RESISTANCE MECHANISMS AGAINST THE PSEUDOSTEM WEEVIL *Odoiporus longicollis* OLIVIER (COLEOPTERA : CURCULIONIDAE) IN BANANA

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "**Resistance mechanisms against the pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae) in banana**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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Certified that this thesis, entitled "**Resistance mechanisms against the pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae) in banana**" is a record of research work done independently by Ms.N. Lalitha, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CERTIFICATE

We, the undersigned members of the Advisory Committee of **Ms.N. Lalitha,** a candidate for the degree of **Master of Science in Agriculture** with major in **Agricultural Entomology**, agree that the thesis entitled "**Resistance mechanisms against the pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae) in banana**" may be submitted by Ms.N. Lalitha, in partial fulfilment of the requirement for the degree.

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In loving

memory Of my

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Introduction

1. INTRODUCTION

Banana is one of the most important fruit crops grown in Kerala and it occupies an area of 72,861 ha, with a total production of 5.7 lakh tonnes. The varieties grown are Nendran (AAB), Palayankodan (AAB), Rasthali (AAB), Njalipoovan (AB), Robusta (AAB), Vannan (AB), Pisanglilin (AA),Nendravannan (AB) and others. Most of these varieties are grown under a crop cafeteria in homesteads, but extensive commercial cultivation is restricted to the multipurpose variety, Nendran(AAB)or French plantain, which accounts for 33 per cent of the total area under banana and contributes to 59 per cent of the total production in the state. Currently,a major problem affecting yield of banana in South India is the incidence of an insect pest, the pseudostem weevil.

The pseudostem weevil, *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae) was first reported by Fletcher as early as in 1914 at Pusa, Bihar. The pest was not considered serious earlier, as the attack of rhizome weevil was more serious and drew the attention of researchers. *O. longicollis* was also reported from Sri Lanka (Speyer, 1918), Java (Frogatt, 1928), Hongkong and Hawai Islands (Hoffmann, 1932), Formosa (Kung, 1955) and China (Luo *et al.*, 1985). Of late, it has become a serious menace to the Nendran growing tracts of Kerala (Sherif and Thomas, 1988). The extent of devastation caused by the pest reached alarming proportions during the nineties.

O. longicollis is a robust reddish brown to black weevil present in the field throughout the year. The attacked plants are generally damaged by the weevil grubs, feeding and riddling through the pseudostem to the peduncle and the badly affected plants snap at the base and fall off the stool in high winds. Almost all the cultivars of the North-Eastern parts of India were reported susceptible. In Kerala, the most important commercial varieties such as Nendran, Palayankodan and Rasthali are more prone to stem weevil.

The outward traces of attack are holes of two mm diameter at approximately equal distance apart, on the pseudostem. These scars later become holes and can be easily seen if the dried outer sheaths are removed. In older plants the holes were seen up to a height of six feet. The damage due to larval tunnelling results in the splitting up of pseudostem, stunting and failure to produce fruits.

The package of practices recommendation (Anonymous, 1996) to control the infestation is to drench with chlorpyriphos (0.03%), quinalphos (0.05%) or carbaryl (0.02%) within the leaf whorls or on the leaf sheath at monthly intervals during March-September. Abraham and Thomas (1995) suggested mud slurry as a base and carrier of insecticide for swabbing of banana pseudostem, to control the pest. The insecticides proved effective on swabbing were 0.25 per cent carbaryl, 0.1 per cent chlorpyriphos or 0.5 per cent neem oil.

Several practical difficulties in implementing control measures are encountered. The pest is an internal borer and difficult to be detected in the early stages. Systemic insecticides cannot be recommended safely at the peduncle formation stage as they impose residual problems. The time of attack of the pest coinciding with initiation of the flower bud and the nature of the host plant where the ripe fruit is consumed raw makes chemical and other conventional methods of management ineffective and almost impractical.

The perennial homestead system of cultivation of banana, grown as an intercrop also provides an ideal habitat for the pest to hibernate or survive throughout the year.

The role of host plant resistance in fruit plants like banana is more paying and of permanent nature owing to its vegetative propagation. It is also essential that the bases of resistance, both morphological and chemically mediated be understood. In the recent past a few attempts have been made to utilize genetic engineering for

incorporating pest resistance. Isolation of new sources of genetic diversity by exploiting biotechnology in host plant resistance would be more promising.

