(T.longibrachiatum) showed the maximum inhibition compared with other *Trichoderma* species.

Production of diffusible antibiotics. This study was done following the method of Dennis and Webster (1971a). The plates were incubated for two days and the colony diameter and sporulation of Af 11-4 were compared with the control. Fifteen of the 39 *Trichoderma* isolates showed inhibition of Af 11-4 colony by producing diffusible antibiotics compared with the control. Colony diameter of Af 11-4 in the control plate was 55 mm compared with 10-50 mm in plates with *Trichoderma* isolates. Isolate T 29 *(T.pseudokoningii),* T 42 *(T.harzianum),* and T 83 *(T.koningii)* showed significant inhibition of Af 11-4 growth.

Hyphal interaction. This study was done following the dual culture method (Dennis and Webster 1971c). A block of cellophane (10 mm x 20 mm) was cut from the juncture of the two colonies and mounted in trypan blue-lactophenol, and examined under microscope for hyphal interactions. Isolates T 16 (71 *viride),* T 109 *(T.harzianum),* and T 188 (T.*viride)* showed clear hyphal coiling with Af 11-4 mycelia.

The data of inhibition zone and morphological characters were subjected to average linkage cluster analysis using Euclidian distance as dissimilarity association of GENSTAT Statistical Package (Rothamsted Experiment Station, Herpenden, Herts, UK). The dendrogram prepared from the above classified the 39 *Trichoderma* isolates into six groups (Fig. 1). Further studies are in progress to determine the biological control potential of these isolates against *A. flavus.*

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Mechanisms of Resistance to Groundnut Rosette

P J A van der Merwe[1], P Subrahmanyam[1], F M Kimmins[2], and J Willekens[3] (1. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), PO Box 1096, Lilongwe, Malawi; 2. Natural Resources International, Chatham, Kent, ME4 4NN, UK; 3. NRI/University of Greenwich, Chatham, Kent, ME4 4TB. UK)

Groundnut *(Arachis hypogaea)* is an important crop in sub-Saharan Africa (SSA) and is mostly grown by smallholder farmers as a subsistence crop under rainfed conditions. Groundnut rosette is endemic to SSA and the impact of the disease can be devastating under the conditions that favor epidemics (Subrahmanyam et al. 1991, 1997, Naidu et al. 1999a). During the 1999/2000 cropping season in Malawi, the average rosette incidence on a national scale was 21.1%, estimated to cause crop losses of nearly US$ 10 million.

The causal agents of rosette are groundnut rosette assistor virus (GRAV), groundnut rosette virus (GRV), and satellite RNA. GRAV is asymptomatic but it acts as a helper virus in vector transmission of GRV and its satellite RNA as they must be packaged in the coat protein of GRAV to form particles that can be transmitted by the aphid, *Aphis craccivora.* The satellite RNA is largely responsible for symptom expression and depends on GRV for replication while GRV depends on satellite RNA for aphid transmission. However, GRV can replicate independently. All three agents must occur together for transmission by the aphid vector and subsequent disease development. Prolonged probes by the aphid vector are

needed for the inoculation of GRAV-containing particles into the phloem, where the virus can replicate, while particles containing GRV and satellite RNA can be inoculated into mesophyll cells during short probes (Naidu el al. 1999b). Detailed studies are in progress to study the complexities of vector transmission and mechanisms of aphid resistance. GRV can be readily transmitted by mechanical inoculation and by grafting (Reddy 1985).

Methods have been investigated to manage groundnut rosette including pesticide applications to control the aphid vector and cropping practices, which enable the groundnut crop to escape or evade aphid infestation in the early stages of crop establishment. Given the economic and labor constraints of smallholder groundnut farmers in SSA, host-plant resistance is the most cost effective and environmentally-benign way to control any groundnut disease including rosette. Sources of resistance to rosette were first identified in late maturing Virginia types in West Africa. The West African sources of resistance are directed against GRV and its satellite RNA (Bock et al. 1990). Several breeding lines with field resistance to rosette were developed at ICRISAT-Malawi. One of the improved varieties, ICGV-SM 90704, was released as Serenut 2 in Uganda in 1999. It was also released in Malawi in 2000. Resistance to *A. craccivora* was first identified in the genotype EC 36892 by Padgham et al. (1990). They showed that resistance limited population increase of the aphid. Subrahmanyam et al. (2000) identified a short-duration rosette resistant genotype, ICG 12991, from the world germplasm collection. Naidu et al. (1999c) later established aphid resistance in ICG 12991. The objective of this study was to understand the differential expression of rosette resistance in some groundnut genotypes by the vector and mechanical transmission of the virus complex.

