 µg and 1 mg (Jansson *et al.* 1992a). In a mark release recapture studies in Florida, Mason *et al.* (1990) reported that number of males recaptured decreased with an increase in release distance and decreased with a decrease in dosage of sex pheromone (99.9%) [(Z)-3-dodecen-1-ol-(E)-2-butenolate] at each corresponding distance. Males of *C. f. elegantulus* were capable of traversing distances of at least 280 m in 16 h.

Talekar and Lee (1989) reported that female sex pheromone (Z)-3-dodecenyl (E)-2-butenolate identified in *C. f. elegantulus* was also active in *C. formicarius*. A trap baited with 0.1 mg sex pheromone attracted significantly more number of males than a trap baited with 5 virgin females in Taiwan. A linear dose response was observed between pheromone concentration of 0.001-1 mg/trap and the number of weevils captured in a period of 4 weeks. A water trough with openings on all sides proved to be suitable and economical. A 1 mg sex pheromone sample could capture more than 94.7% of marked males from 10 m and 11.4% from 100 m in 24 h. A 10 µg sample was uniformly active for at least 1 month in the field. Basic aspects such as the optimum concentration of pheromone to be used in field control, the effective attraction range of sex pheromone in the field and the temporal activity rhythm of males of *C. formicarius* were studied in sweet potato fields in Thiruvananthapuram, Kerala, India. Of three concentrations of pheromone tested (0.25, 0.5 and 1.0 mg), 1.0 mg attracted maximum number of weevils and the efficacy of the pheromone declined after 3 months. Mark/recapture studies revealed that all *C. formicarius* released 10 m from the trap were attracted to the pheromone source within 3 days. Trap catches indicated that *C. formicarius* was more active during 18.00 and 06.00 h in both May and October (Pillai *et al.* 1994).

The SPW sex pheromone used in China at 2 traps/667 m<sup>2</sup> (30 traps ha<sup>-1</sup>) with an interval of 15 m between traps reduced tuber damage by 8.5-10.1% whereas in control the damage was 53.1-58.2% (Li Zheng 1998). In Indonesia, marketable yield of sweet potato and damage from weevils were higher during the dry season with a tuber damage of 8.2%, reducing marketable yield from 3.32 to 2.50 kg m<sup>-2</sup>. During the wet season, damage was dropped to 2.1% (Braun and Fliert 1999). Pillai *et al.* (1996) reported that trap containing synthetic sex pheromone [(Z)-3-dodecen-1-ol (E)-2-butenolate] at 1 mg concentration installed in sweet potato field at 1 trap 100 m<sup>2</sup> (100 traps ha<sup>-1</sup>) area was found to be highly effective for mass trapping the male SPW leading to significant decline in the population build up and consequent yield increase. The tuber damage in the pheromone treatment was only 7% against 45.7% damage in the control. The marketable tuber yield was 9.0 t ha<sup>-1</sup> in the treatment while in the control the yield was only 4.7 t ha<sup>-1</sup>.

Integration of sex pheromone traps with insecticides or entomopathogenic fungi were successfully used in recent times. Yasuda *et al.* (2004) developed a new pheromone formulation to increase exposure time to insecticide for control of the SPW. The formulation was a combination of sex pheromone, (Z)-3-dodecenyl (E)-2-butenolate, and an insecticide (MEP) impregnated into a blue ball (2 mm in diameter) made of the diatomaceous soil. The male weevils were attracted to the visual stimulation in addition to the sex pheromone. The attracted males located and tried to mate with the ball. They were thereby efficiently exposed to the insecticide for a longer time. The concentration of the new formulation was extremely low compared to the conventional formulations and, therefore, lowers the cost of application. Hwang (2001) reported that use of pheromone baited traps in the integrated management of SPW is estimated to save 1 to 3 applications of insecticide. In Taiwan, use of pheromone baited traps placed at a density of 4 traps 0.1 ha<sup>-1</sup> reduced damage caused by SPW by 57-65%. Use of such traps in combination with the pre-planting application

of chlorpyrifos (Dursban) granules at 2.25 kg a.i. ha<sup>-1</sup> reduced SPW damage by 62-75%. The effect was comparable to that of two applications of chlorpyrifos (80-85%), one applied just before planting and the other at the time of earthing up. Insecticide efficacy tests on SPW revealed that chlorpyrifos was more effective, persistent and was most effective when it was applied before planting than at the time of earthing up.