The present study aims at exploiting the self-defense mechanisms exhibited by the commercial cultivar 'Nendran' against the pest *O. longicollis*. The elucidation of induced allelochemicals in curbing the attack in the second and or further generations of the plant would facilitate identification of probable resistance mechanisms.

In vitro regenerated progenies of infested mother plants might offer resistance to the weevil due to probable transport of allomonal semiochemicals from the mother to the suckers. A moderate or partial resistance obtained from the above-mentioned research, could also act as a pivot around which other control tactics can be integrated to develop an ecofriendly pest management system.

The investigation was hence, focussed on the following objectives.

a. Evaluation of sucker and *in vitro* regenerated progenies of infested Nendran (AAB) for resistance against *O. longicollis*.
b. Identification of morphological, biochemical and anatomical bases of resistance, using Njalipoovan (AB) as the resistant source.
c. To evolve methods for screening resistance under artificial conditions with adult weevils mass reared in the laboratory.

Review of Literature

2. REVIEW OF LITERATURE

The banana pseudostem weevil, *Odoiporus longicollis* Olivier is one of the most important pests affecting the yield of banana severely. The investigations on the biology of the weevil were carried out by Fletcher as early as in 1914 at Pusa Bihar. McSwiney (1920) recorded *O. longicollis* as one of the miscellaneous pests infesting plantain crop in Assam. A minor pest during those years, *O. longicollis* has become major in Kerala and adjoining states in recent years. A brief review of literature available on the biology, nature of damage, behavioral patterns, host resistance and the mechanisms of plant resistance is attempted here.

2.1 Biology and nature of attack

Pinto (1928) recorded that life cycle of the stem weevil occupied 26-30 days with the egg, larval, pre-pupal and pupal periods ranging from 3-4, 11-18, 3-6 and 7-8 days respectively under laboratory conditions. Frogatt (1928) found that *O. longicollis* oviposited in the cut ends of the pseudostem or where an injury permitted its entry into the centre of the stem. The larva started feeding immediately in and around the bunch stalk and later spread through tissues of the leaf bases. Pupation took place in a thick tight cocoon made out of the fibres from the leaf bases. Hoffmann (1932) reported that the weevils readily bred under laboratory conditions and completed its life cycle in about 6 weeks. He also observed that in the field, it bred almost throughout the year probably having several generations annually. The longevity of the adult weevil which bored into the pseudostem was found to be extending upto two years.

The detailed investigations on the biology of *O. longicollis* conducted by Kung (1955) showed that the weevil had four or more generations in an year and all the stages continued their development even during the winter periods. The eggs were usually laid singly in the stems under the leaf sheaths particularly in weak or

injured parts or at the cut ends of the pseudostem. The female laid upto 11 eggs with an average of six per mating. The larvae bored into the stems particularly in the upper parts and also damaged the fruit stalks. Pupation was near the surface of the stems in cocoons formed from the fibres. According to him the egg, larval, pre-pupal and pupal stages lasted for 5-12 days, 3-6 weeks, 3-7 days and 3-13 days respectively during the period from February to April and newly emerged adults remained in the cocoon for 7-14 days. During September to October, the egg and larval stages lasted for 3-5 and 25-27 days respectively. The ratio of males to females among the adults collected in the field was about 2:3.

Kung (1964) recorded that larvae passed through 4 to 7 instars. Pupation takes place inside a fibrous cocoon in the pseudostem for a week. All stages were found in maximum number in older plants after harvest (55%). Plants at fruiting harboured 30.2 per cent while before blooming it was 14.8 per cent of the total weevil population. The population density was found inversely proportional to the vigour of the plant. The weevil breeds throughout the year but its incidence peaks during summer and monsoon.

Shukla and Kumar (1969) conducted a detailed study and found that 70 per cent of plantains were infested in Uttar Pradesh. The only outward traces of the damage reported were holes of two mm diameter approximately at equal distance apart upon the pseudostem. He also observed that in older plants the holes were seen up to a height of six feet. The damage due to larval tunnelling resulted in the breaking up of at soil level, stunting and failure to produce fruits (Edward *et al.*, 1973; Luo *et al.*, 1985).

The biology of banana pseudostem weevil was worked out by Jayasree (1992). She found that the attack by the weevil was prevalent throughout the year and incidence usually started around four months from planting, coinciding with the inception of peduncle in the growing primordia. Field survey throughout Kerala and

neighbouring districts of Karnataka and Tamilnadu have confirmed the above finding (Aravindakshan, Personal Communication).

