Session 7D

Efficacy of Biological Control, Using Living Organisms and Natural Products

Effective Biocontrol/socioeconomic Benefit

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Socio-economic benefits of some classical biological control projects in Africa

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Once society decides that an invading species should be combated by biological control, usually after all other measures have proven futile, foreign exploration is initiated to find specific antagonists in the purported home land of the introduced pest. The collected species are quarantined for the study of possible negative effects. Today, polyphagous species, antagonists of useful species, but also those harming unspecified indigenous organisms are excluded. No vertebrate or mollusc predators can be introduced anymore. The species are studied, released, and monitored for their establishment, spread, impact on the target organism and impact on society, including economic impact. The permanent lowering of the population density of the pest is accepted as impact. For an understanding of the mechanisms, life table analyses are needed. Further insights were gained by studying the host-seeking behaviour of ovipositing females. Such data form the basis for population dynamics models, whereby model runs with and without introduced enemy give a direct measure of impact. Such models can be expanded to describe the economic impact. Here we describe impact studies of a different type concerning three biological control projects, executed by IITA in collaboration with numerous international and national partners.

1) Cassava mealybug of South American origin was introduced in the 1970s, and spread across the continent. Between 1981 and 1995, a specific encyrtid parasitoid (*Anagyrus lopezi*) has been released in about 150 localities and monitored across most of sub-Saharan Africa. Within two to four years of its establishment, mealybug populations collapsed, peaks were lowered 10 times, with complete control recorded in about 95% of fields. The plants of the remaining 5% of fields invariably grew on pure sand without mulch. By 1995, the parasitoid had reached all infested areas on the continent; the mealybug itself has not yet spread to Madagascar and further to Asia (Neuenschwander, 2001). The economic analysis (Zeddies *et al.*, 2001) consisted in tabulating, country per country, the size of the cassava producing area, split into forest, savannah and highlands, and calculating the potential production. For each country, the year the mealybug first appeared was registered and computed with an 80% harvest loss. Within five years, these losses were computed to decline by half because farmers adopted more tolerant varieties and indigenous general predators (mostly coccinellids) attacked peak populations. Yield losses were tabulated according to field data to reach a 10-times reduction, resulting in a remaining loss of 4% for the savannah, for instance. The reduced loss in dry weight was translated into $-terms according to four scenarios: a) farmers produce more cassava by expanding their fields, b) cassava is imported, c) the food gap is compensated for by maize, and/or d) the food gap is filled by imported maize through food aid. All calculations were computed with a 6% discount rate, the investment base being 1994. Calculations were tabulated for 40 years; recalculations covering 100 years gave only marginally different results. Other sensitivity analyses assumed changes in prices, discount rates, and even a never documented loss of efficiency of *A. lopezi*. The results were compared with equally discounted costs by IITA and its collaborators and the governments (farmers incurred no costs!). The results, covering 27 countries, indicated total benefits of between about nine and 14 billion US$ and benefit:cost ratios of 170 to 430, depending on different scenarios.

2) Mango mealybug, of Indian origin, invaded West Africa in the 1980s. Between 1987 and 1993, two specific encyrtid parasitoids, *Gyranusoidea tebygi* and *Anagyrus mangicola,* were released on 40 occasions and spread to 14 countries. The parasitoids drove their host to local extinction. Behavioural studies revealed how the two species co-existed. The socio-economic study (Bokonon-Ganta *et al.*, 2002) was based on three surveys, from 1989 to 1991, of 300 mango producers, selected at random from registers in all six provinces. Respondents who had only young trees and the lowest score on trustworthiness were excluded, leaving 142 representative producers. In 1999, a final survey was made covering the same trees to assess the impact of the biological control program. Since no new technologies (irrigation, fertilizer, varieties) had been introduced, changes in production were attributed to the observed changes in mealybug densities. Calculations were based on a 10% discount calculated over 20 years. The results indicated a gain due to biological control of $328 per year per producer or, extrapolated over all of Benin over 20 years, of $531 million, with a benefit:cost ratio of 145. Other countries profited considerably as they did not have to bear the costs of research, parasitoid production, release, and monitoring.