In Bangladesh, water pan traps with synthetic pheromone, (*Z*)-3-dodecen-1-ol (*E*)-2-butanoate was used. Dipping the sweet potato vines in 0.01% a.i. phenthoate (Elsan 50EC) before planting combined with mass trapping recorded the lowest number of sweet potato roots (7.08%) compared to mass trapping only (9.18%) and highest in the control plots (17.38%) (Islam *et al.* 1992). Pheromones combined with one application of carbosulfan on 45 days after planting is more effective in controlling the weevils than the insecticide treated 3 times (Kawasoe *et al.* 1993). Pheromone traps (40/ha) reduced sweet potato root damage by 65% and use of chlorpyrifos at 2.25 kg a.i. ha<sup>-1</sup> in addition to the pheromone reduced the damage by an additional 10% over the pheromone treatment (Hwang and Hung 1991).

Stereoisomerically pure pheromone is always not essential to trap the insects (Sureda *et al.* 2006). In a double choice olfactometer, males showed maximum activity between 4<sup>th</sup> and 8<sup>th</sup> h of scotophase at a dose of 50-1000 ng of pheromone. In field tests, a correlation between the contents of the *Z*, *E* isomer in the pheromone formulation and activity was noticed, and baits containing this isomer of stereoisomeric purity above 94% showed highest attraction. The presence of 5% of the *Z*, *Z* isomer in the lure did not induce any synergistic or inhibitory effect and the alcohol precursor of the pheromone was inactive. The results showed that use of a stereoisomerically pure pheromone may not be necessary in pest control strategies. Yasuda *et al.* (1992) reported addition of up to 10% of the (*E*, *E*)-isomer of synthetic sex pheromone (*Z*)-3-dodecenyl (*E*)-2-butenate (1 mg/dispenser) did not inhibit the activity of the pheromone and was attractive to males of *C. formicarius* in a sweet potato field in Japan for more than one month. As the pheromone is used to monitor weevils both in weevil free and infested regions, the use of sex pheromone [(*Z*)-3-dodecen-1-ol-(*E*)-2-butenate] with a purity level above 99% is most appropriate (Jansson *et al.* 1992a).

Yen and Hwang (1990) found improved synthesis of sex pheromone wherein the carcinogenic solvent HMPT is replaced with DMPU. Lo *et al.* (1992) reported an alternative synthesis of (*Z*)-3-dodecen-1-yl (*E*)-2-butenate without using carcinogenic ethylene oxide and hexamethyl phosphoric triamide (HMPT). By coupling the tetrahydropyranyl ether of 3-butyn-ol with 1-bromooctane with sodamide in liquid ammonia, 12-(2-tetrahydropyranyloxy)-9-dodecynoic acid was obtained; subsequent hydrolysis and semi hydrogenation afforded (*Z*)-3-dodecen-1-ol. The alcohol was then reacted with crotonyl chloride to give the desired crotonate with a total yield of 29.8%. Males of *C. f. elegantulus* were strongly attracted to the synthetic sex pheromone and attraction to polyethylene tube dispensers was better than attraction to a rubber septum dispenser. Another sex pheromone *trans*-2-butenic acid-*cis*-3-laurylene ester was also used to trap *C. formicarius* (Chen *et al.* 2001) and its effect lasted for 110 days and up to 15 m. After trapping for one year, the number of male weevils was reduced by 55.4 and 57.7% in Putian and Hui'an respectively, whereas in control the respective values were 69.5 and 72.6%. A dosage of 10 µg of pheromone formulated on methylene chloride extracted rubber septa is adequate for monitoring weevils in southern Florida and the Caribbean. When pheromone is formulated on methylene chloride extracted rubber septa, septa can be used continuously for at least 30 days or longer without a substantial decrease in attractiveness (Jansson *et al.* 1990).