2.2 Host plant resistance

Host plant resistance refers to the heritable qualities of a cultivar to counteract the activities of insects so as to cause minimum per cent reduction in yield as compared to other cultivars of the same species under similar conditions. The emphasis in this definition is not on the absolute yield obtained from the so called resistant variety as compared to the susceptible one, but on the per cent decrease in yield vis-a-vis the yield obtained without the attack of the insect. It means that a cultivar may yield poor but carries the genes for resistance and on the contrary a cultivar may yield good without having any genes for resistance (Dhaliwal and Dilawari, 1993).

2.2.1 Mechanisms of resistance

Painter (1951) grouped mechanisms of resistance into three main categories, viz. non-preference, antibiosis and tolerance.

2.2.1.1 Non-preference

Non-preference refers to the response of the insect to the characteristics of the host plant which make it unattractive to the insect for feeding, oviposition or shelter. As the term 'non-preference' pertains to the insect and not to the host plant, Kogan and Ortman (1978) proposed the term 'antixenosis', derived from the Greek word *xeno* (guest) which describes the inability of a plant to serve as a host to an insect herbivore.

Antixenosis signifies that the plant is considered as undesirable or a bad host. Antixenosis may result from certain morphological characteristics or the

presence of allelochemicals in the host plant (Kogan, 1982). In field plantings, non-preferred varieties frequently escape infestation and even when insects are caged on non-preferred hosts, they lay fewer eggs and thereby develop smaller populations than those caged on susceptible varieties (Pathak and Dhaliwal, 1986).

2.2.1.2 Antibiosis

Antibiosis refers to the adverse effect of the host plant on the biology (survival, development or reproduction) of the insects and their progeny infesting it. All these adverse physiological effects of permanent or temporary nature following ingestion of a plant by an insect are attributed to antibiosis.

The insects feeding on resistant plants may manifest antibiotic symptoms varying from acute or lethal to subchronic or very mild. The most commonly observed symptoms in insects include larval death in first few instars, abnormal growth rates, disruption in conversion of ingested food, failure to pupate, failure of adults to emerge from pupae, abnormal adults, decreased fecundity, reduction in fertility, restlessness and abnormal behaviour. These symptoms may appear due to various physiological processes, viz. presence of toxic substances, absence or insufficient amount of essential nutrients, nutrient imbalances, presence of antimetabolites and enzymes adversely affecting food digestion and utilization of nutrients (Kogan, 1982).

2.2.1.3 Tolerance

Tolerance refers to the ability of the host plant to withstand an insect population sufficient to damage severely the susceptible plants. It is generally attributable to plant vigour, regrowth of damaged tissues, resistance to lodging, ability to produce additional green matter, utilisation of non vital parts by insects and compensation of growth of neighbouring plants (Kogan, 1982).

However, tolerance is not likely to provide a high level of resistance and could be useful in combination with other mechanisms of resistance. Moreover, tolerant varieties do not depress insect populations nor do they provide any selection pressure on the insects. Thus they can prove very useful to prevent the development of insect biotypes (Tingey, 1981; Velusamy and Heinrichs, 1986).

There is hardly any information available with regard to the varietal reaction of banana plant to the pseudostem weevil infestation in Kerala. The scanty reports from elsewhere are therefore reviewed hereunder.

2.2.2 Resistant and susceptible sources

Frogatt (1928) recorded that *O. longicollis* attacked all the varieties of banana and bred in the stem tissues after the bunch had been cut or after the plant got killed with the attack of rhizome weevil.

Dutt and Maiti (1973) reported that the banana varieties Martaman, Champa, Kanchakela and Kabuli were the most susceptible ones to the weevil infestation in West Bengal. The weevil preferred their oviposition sites on pseudostems with a circumference of 25-60 cm and a height up to 125 cm in tall varieties such as Martaman, Champa, Kanchakela and height of 100 cm in dwarf varieties such as Kabuli.

A field survey carried out by Isahaque (1978) in Assam revealed that the varieties Malbhog and Chenichampa were highly susceptible both in the extent of damage and population density. The variety, Bhimkel was completely free from attack while Kaskel was found to be moderately resistant. Resistance in these varieties was ascribed to their broad, thick and compact leaf sheaths and pseudostems.

Visalakshi *et al.* (1989) reported that Nendran and Red Kappa were highly susceptible in Kerala. The life cycle was completed in about 42 days and adult longevity was 90-120 days.

Jayasree (1992) found that Njalipoovan had very low levels of attack of *O. longicollis* as compared to other varieties in Kerala conditions.