Grafting experiments

Grafting rosette-infected branches (scions) onto a healthy rooted plant (stock) can result in virus transmission without the vector. Three genotypes (ICGV-SM 90704, ICG 12991, and JL 24) were sown in pots in a glasshouse in 10 replications arranged in a randomized complete block design. Branches from rosette-infected plants (cv Malimba) were used as scions and grafted onto 23-day-old healthy stocks of the three varieties. The plants were checked daily for disease symptoms. Eighteen days after grafting all the new shoots of ICG 12991 and JL 24 stocks showed severe rosette symptoms. This indicated that the two lines were susceptible to GRV and satellite RNA, although no testing was carried out for GRAV.

These results of the reaction of ICG 12991 contradicted field trial observations in 1997-2001. Under high rosette incidence at the Chitedze Research Station in Malawi, ICG 12991 and JL 24 expressed 1% and 45% disease incidence respectively (van der Merwe and Subrahmanyam 1997) compared to the severe symptoms (100%) observed in grafting experiments. Forty days after grafting, however, only 20% of ICGV-SM 90704 plants showed mild rosette symptoms. This can be explained since ICGV-SM 90704 is resistant to GRV and its satellite RNA and the resistant reaction of this variety has been consistent under laboratory and field conditions (trials from 1994 to 1996). Across 24 sites in Malawi ICGV-SM 90704 showed 2% rosette incidence compared to the local variety Chalimbana which showed an average of 42% incidence (Chiyembekeza et al. 2000).

Aphid performance on the test genotypes

The grafting experiment showed that ICG 12991 is susceptible to GRV and satellite RNA. The differences in the rosette incidence recorded from graft transmissions and the field observations may involve resistance to *A. craccivora.* Therefore an experiment to assess vector performance on the three test genotypes was carried out.

ICGV-SM 90704, ICG 12991, and JL 24 were sown in pots in a glasshouse and 30 days after sowing, young leaves were exposed to five viruliferous *A. craccivora* alatae (winged). After inoculation each pod was covered with a crisp bag. Seven replications were arranged in a randomized complete block design. Aphids were counted 10 days after infestation (DAI) on each plant and plants were sprayed with dimethoate. Exposed plants were left in a glasshouse up to 60 days after infestation to record rosette symptoms. Results indicated highly significant differences *(P* <0.001) in aphid population counts between the three varieties. At 10 DAI, increased numbers of aphids (alatae plus nymphs) were observed on ICGV-SM 90704 and JL 24 with an average of 93 and 96 aphids per plant respectively. In contrast aphid number on ICG 12991 fell from 5 to 3 per plant. The increased numbers of aphids on both ICGV-SM 90704 and JL 24 indicate susceptibility to aphids while the reduction on ICG 12991 is an indication of resistance to aphids. These results support the report by Minja et al. (1999). There were also significant differences in disease expression al 60 DAI since JL 24 showed 100% disease incidence while no symptoms were noted on ICG 12991. Once again, only mild symptoms were observed on ICGV-SM 90704.

The mechanism of resistance to groundnut rosette is different in ICG 12991 compared to ICGV-SM 90704

and it is proposed that under field conditions, aphid resistance is responsible for low rosette incidence in ICG 12991, while resistance to GRV is responsible for low rosette incidence in ICGV-SM 90704.

It is difficult from the present screening trials to distinguish between virus and vector resistance because the criterion used is based on presence or absence of rosette symptoms and does not involve aphid population counts. Given the large numbers found in aphid colonies (range 2-1,000 individuals) and the relatively small size of the individual insects, such counts will not be practical, although rough estimations of colony sizes can be made (W W Page, NRI/University of Greenwich, UK, personal communication). However, genotypes with resistance to both virus and the vector are expected to be more stable than those to either one of the components. It is therefore suggested that field screening tests using disease incidence as a measure of resistance should be complemented by laboratory screening to the vector. Diagnostic methods are now available for the detection of each of the three causal agents by using reverse transcription-polymerase chain reaction (RT-PCR) (Naidu et al. 1998). RT-PCR is an efficient and specific method for detecting the asymptomatic GRAV in groundnut although it can also be detected using a triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) (Rajeshwari et al. 1987). Studies are also under way to identify molecular markers associated with resistance to GRV and possibly the aphid vector (L Herselman, Agricultural Research Council, South Africa, personal communication). Application of these techniques should facilitate the rapid identification of resistance to rosette or the mechanism governing the resistance.