## BOTANICALS

Foliage of *Croton linearis* contain diterpene which showed insecticidal activity to adult *C. f. elegantulus* (72 h LD<sub>50</sub> was 0.32 µg insect<sup>-1</sup>) (Alexander *et al.* 1991). Williams and Williams (1997) isolated sesquiterpene furan, epingaione by guided isolation of the crude ethanol extract of the leaves and stems of *Bontia daphnoides* and is found to have insecticidal properties to *C. formicarius*. The 72 h LC<sub>50</sub> for the furan was 20.80 µg insect<sup>-1</sup>, while those for a nicotine rich extract of tobacco and a commercial organophosphate insecticide, dimethoate, were 32.00 and 9.54 µg insect<sup>-1</sup>, respectively. Williams (1999) evaluated the toxicity of *Rhizophora mangle* (Rhizophoraceae) crude extract and was found most toxic to *C. formicarius* (LC<sub>50</sub> of 70 µg insect<sup>-1</sup>) and the extract is reported to contain triterpenoids. Tobacco decoction (1% a.i.) was toxic to SPW and effective for a period of two weeks (Palaniswami and Mohandas 1996).

Botanical pesticides are effective against insect pests *in vitro* and have their toxic effects on target pests under specific climatic conditions. Pacheco *et al.* (2000) studied the adulticide effect (spray method) of the new bioinsecticide NeemAzal 5 (Trifolio-M, Lahnu, Germany), at a dose of 0.05 % a.i. v/v and found that it did not result in any mortality of adult male or female *C. f. elegantulus* but there was significant reduction in the number of eggs laid and feeding activity. Collins *et al.* (2000) isolated four structurally novel isomeric sesquiterpenes from the aerial parts (leaves and stems) of *Capraria biflora*. Caprariolides A (1), B (2), C (3) and D (4) have been determined by NMR spectroscopy experiments to be the (3*S*,5*S*,9*R*), (3*R*,5*S*,9*R*), (3*R*,5*R*,9*R*) and (3*S*,5*R*,9*R*) isomers of 7-(furan-3'-yl)-3,9-dimethyl-1-oxaspiro [4.5] dec-6-en-2-one, respectively. Both 1 and 2 were found to exhibit insecticidal activity against adult *C. f. elegantulus*.

Buchanan *et al.* (2000) incubated cadina-4,10 (15)-dien-3-one with *B. bassiana* ATCC 7159 which resulted in the production of nine novel sesquiterpenes. These metabolites were identified as (4*S*)-cadin-10(15)-en-3-one, (4*S*)-3 α-hydroxycadin-10(15)-ene, (4*R*)-3 α-hydroxycadin-10(15)-ene, (4*S*)-3 β-hydroxycadin-10(15)-ene, (4*S*)-3 β-hydroxycadina-10(15),12(14)-diene, (4*S*)-13-hydroxycadin-10(15)-en-3-one, (4*S*)-12-hydroxycadin-10(15)-en-3-one, (4*R*)-3 β,14-dihydroxycadin-10(15)-ene and 3 α-hydroxycadina-4,10(15)-diene. The allylic alcohol 3-α-hydroxycadina-4,10(15)-diene was also biotransformed to afford cadina-4,10(15)-dien-3-one, (4*S*)-cadin-10(15)-en-3-one and (4*S*)-12-hydroxycadin-10(15)-en-3-one. These metabolites showed insecticidal activity against *C. f. elegantulus*.

Collins *et al.* (2001) isolated squamulosone (aromadendr-1(10)-en-9-one) from the plant *Hyptis verticillata* and incubated with the fungus *Curvularia lunata* (*Cochliobolus lunatus*) ATCC 12017 in two different growth media. Six metabolites were isolated from each medium, with five of the products being common to both fermentations. All seven metabolites are novel. The insecticidal activity of these aromadendranes was evaluated against the SPW *C. f. elegantulus*. Squamulosone was an effective insecticide after 48 h at a concentration of 60 mg/insect, surpassed only by its 13-hydroxy derivative. All metabolites effected high mortalities after 72 h. It would appear that the insertion of a hydroxyl group at C<sub>13</sub> increases the toxicity of the terpene, however, the opposite is seen when this substituent is placed at C<sub>2</sub> or C<sub>14</sub>. *C. lunata* is well known for its efficient 11b-hydroxylation of steroids (Holland and Riemland 1985; Mazumder *et al.* 1985; Chen and Wey 1990), but it has also been used to transform a number of terpenes (Azerad 2000) including *R*-(+)-pulegone (Ismaili-Alaoui *et al.* 1992), (*R*)-hydrophenanthreneone (Hammoumi *et al.* 1993), a number of 1,6-difunctionalised eudesmanes (Garcia-Granados *et al.* 1991) and an aromadendrane (de Lima *et al.* 1999). Previously, a number of aromadendranes have been incubated with fungi including *Cephalosporium aphidicola* (Hanson *et al.* 1994a), *Glomerella cingulata* (Miyazawa *et al.* 1994, 1995), *Mucor plumbeus* (Guillermo *et al.* 1997), *Beauveria*