2.2.3 Assessment and evaluation of resistance

Interaction between host plants and insects are spread over a wide spectrum of intensity. In terms of the host plant, lesser the population of the insect and/or lesser the damage they cause to the plant, more resistant the plant is likely to be. On the other hand, from the point of view of the insect, interaction varies from totally unsuitable host to completely suitable for growth and development of the insect (Horber, 1980). Therefore, intensity of resistance is a relative term and should be discussed in relation to a susceptible cultivar of the same species.

Charles *et al.* (1996) devised a scoring technique based on the total number of feeding holes on leaf sheaths, recorded, during the time of observation. The plants were rated as 0 - no infestation, 1 - slight infestation (1-5 holes), 2 - moderate infestation (6-15 holes), 3 - heavy infestation (16-45 holes), 4 - severe infestation (more than 46 holes).

2.3 Tissue culture

Tissue culture has been used for developing insect resistant plants in two ways, viz., screening for insect resistance at cellular level and by exploiting variation in regenerants. Recently many attempts have been made in the field of biotechnology to develop resistant plants.

2.3.1 Allelochemics in cell and tissue culture

Fowler (1983) showed that the plant callus tissues may be produced in cellular suspension and that can produce allelochemicals such as alkaloids, benzoquinones, furanocoumarins, proteins and tannins. As with the production of nutrients, the possibilities exist for entomologists and phytochemists to use plant tissue culture to obtain allelochemicals more efficiently than by production of whole plants. Kogan and Schroeder (1989) used soybean cell culture to study the allelochemistry of induced resistance in soybean foliage.

Kreuzaler *et al.* (1983) exploited the possibilities of accumulating the antimicrobial compounds in localised areas of infected plant tissues. Kuhn *et al.* (1984) studied the structure and pathways of biosynthesis of a number of enzymes such as phenyl alanine ammonia lyase and chalcone synthase. These have been cloned for different species. Similarly, studies can be made on allelochemicals produced by the host against the attacking insect.

Duffey and Felton (1989) showed plant enzymes and their end products as inducible elements of pest resistance. The potential use of such enzymes and their end products as basis of resistance against pests was discussed.

2.3.2 Screening for resistance *in vitro*

Matsumoto and Yamaguchi (1990) reported two lines derived from protocorm like bodies of banana, *Musa* AAA group, Cavendish subgroup as showing a higher tolerance to aluminium stress.

Sarah *et al.* (1992) reared *Radopholus similis* on carrot discs *in vitro* and inoculated in micropropagated plantlets of banana. The five varieties which have been tested following this technique ranged between a high partial resistance and a high susceptibility. These results corresponded to preliminary observations in field collections, indicating *in vitro* screening for resistance as a reliable method.

A rapid method for screening against panama disease in tissue culture produced banana plants was described by Beer and De Beer (1992). Three month old plants were routinely inoculated with *Fusarium oxysporum* f. sp. *cubense* under their roots in bags. Evaluation carried out 4 weeks later showed a 5.25% rate of uninfected corms. This rate coincided with the degree of tolerance occurring between different genotypes. Plants with symptoms were destroyed while those without were further proliferated.

Tissue culture and gamma irradiation of commercially non-viable banana cultivar resistant to Fusarium wilt (race 4) produced a mutant population with improved height, earlier bunching and better cold tolerance than standard Cavendish types. The plants also retained race 4 resistance of the parent (Smith *et al.*, 1993).

Morpurgo *et al.* (1994) produced shoot tip cultures from susceptible and resistant banana clones *in vitro* with different concentrations of Fusaric acid and fungal crude filtrates or inoculated with a conidial suspension of *Fusarium oxysporum* f. sp. *cubense* to assess any correlation between *in vivo* and *in vitro* behaviour. These authors suggested that the use of crude filtrate of non-host specific toxin (Fusaric acid). in a screening programme for selecting a novel resistant genotype of Musa is not feasible. But when peroxidase activity was used as a parameter to discriminate between susceptibility and tolerance, results were in good agreement with field response of host plant to pathogens. Early enzymatic activity increased in the incompatible host pathogen interaction but not in the compatible interaction.

Ahloowalia (1995) reported that *in vitro* methods allow induction and expression of recessive mutations in the haploids, producing homozygous doubled haploids.