3) Waterhyacinth, a floating water weed of South American origin, invaded Benin in the late 1970s. From 1991 to 1993, three specific natural enemies were released in Benin, of which only the weevil *Neochetina eichhorniae* became important. This was part of an Africa-wide effort involving many institutions, which resulted also in the dramatic control of water hyacinth on Lake Victoria. In Benin, a local reduction to 5% cover was documented; but overall impact was slow, reducing water hyacinth from a serious to a moderate pest. In 1999, the economic impact in Benin was assessed based on interviews of 192 households in 24 randomly chosen villages in the Ouémé valley (de Groote *et al.,* 2003). Infestation by water hyacinth had reduced income of about 200 000 people by $84 million. The increase of water cover was credited with an increase in income of $30 million per year and the accumulated present value amounted to $260 million for Benin alone. Additional benefits to other countries and to the environment, human health, and still increasing biological control impact were not yet taken into account.

In Africa, biological control of the few projects that have been evaluated with modern economic techniques has contributed substantially to plant protection, on a scale comparable to the benefits from vast crop improvement programs (Neuenschwander, 2004). Many more programs resulted in total or partial control of pests and weeds. Yet, institutional support for this approach is generally declining; why?

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Socio-economic implications of cashew nut shell liquid and cypermethrin in protecting cowpea, *Vigna unguiculata* (L.) Walpers against field insect pests

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Introduction

Cowpea is an important crop in tropical regions particularly in West and Central Africa. The green parts of the plant are used as a vegetable or as fodder for cattle. The seeds contain a high amount of protein and B-vitamins and help to prevent starvation among low resource farmers and the poor urban population. Cowpea is a crop that is vulnerable to insect pests at almost every stage of its development. Insect infestation causes a change in protein quality, a reduction in the contents of vitamins of the B-complex, starch, energy and non-reducing sugars while contents of crude fibre, cellulose, hemicellulose, lignin and uric acid increase. On this basis, several approaches have been adopted in its control. Research had centred primarily on the use of synthetic insecticides but in recent years it has been turning more towards selective natural pesticides, including plant extracts that are generally perceived to be safer than synthetic pesticides. The use of cashew nut shell liquid (CNSL) has been gaining more attention due to its active phenolic compounds, anacardic acid and cardol which also have corrosive and abrasive properties. It has been demonstrated that low concentrations of about 0.5% ethanolic extract of CNSL could be effective in preventing oviposition in *Callosobruchus maculatus*, Ofuya & Fayape (1999) as well as in the protection of okra *Abelmoschus esculentus* L. against *Podagrica* beetles (Olotuah, 2003). This paper reports the results of two field trials at Adekunle Ajasin University, Akungba-Akoko on the comparative control of cowpea using a protectant of plant origin, CNSL and a synthetic insecticide, Cymbush 10EC (100g cypermethrin per litre).

Aims and objectives

To determine a suitable protective concentration of CNSL that is non toxic to plant foliage, to test its efficacy in controlling field insect pests of cowpea (*Vigna unguiculata*) and to determine the effect of the selected formulation on the crop as well as its yield. Also to compares the performance of the selected concentration of CNSL to a recommended synthetic insecticide, cypermethrin, and estimate a cost-benefit analysis of using CNSL.

Materials and methods

CNSL was obtained from a cashew-processing factory in Owo, Ondo State, Nigeria. Ethanolic formulations of 0.01%, 0.1%, 0.25%, 0.5% and 1.0% were made for screening in the screen house and the most effective concentration (1.0%) was subsequently transferred to the field for evaluation with cypermethrin in controlling cowpea against insect pests. Normal agronomic practices were observed and randomized block design was used. Cypermethrin was applied at a recommended reduced dosage of 25 ml in 20 litres of water for comparison with the selected 1.0% CNSL. Spray treatment was adopted fortnightly for eight weeks. Natural infestation of experimental plots by the insects was not monitored. The leaves were assessed for the presence of *Ootheca mutabilis*, *Aphis craccivora* and *Maruca testulalis*, while 20 flowers were randomly selected from the outer rows of the two spray treatments to determine insect presence or injury. At maturity, ten pods were randomly harvested from the two spray treatments plots to determine insect entry or exit holes, from which scores were obtained for the pod load and pod damage. The performance of CNSL was expressed on the pods using a pod evaluation index, [PL x (9 –PD)], developed at IITA where scores were taken on Pod Load (PL) which measures the degree of successful pod production and Pod Damage (PD) as represented by entry holes and the presence of frass, both using a 1 – 9 scale (1 representing no damage while 9 represents 100% damage) which is also applicable to pod load.