*densa* and *B. bassiana* (de Lima *et al.* 1999) and also with a limited number of bacteria (Abraham *et al.* 1992).

Jayaprakash *et al.* (2000) demonstrated the insecticidal effects of petroleum ether fraction of cassava seed extract against *C. formicarius*. Of the three concentrations tested on *C. formicarius*, the mortality was recorded as 17.7 and 86.7% on one day after treatment (DAT) in the batches treated with 1.0 and 5.0%, respectively. On 7 DAT, the mortality in the treatments with 0.1 and 1.0% was 40.0% each and it was complete in the treatment with 5.0%. However, only 22.0% weevils were found dead in the control batch. Facey *et al.* (2005, 2006) stated that the essential oils of leaves and stems as well as fruits of *H. capitata* are dominated by oxygenated sesquiterpenes (>50%) with viridiflorol being the major component. Both oils exhibited comparative insecticidal activity against *C. f. elegantulus*, with 72 h LD<sub>50</sub> of 55 µg insect<sup>-1</sup> (leaves and stems) and 60 µg insect<sup>-1</sup> (fruits). However, the fruit oil had slower action at 24 and 48 h compared to the oil from leaves and stems.

Hexane extracts of leaves and stems of *Cleome viscosa* L. also showed pyrethroid type of contact insecticidal activity on adult *C. f. elegantulus* with 60% knock down effect (Williams *et al.* 2003). However, most of these insects (50%) recovered after 48 h. Formulation of *C. viscosa* extract with piperonyl butoxide (PBO) (0.05% w/v in acetone) enhanced its toxicity to *C. formicarius*. The LD<sub>50</sub> determined for the extract on *C. formicarius* when applied in acetone was 89.0 µg insect<sup>-1</sup>, compared with 43.0 µg insect<sup>-1</sup> for the PBO formulated extract. Their findings further support that the extract could be mediating its weak insecticidal activity via a disruption of the mixed function oxidase (MFO) enzyme system, which includes the P450 detoxification enzyme complex because PBO is a known selective inhibitor of these MFO enzymes (Bowers 1984).

## CHEMICALS

Chemical insecticides were evaluated for the management of weevil which were used in the form of sprays, application at the base in the form of granules and also as vine dipping. Rajamma (1990) reported that fenvalerate (0.03%), permethrin (0.03%) and deltamethrin (0.0003%) were the most effective insecticides; 0.05% heptachlor, chlordane and fenthion, and 0.1% carbaryl plus molasses were found effective to control *C. formicarius*. In another study, dieldrin was found to be most effective compared to the untreated control (46.71%) (Islam *et al.* 1991b), though dieldrin is no more available in developing countries for their use on crops. Rajamma and Pillai (1991) showed that 0.05% fenthion or endosulfan sprays at monthly intervals with or without pruning or soil application of carbofuran or phorate granules at 1 kg a.i. ha<sup>-1</sup>, 45 days after planting were all effective in reducing infestation by *C. formicarius* and increasing marketable tuber yields. Phorate treatment resulted in the greatest cost benefit ratio. Dalvi *et al.* (1992) reported that soil application of carbofuran 3G and phorate 10G at 1.0 kg a.i. ha<sup>-1</sup> (applied at 45 DAP), and drenching with aldrin, fenthion or endosulfan at 0.05% (on 30, 50, 70 DAP each) were equally effective at reducing infestations. However, all the spray treatments failed to control the pest effectively. Significantly higher yields were recorded for treatments with carbofuran 3G (106.94 q ha<sup>-1</sup>) and phorate 10G (91.71 q ha<sup>-1</sup>). Field trials carried out in sweet potatoes cv. LA 206 in Jamaica showed that out of 5 insecticides tested against *C. formicarius*, heptachlor (23 EC) and chlordane (73 EC) were the most effective (Williamson and Murray 1993). Teli and Salunkhe (1993, 1994b) found that dipping of vine cuttings in insecticide solution before planting and spraying the crop 1 month after planting, and a further 3 times at 3 week intervals with cypermethrin or fenvalerate at 375 g a.i. ha<sup>-1</sup>, was most effective in reducing damage caused by the insect. The best cost:benefit ratio (1:1.19) was recorded for fenvalerate followed by cypermethrin and fenthion. Similarly many others have reported some more insecticides to achieve better control of SPW