2.3.3 Variation in regenerants

Plants derived from callus tissue may possess sufficient genetic variability to show insect resistance. Such variation in corn, sugarcane, rice and oat callus has been used to select for disease resistance, agrochemical tolerance and improved agronomic traits. Attempts to identify insect resistance in tissue-cultured plants are still at infancy.

White and Irvine (1987) produced 2000 somaclonal variants of sugarcane callus tissue from a cultivar susceptible to the sugarcane borer, *Diatraea saccharalis*. Regenerated plants grown and infested in field plots exhibited random variation in the amount of borer damage incurred. Results showed increased levels of borer resistance in tissue-cultured plants as compared to their parents.

The tissue culture techniques to develop regenerated plants from three bermuda grass *Cynodon dactylon* genotypes, viz. Brazos, Grazer and OSULCB W26 were used to enhance the level of resistance to insects (Croughan and Quisenberry, 1989). The regenerated plants were compared with original genotypes for resistance to fall armyworm. Two lines Brazos R_3 and OSULCB W26-R_2 from the seven regenerated plants tested had increased resistance to fall armyworm.

Isenhour *et al.* (1991) evaluated sorghum genotypes regenerated from tissue culture under field and laboratory conditions for resistance to leaf feeding by fall army worm. Two regenerated lines were identified as having significantly higher level of resistance to fall army worm feeding compared with non-regenerated and susceptible parents. Laboratory studies measuring growth and development of fall armyworm were conducted with meridic diets containing dried sorghum foliage from regenerated or non-regenerated plant material. Free choice studies revealed a significant degree of non-preference for the R_3 line that has shown the greatest adverse effect on fall armyworm growth in the developmental studies. These results

indicate that tissue culture induced variation can be used as a viable means of generating new sources of genetic diversity for use in crop improvement.

Attempts to transfer insect resistance to cultivated species of potato, have been unsuccessful through traditional back crossing programme. There appears to be restricted recombination between the wild species and the cultivated genome (Mehlenbacher *et al.*, 1983; Kalazich, 1989). Lentini *et al.* (1990) tried to generate reassortment of genes already present in the mother potato plants. Progenies were regenerated from petiole calli of interspecific hybrids. Callus culture was used to generate genetic changes to overcome the restricted recombination between the two genomes. Two plants out of 58 (3.5%) from calli of Hybrid J114-1 showed stable and heritable differences from the hybrid over two cycles of evaluation in the field. One regenerant showed insect resistance and increased marketable yield (approximately two fold) in the field. These results suggest that a period of callus culture followed by plant regeneration may aid in the introgression of desirable traits from wild species into crop plants.

2.4 Pseudoresistance

Pseudoresistance refers to the apparent resistance resulting from transitory characters in potentially susceptible host plants (Painter, 1951).

Induced resistance is the qualitative or quantitative enhancement of defense mechanisms in plants against pests in response to extrinsic physical or chemical stimuli (Kogan and Paxten, 1983). This is a nonheritable resistance where host plants are induced to impart resistance to tide over the pest infestation. A number of mechanisms appear to be involved in induced resistance, viz., physiological conditions, nutrient concentrations, allelochemical concentrations and production of phytoalexins.

2.5 Biochemical bases of plant defenses

The chemicals involved in host defense were termed as secondary substances and these metabolites are produced in plants through secondary metabolism. However, their role in insect-plant interactions was first reported by Verschaffelt (1910) involving mustard oil glucosides and pierid butterfly larvae. Later, Dethier (1954) came out with the evidence of involvement of secondary chemicals in selecting food plants by *Papilio* larvae.

The classical example of successful plant defense was explained by Klun and Robinson (1969) on maize against *Ostrinia nubilalis*. The borer resistence was dependent upon the presence of an effective concentration of DIMBOA, the resistance factor.

Ghosh (1960) associated high moisture content and succulent nature of the plant with susceptibility to stem borer in rice.

Whittaker and Feeny (1971) termed secondary substances as allelochemicals indicating a shift in the stand of workers who probably started realizing the adaptive significance of the chemicals in plant defense against herbivores. Subbarao and Perraju (1976) also opined high crude fibre and low content of moisture to impart resistance against stem borer.

A wide array of chemicals including inorganic chemicals, primary and intermediary metabolites and secondary substances are known to impart resistance to a wide variety of insect pests (Norris and Kogan, 1980). However, the role of these compounds in insect plant interactions is greatly variable. These compounds act as allomones, kairomones or as synomones (Harborne, 1982; Ananthakrishnan, 1990). The allomonal effects on insects range from mild repulsion to reduced fecundity and longevity, and even toxicity (Dhaliwal and Bathal, 1992). These compounds are either constitutive or induced by environmental factors or

mechanical injury including that from herbivory. Kairomonal effects range from mild, short term attraction to powerful, long term feeding or ovipositional excitation. These compounds are mostly constitutive.