The efficacy of the two spray treatments was compared using Henderson Tilton's formula: % Efficacy = (1 – (Ta/Ca) × (Cb/Tb)) × 100 where Tb = Infestation in the treated plot before application (1.0 % CNSL or cypermethrin); Ta = Infestation in the treated plot after application (1.0% CNSL or cypermethrin); Cb = Infestation in the check plot before application; Ca = Infestation in the check plot after application

All data were subjected to ANOVA and means compared using LSD values at the 5% level of probability of Tukey's Honestly Significance Test. Counts were normalized by square root and arc sin transformations $(x + 0.5)^{1/2}$

Results and discussion

1.0% CNSL selected for comparison was observed to show the highest mortality rate with minimal toxicity to the leaves in the screen house. Table 1 shows that, at the different levels of infestation in cypermethrin and extract protected plants, there were no significant differences $P < 0.05$ in the yield and yield parameters while the highest infestation was observed in the control. Thus, a direct relationship exists between insect count and other parameters. In its local extraction, 50kg of cashew nut costs £3 from which at least two litres of the pure liquid can be extracted from the shell and ethanol costs less than £1 per litre. This is much more economical than cypermethrin that costs about £10/litre. In protecting an equal area of plot, the use of CNSL is cost effective than the use of cypermethrin. Moreover, cashew nuts are readily available and its liquid, although viscous, has not been reported with toxic residues in crops. It has also been tested and found successful as bactericide and confirmed to be active in pest control. For optimal use, since the liquid does not dissolve in water, it is recommended that in the future, other solvents could be evaluated.

Table 1: Cumulative means of parameters of Cowpea in the two field trials

Treatment	Mean insect count	% Flower infestation	Pod load	Pod damage	Pod evaluation index (Ipe)	Mean seed count	Converted (Ipe) Values
1.0 % CNSL	$14.2±3.6^a$	$0.1±0.0^a$	$7.5±0.5^b$	$1.7±0.1^a$	$54.5±2.5^b$	$1598±14.4^b$	6.8^b
Cypermethrin	$7.6±3.7^a$	$0.06±0.0^a$	$8.0±0.1^b$	$1.5±0.1^a$	$60.1±3.6^b$	$1624±12.5^b$	7.5^b
Control	$47.2±2.5^b$	$0.28±0.1^b$	$5.0±0.0^a$	$2.4±0.0^b$	$32.6±2.1^a$	$488±6.8^a$	4.1^a

Means with the same letter are not significantly different (5 %, Tukey's test).

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Evaluation of the efficacy of an indigenous Peruvian entomopathogenic nematode *Heterorhabditis* sp. to control the Andean potato weevil *Premnotrypes suturicallus* Kuschel under field conditions

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Introduction

The Andean potato weevil (APW) (Coleoptera: Curculionidae) is one of the most important pests of potato (*Solanum* sp.) and other root and tuber crops cultivated in the Andean region; its distribution extends from Argentina to Venezuela covering a wide mountainous territory about 5,000 km in length between 2,800 and 4,700 m a.s.l. Most of the APW species occur in the highlands of Peru and Bolivia, which are considered the centre of origin. The species *Premnotrypes vorax* Hustache, *P. suturicallus* Kuschel and *P. latithorax* (Pierce) are the most important species with regard to distribution, predominance and damage caused. Most damaging are APW larvae, which feed and develop in potato tubers. At present, the application of highly toxic insecticides (1a and 1b according to the WHO classification) is the most frequent control method applied by farmers. Mean damage rates vary between 16 and 45% while without insecticides up to 100% of tubers might be damaged. In 2003, an entomopathogenic nematode of the genus *Heterorhabditis* was discovered in Peru that proved to be highly pathogenic to all developmental stages of *P. suturicallus*. We tested the hypothesis that entomopathogenic nematodes could be integrated into APW management programs. A series of experiments were performed in the Central Highlands of Peru over a period of three years to determine the efficacy under (i) semi-field conditions with controlled numbers of APWs released per potato plant, and (ii) under field conditions with natural APW infestation and applications of nematodes in aqueous suspensions and infected cadavers.