(Mason *et al.* 1991; Yoshida *et al.* 1992; Sinha 1994).

Palaniswami and Mohandas (1996) suggested soil drenching on 50 and 80, 60 and 90, and 50, 65 and 80 days after planting was equally effective in suppressing the incidence and intensity of weevil damage. Among the single soil drenching, application on the 65<sup>th</sup> day was found to be most effective against the weevil. Persistent toxicity of endosulfan, fenthion and fenitrothion (all at 0.05% a.i.) was high (> 52%) even at four weeks after treatment, while it remained only for two weeks for the tobacco decoction (1% a.i.). The growth stages of the crop did not have any influence on the persistent toxicity of the insecticide. When endosulfan and fenthion were applied in sprays at 500 g a.i. ha<sup>-1</sup>, and carbofuran and phorate as soil applications at 1 kg a.i. ha<sup>-1</sup>, to sweet potatoes in Kerala, India, against the *C. formicarius*, the weevil populations in 2 kg samples of tubers were 120.3, 73.6, 83.6 and 140.2, respectively, compared to 388.4 in control treatment. When endosulfan and fenthion treatments were combined with pruning, it resulted in 92.7 and 86.4 weevils sample<sup>-1</sup>. Corresponding increases in the marketable tuber yield are also observed (Rajamma and Pillai 1987).

Misra *et al.* (2001) mentioned that combination of vine dipping of 0.05% monocrotophos 40 EC and three foliar sprays of 0.05% endosulfan 35 EC was found very effective than that of basal application of phorate 10 G followed by vine dipping of 0.05% monocrotophos. The maximum marketable tuber yield (195.33 q/ha) was also obtained when vines were dipped in 0.05% monocrotophos along with 3 foliar sprays of 0.05% endosulfan at three equal intervals. The spraying of endosulfan at 0.05% was proved quite effective and gave the highest return (Rs 10.10 per rupee invested). The crop which received foliar sprays one month after planting had better protection and produced quality tubers, thereby resulting in the maximum profit over cost incurred compared to insecticidal treatments.

In Taiwan, comparative toxicity and persistence of some commonly used insecticides were tested by mixing them in a sandy loam soil infested with SPW adults (Hwang and Hung 1992, 1994). Chlorpyrifos and fensulfthion granules were more toxic to SPW adults than other insecticides tested, while their persistence was about ten months in the laboratory. Carbofuran granules were of medium toxicity to SPW adults and had a persistence of about six months. Terbufos and phorate granules were less toxic to SPW adults and their persistence was only one or two months. Hwang (2007) found that a moisture content of 5-15% and a pH value of 4-8 in sandy loam soil do not influence the toxicity of insecticide to SPW adults, although it was reduced if the soil had zero moisture content. Sun shine does not affect the efficacy of chlorpyrifos, fensulfthion or carbofuran against SPW adults, but may decrease the efficacy of terbufos and phorate.