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Entomology

Biopesticidal Effect of Neem and NPV on Production Potential and Behavior of *Rhynocoris marginatus* to Groundnut Pest *Spodoptera litura*

K Sahayaraj (Crop Protection Research Unit, Department of Zoology, St. Xavier's College, Palayamkottai 627 002, Tamil Nadu, India)

Rhynocoris marginatus is an important reduviid natural enemy of groundnut (*Arachis hypogaea)* pests (Sahayaraj 2000). It can be used in the groundnut pest management (Sahayaraj 1999). Botanicals, particularly neem products and nucleopolyhedroviruses (NPVs) are ideally suitable for integrated pest management (IPM). Generally, biopesticides are considered safe to natural enemies of the target pests. However, recent studies (Jhansilakshmi et al. 1998) have shown that biopesticides are toxic to some natural enemies. It is therefore necessary to understand the effect of biopesticides on predation potential of *R. marginatus.* This paper presents the results of laboratory experiments on the effect of biopesticides Vijayneem, Nimbicidine, and NPV (Spodoptera) [NPV(S)] were evaluated for their effect on *R. marginatus* behavior and biocontrol potential.

Both the predator and pest were collected from Tirunelveli district, Tamil Nadu, India. The predator was maintained on *Spdoptera litura* larvae. The 5. *litura* larvae were maintained on groundnut leaves under laboratory conditions (28 ± 2°C, 70-80% relative humidity, and 11 h photoperiod) in 500 ml plastic containers. The healthy culture of *S. litura* was maintained by following proper sanitary conditions. Two neem formulations (Nimbicidine and Vijayneem) were used in this study. The spreader Teepol (0.5%) was added to each biopesticide (Venkadasubramanian and David 1999). In all bioassays, leaves (2 g) of groundnut cv TMV 1 were used against 5. *litura.* The leaves were immersed in the biopesticide suspension and dried in shade and then used to feed *S. litura.* The glass olfactometer used to study the insect behavior consisted of a central chamber (2.5 cm upper diameter, 6.5 cm lower diameter, 6 cm height), from which two equally spaced glass tubes projected outward. A beaker of 9 cm height and 7 cm width was fitted on the terminal opening of each tube, and closed with a muslin cloth.

The biopesticide treated groundnut leaf fed *S. litura* acceptance by different stages of *R. marginatus* was evaluated by a no-choice experiment. One-day-old nymphal instars (first, second, third, fourth, and fifth) and adults of *R. marginatus* were tested for their response to biopesticide treated groundnut leaf fed *S. litura* third instar larvae. Two biopesticide treated groundnut leaf fed S. *litura* larvae were introduced in one beaker, and water treated leaf fed *S. litura* in the opposite beaker as a control. The beakers were covered with muslin cloth and the prey *S. litura* larvae allowed to move undisturbed for five minutes. Two first instar *R. marginatus* were then introduced into the central tube and the feeding events were recorded for one hour. The successful approaching and predatory response was recorded. The predatory response was defined in terms of an Access Proportion Index (API) (Yasuda and Wakamura 1996). The API was calculated as given below.

API = NS - NC/NS + NC, where NS = number of animals choosing the sample side, and NC = number of animals chosing the control side. Each experiment was replicated fifteen times with different life stages of the predator.

In order to assess the biocontrol potential of *R. marginatus* life stages, *S. litura* third instars were allowed to feed on biopesticide treated leaves for 24 h. One-day-old first instars of *R. marginatus* were placed in a plastic container (500 ml capacity) and the biopesticide treated groundnut leaf fed *S. litura* were provided to them. After 24 h, number of prey eaten by *R. marginatus* was recorded. Predatory efficiency was assessed in terms of number of prey killed and consumed in 24 h. Second, third, fourth, and fifth nymphal instars and adults were provided with four, six, eight, ten, and twelve *S. litura* larvae per day, respectively. Fifteen replications with one predator were maintained for each life stage.

Results of prey (third instar *S. litura*) acceptance by *R. marginatus* are presented in Table 1. First, second,