Material and methods

The semi-field trial was conducted at CIP's experimental Station in Huancayo, at 3,200 m a.s.l. The on-farm field experiment was realized in El Mantaro, Jauja at 3,300 m a.s.l. Both locations are in the Department of Junin, Peru. The semi-field experiments were conducted in 1 m^2 field plots, which were covered with 1 m^3 metal-framed boxes fully covered with nylon mesh. In each plot four seed tubers of the potato variety Revolution were planted. After emergence, each plot was infested with 12 couples of recently emerged APWs. The field experiment was conducted on-farm with a high natural infestation by APW. Minimum air temperature reached 4.7°C with maximum values of 18.5°C and rainfall of 706 mm during the cropping season from November to May. Seed tubers of the potato variety Tomasa were planted in plots of 5 x 3 m (15 m^2) with five rows and 10 tubers per row. All experiments were carried out in a complete randomized block design with four and five repetitions, respectively. The treatments consisted of (i) nematodes applied in aqueous suspension at a rate of 50 infective juveniles/cm^2, (ii) nematodes applied in two infected cadavers of the wax moth *Galleria mellonella* (L.) per plant, (iii) insecticide application with Carbofuran at a rate of 2.5 ml/l, (iv) untreated control with an application of water.

The aqueous suspension of nematodes was applied at the neck of the potato plants with a manual backpack sprayer using 2 and 5 l of water for the 1 m^2 and 15 m^2 field plots. The first application was carried out at the time of tuberization and repeated in 30-day intervals with a total of three applications. Parameters assessed at harvest included tuber infestation and APW larvae infection with nematodes.

Results

In both experiments, the native nematode *Heterorhabditis* sp. applied in suspension or as cadavers of *G. mellonella* larvae significantly reduced tuber damage and plant infestation by APW larvae (Table 1). Compared to the control, in both experiments tuber damage could be reduced by 65% and 41.4% and the larval infestation by 76.9% and 53.2%, respectively. The proportion of nematode-infected larvae reached 57.4% in the semi-field experiment and 34.9% in the field experiment.

Table 1. Efficacy of *Heterorhabditis* sp. in controlling APW *P. suturicallus* applied as suspension (50 infective juveniles/cm^2) or cadavers of infested *G. mellonella* larvae.

Treatments	Infested tubers (%)			Efficacy (%)	No. larvae/plant			Efficacy (%)	Nematode -infected larvae (%)
Semi-field experiment									
Suspension	20.8	b	(5.4)*	65.0	3.3	b	(1.5)*	76.9	57.4
Cadavers	26.5	b	(6.2)	55.4	4.8	b	(0.8)	69.6	45.4
Carbofuran	24.7	b	(7.4)	58.7	6.3	b	(1.7)	56.0	
Control	59.5	a	(5.9)		14.3	a	(3.9)		
Field experiment									
Suspension	42.9	b	(9.2)*	41.4	7.3	bc	(1.8)*	53.2	34.9
Cadavers	47.9	b	(10.4)	34.6	9.9	ab	(2.2)	36.4	34.0
Carbofuran	6.4	c	(1.9)	91.3	1.9	c	(0.2)	93.1	
Control	73.2	a	(7.5)		15.6	a	(3.8)		

*Standard Error. Means with the same letters are not significantly different at $P \leq 0.05$

Discussion

For the use of entomopathogenic nematodes in APW control two strategies are potentially suited: (i) reducing the APW population during the potato-growing season by infecting different larval stages, thereby reducing tuber damage at harvest, as demonstrated in the experiments, and (ii) by infecting L4 larvae after harvest especially in soils of former potato heaps which are important over-wintering and infestation sources of APW. While for the second strategy a community-level production may be sufficient (which we are going to test), the first strategy requires large-scale production technologies for nematodes. Future research will assess application frequency and timing with the objective of reducing the amount of inoculum. *Heterorhabditis* sp. also proved to be pathogenic to species of the potato tuber moth complex (e.g., *Phthorimaea operculella* Zeller). This could make its application in potato more economic. Furthermore, small-holder potato production systems of the high Andes could profit from a wider use of nematode-based bioinsecticides in high-value crops, like asparagus (*Asparagus officinallis* L.) along the Peruvian coast. Recently, *Heterorhabditis* sp. and *Steinernema* sp. have been identified from this region.