Hwang (2001) used pheromone baited traps at a density of 4 traps/0.1 ha and it reduced tuber damage by 57-65% by *C. formicarius*. Use of such traps in combination with the pre-planting application of chlorpyrifos (Dursban) granules at 2.25 kg a.i. ha<sup>-1</sup> reduced damage to tubers by 62-75%. The effect was comparable to that from two applications of chlorpyrifos (80-85%), one applied just before planting and the other at the time of earthing up. Chlorpyrifos application before planting was more effective than application at the time of earthing-up. The use of pheromone baited traps in the integrated management of SPW is estimated to save 1 to 3 applications of insecticide. This involves a saving of several hundred US dollars worth of insecticide. Palaniswami *et al.* (2002) reported that endosulfan, fenthion and fenitrothion each at 0.05% concentration applied as soil drench on 50 and 80 DAP were effective against *C. formicarius*, and their residues in tubers at harvest were lower than the detectable levels. The application of insecticides did not have significant effects on actinomycetes, bacteria and fungi in sweet potato fields. Chiranjeevi *et al.* (2002) found that dipping of vines (45 cm long) before planting, followed by spraying on 30 and 45 DAP, with monocrotophos (0.05%), dimethoate (0.1%), chlorpyrifos (0.05%),

acephate (0.05%) and fenvalerate (0.001%) effectively reduced the vine and tuber damage by *C. formicarius* and increased tuber yields in Hyderabad. Among the insecticides, fenvalerate and monocrotophos were highly effective with cost:benefit ratio of 1:1.9 and 1:1.6, respectively. As few of the insecticides described above were banned in the recent years in India, search for new molecules with very low mammalian toxicity and environmental contamination is important.

## IRRADIATION

Irradiation with gamma rays from different sources is one of the most frequently practiced method for sterilization of the SPW in tubers meant for export purposes and for long storage. Release of irradiated weevils formed the major part of its eradication programmes. Reproduction of over 30,000 adult SPW (*C. formicarius elegantulus*) was prevented with 165 Gy. (Hallman 2004). Dose response tests indicated that the most radiotolerant stage occurring in roots was adult *C. f. elegantulus* (summers). In large scale confirmatory tests, irradiation of 60,000 *C. f. elegantulus* adults at a dose of 150 Gy resulted in no production of F<sub>1</sub> adults, demonstrating that this dose is sufficient to provide quarantine security (Follett 2006).

Effects of gamma radiation on gametogenesis and embryogenesis in SPW were studied histologically (Sakurai *et al.* 1994; Sakurai *et al.* 1998). Spermatogenesis was inhibited by the treatment; the spermatogonia and spermatozoa almost completely collapsed and the number of spermatozoa in the seminal vesicles markedly decreased. In the spermatheca of normal females which had mated with irradiated males, spermatozoa were few. In eggs laid by normal females which had mated with irradiated males, the normal embryogenic process was inhibited; the deposition of cleavage nuclei was followed by the degradation of the blastoderm, vacuolation of the ooplasm and necrosis of the egg cells, showing that gamma radiation resulted in a few abnormal spermatozoa in the males, which caused dominant lethal mutations in fertilized eggs. Gamma radiation of the females resulted in complete inhibition of oogenesis. Oocytes did not develop and pycnosis and collapse of nurse cell nuclei occurred, which resulted in inhibition of oviposition in sterilized females. The susceptibility of reproductive tissue to gamma radiation was greater in females than in males. In the midgut of irradiated adults, an abnormality was observed in the columnar cells of the epithelium. The midgut epithelium cells were damaged by the gamma radiation, which might cause a lowering of nutritive assimilation and result in a short lifespan of irradiated adults. Hayashi *et al.* (1997) reported the longevity of three groups of SPW adults (irradiated with 80 Gy of gamma radiation) which emerged 29-30, 31-32 and 33-34 days after oviposition as 11.0, 11.2 and 11.2 days respectively in males, and 11.6, 12.6 and 14.4 days respectively in females. Detection rates of fluorescent dye were 60.5% in males and 55.2% in females, indicating that irradiated females live longer than males. Hayashi *et al.* (1994) and Ito *et al.* (1993) also demonstrated that SPW adults that emerged from 4 and 6 day-old pupae irradiated at 40 or 60 Gy were completely sterilized. Longevities of adults from 4 and 6 day old pupae were 19 and 31 days for pupae irradiated at 40 Gy, respectively, and 12 and 28 days for those irradiated at 60 Gy, respectively. The longevity of adults irradiated during the pupal stage at any dose was significantly reduced to 33-50% of un-irradiated controls (Ito *et al.* 1993). Adults of *C. f. elegantulus* irradiated with a target absorbed dose of 150 Gy (maximum absorbed dose was 165 Gy) lived for 32 days, while at 32 days unirradiated weevils suffered 57% mortality (Hallman 2001). In Japan, SPW were irradiated with gamma ray 80 Gy on the 27<sup>th</sup> to 28<sup>th</sup> days after oviposition and released up to September 1995 and during September 1996 only a few unmarked weevils were found in sex pheromone traps which indicated that all the irradiated and marked adults were eradicated within one year in a

13 ha restricted area (Setokuchi *et al.* 2001).

Eggs and 3<sup>rd</sup> instar larvae of SPW present in the roots of sweet potato cv. 'Jewel' are more susceptible to gamma radiation when they are exposed to 100 to 1000 Gy. Dose-mortality data were analysed by logistic, Gompertz and normal probit probability density functions with and without logarithmic conversions. Gompertz probability density function analysis gave the best (99.9968%) mortality estimates (and 95% FL) against stages of *C. formicarius* in roots: 37 Gy (35-42 Gy) for eggs (no log 10 conversion); 73 Gy (53-132 Gy) for 1<sup>st</sup> instar larvae (log 10 conversion); 38 Gy (31-55 Gy) for 2nd-instar larvae (log 10 conversion); 28 Gy for 3<sup>rd</sup> instar larvae (no log 10 conversion) and 1497 Gy (963-2229 Gy) for pupae (log 10 conversion). Pupae irradiated with more than 800 Gy produced no adults when efficacy tests were done. Irradiating adults in roots with 1000 Gy did not prevent adult emergence. However, males exposed to 150 Gy and females exposed to 300 Gy were sterile (Sharp 1995).

Irradiation of sweet potato with white and orange flesh differ significantly in their taste and appearance (McGuire and Sharp 1995) which seems to throw light on the use of irradiation as one of the best control strategies for production of orange flesh sweet potato varieties since they are reported to be more susceptible to SPW.

## CONCLUSIONS

Sweet potato farmers are suffering from quantitative and qualitative loss of tubers because of *C. formicarius* infestation in several countries. For its effective management, identification of durable weevil resistance in the existing germplasm is of paramount importance. The existing screening methods for finding weevil resistance are to be strengthened by new approaches like 'volatile assisted selection' in which plants are selected based on the volatiles they release and the volatiles that are attractant or repellent to the weevil. Studies on understanding the complex infestation pattern of sweet potato vines or tubers and influence of climate change on structural and physiological changes in plant and weevil would result in identification of absolute and/or real resistance in plants. It is also essential to identify compounds from leaves, vines and roots of sweet potato from most susceptible and resistant genotypes that are most attractive to male and female weevils so as to design effective management strategies using push pull strategy. While repellent compounds can be used in the monocrops and attractant compounds can be used in periphery of the crop such that the weevil repelled from the centre of the crop are diverted to the periphery of the crop.

Management of SPW reached an important milestone after the discovery of the sex pheromone and it became an important component in the control or eradication programmes in the West but not in the South. Sweet potato weevil became an important insect pest in the fields of poor and marginal farmers in developing countries like India, where sex pheromones are not commercially produced or available. Identification of SPW resistant germplasm or sources could not be possible even after introduction of *Agrobacterium* plasmids into the cultivated species. The transformed plants were either unable to express the gene of interest or the weevil is tolerating the genes by production of defensive enzymes. Studies are required to identify more sources of resistance and the possible mechanism of action of plasmids in the plant and weevil. With the recent ban of endosulfan in India and the banned/restricted use of monocrotophos, carbofuran, methomyl, quinalphos and several other chemicals on horticultural crops, it is high time to focus on screening of new molecules, combination products and safer formulations in the form of tablets (example, deltamethrin 2.5 g tablet covers 400 m<sup>2</sup> area) for effective management of the weevil. Until the new formulations and interventions are ready, farmers would adopt cultural, botanical and other feasible methods that suit to their incomes and farm holds.

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