Brassica carinata as a biofumigant to control *Phytophthora* spp. in strawberry fields

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Introduction

Biofumigation as a nonchemical alternative method to control soil-borne pathogens is based on the action of volatile compounds produced by the hydrolysis of *Cruciferae*, essentially isothiocyanates (ITCs). Different species of *Brassica* are used as biofumigants because of their different concentrations and types of ITC emission that differ in their toxicity against pathogenic fungi (Angus *et al.* 1994; Kirkegaard & Sarwar, 1999). The objectives of this work were to determine the biofumigant effect of *Brassica* spp. on the *in vitro* growth of *Phytophthora* spp., as well as to evaluate its control of the fungus in soil and its effect on field production of strawberry.

Materials and methods

The following *Brassicas* were tested in an *in vitro* bioassay: *Brassica juncea*, *B. nigra*, *B. carinata*, *B. rapa*, *B. oleracea* and *Raphanus sativus*, selected for their high glucosinalate content. Three sowings, T1 October, T2 January and T3 April, were tested. Different stages of the plant life cycle were selected: vegetative growth (V), preflowering (PF) and formation of siliquas (S). Different amaounts of fresh shoot tissue were macerated in a food processor (5-30 g) and tested in glass jars (Mayton *et al.* 1996). Two *Phytophthora* spp. isolates, P4M and P10M (from I.F.A.P.A Centro 'Las Torres' culture collection) from diseased strawberry plants were used. PDA plates with *Phytophthora* spp. isolates, were inverted into the jars containing the macerated plant tissue and sealed. The colony radial growth was measured daily and the treatments were considered "suppressive" if the radial growth was <50% of the unamended control (Mayton *et al.* 1996).

Field experiments were conducted in an experimental strawberry farm located in Moguer (Huelva, SW Spain). Plots, never previously treated with methyl bromide, were naturally infested with *Phytophthora* spp. Biofumigation was done with the stage and species of *Brassica* most effective obtained in the bioassays results: *Brassica carinata* in siliquas (S) sowing in May (T3). Culture practices in strawberry field biofumigation in Huelva is done in July. At this time, July, *B. carinata* sowing in May (T3) was in S stage. *Brassica* was incorporated into the soil at a rate of 10 $Kg.m^{-2}$ at 10 cm depth and then plastic-covered. A randomized completed block design with eight replications was used. Quantification of *Phytophthora* propagules in soil was done in semiselective P_5ARP medium.

Results and discussion

The results in T1 and T2, showed that *B. juncea*, *B. carinata* and *B. nigra* were the species with major suppressive effect. *B. juncea* had the greater biofumigant effect with the P4M isolate while with the P10M isolate it was *B. carinata* (Table 1). *B. nigra*, *B. carinata* and *B. juncea* were significantly different according to the biofumigant stage. S stage was the most effective. The S stage of *B. carinata* used for biofumigation in the field, was the most effective at all three sowing dates (October – April).

Table 1. Radial growth (% of control) of *Phytophthora* spp. isolates exposed to *Brassica* spp. with all stages. Data are means of four replications. Mean values followed by different letters indicate significantly difference according Tukey's multiple range test, $P < 0.01$.

Brassica spp.	*Phytophthora* spp. isolates	
	P4M	P10M
B. carinata	11.31 a	19.21 a
B. juncea	6.63 a	25.32 ab
B. nigra	13.25 ab	30.07 ab
B. rapa	25.09 bc	34.82 bc
B. oleracea	36.34 cd	47.31 cd
R. sativus	38.97 d	54.05 d

The field trials showed that *B. carinata* reduced the population of *Phytophthora* in soil in relation to the control, especially at first of the season critical moment for the establishment of strawberry plant (1 month after plantation) (Table 2). Moreover biofumigation with *B. carinata* significantly increased strawberry yield and fruit weight relative to the untreated control (Table 2).

Table 2. *Phytophthora* spp. soil population (in November); total accumulated strawberry yield and mean fruit weight from February to May. Data are means for eight replications. Letters indicate a significant difference, Tukey's multiple range test, $P<0,001$.

	Phytophthora spp. soil population (CFU/g soil)	Strawberry yield (g/plant)	Fruit weight (g/fruit)
Biofumigation	0 b	980.25 a	24.73 a
Control	64.62 a	737.73 b	17.32 b

The current work, supported by INTERREG ANDALGHORT Project FEDER, has demonstrated the existence of differences in the biofumigant effect *in vitro* depending on *Cruciferae* tested. *B. carinata* has demonstrated to be effective in the control of *Phytophthora* spp. as alternative to the traditional use of chemicals in strawberry production increased yield and individual fruit weight.

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