Optimal defense theory (Zangari and Rutledge, 1996) predicts that tissues that are unlikely to be attacked by herbivores should have low constitutive amounts of defense and high inducibility, while tissues that are likely to be attacked should have high levels of constitutive defense and low inducibility. Later these authors also artificially damaged roots, leaves and reproductive parts of wild parsnip and found that these parts differ not only in constitutive levels of chemical defense but also in the degree to which these defenses are inducible. Reproductive parts contain the highest constitutive concentrations of a toxic furanocoumarin and xanthotoxin. Roots contain lowest constitutive concentrations but are highly inducible. Leaves are intermediate in both the constitutive amounts of xanthotoxin they contain and inducibility. The probabilities of attack of roots, leaves and reproductive parts were remarkably consistent among populations and years. The relationships between patterns of defense and attack were consistent with predictions based on optimal defense theory.

2.5.1 Phytoalexins

Phytoalexins are induced by the plant pathogens and these have been shown to have effects on the insect herbivores. Even insect attacks on the plants may also result in the production and accumulation of phytoalexins. Hart *et al.* (1983) have studied the production of phytoalexins in response to Mexican bean beetle and soybean looper in soybean. The production of phytoalexins deterred the feeding of Mexican bean beetle on cotyledons of soybean. However, there was no effect on the larvae of soybean looper.

Four new phenalenone type phytoalexins, named musanolones were isolated from rhizomes of banana which were infected with *Fusarium oxysporum* F. sp. *cubense*(Luis *et al.*, 1996). They revealed that these musanolones strongly inhibited the growth of the germination tube of *F. oxysporum*.

Binks *et al.* (1997) isolated a phenalenone type phytoalexin from the nematode infected roots of banana plants.

2.5.2 Allelochemical concentrations

Allelochemicals are mostly stored in the plants separately away from the site of metabolism. However, activity of these compounds is induced by any injury to the plant like herbivore feeding or even through the autolysis of the plant cells. Wounding may induce changes in phenol (Rhodes and Wooltorton, 1978), lipid (Galliard, 1978) and protein (Davies and Schuster, 1981) metabolism.

Edwards and Wratten (1983) classified the phytochemical responses evoked by mechanical disruptions as (i) cellular chemical changes, (ii) changes in cells adjacent to the damaged tissues and (iii) generalised changes apparent in a plant part or the entire plant.

Allelochemicals that appear to play a dominant role in host plant resistance are phenolic compounds including flavonoids and aromatic acids; proteinaceous compounds like protease inhibitors, glycosidase inhibitors and phytoagglutamine, the amides, lipids, saponins, lignins and tannins (Hedin, 1986; Dhaliwal and Bhathal, 1992).

2.5.3 Phenols

Rhodes and Wooltorton (1978) revealed that wounding induced the oxidation of plant phenols to produce toxic quinones and synthesis of mono and di

phenols. Peraiah and Roy (1979a) reported that there was high phenol content in growing points of paddy before infestation of BPH but its content after infestation in susceptible varieties decreased. The phenolic contents of resistant varieties increased during early infestation. Phenol levels increased following damage by lygus bug to Chinese cabbage and sugar beet (Hori and Atalay, 1930) and cotton (Guerra, 1981). One of the most exciting reports is about the damage to poplar and sugar maple tree foliage that increased the total phenol content of foliage of adjacent, non connected trees suggesting that plants are capable of communicating through chemicals regarding wounding (Baldwin and Schulz, 1983; Perry and Pitman, 1983; Rhoades, 1983). Abrasions on the cotton cotyledons transported secondary compounds into the leaves systemically (Karban and Carey, 1984). Smith (1988) suggested that mild wounding via. defoliation, abrasion or infection appears to elicit a general plant response that is beneficial to the plant but detrimental to the insect.

Recent studies conducted by Butter *et al.* (1992) on total phenols and ortho-dihydroxy phenols indicated negative correlation between whitefly population and these chemical compounds. The chemical compounds produced as a result of proliferation of parenchymatous tissues in Sanguineum cotton caused mortality of pink boll worm.

2.5.4 Proteinaceous compounds

Green and Ryan (1972) first discovered that mechanically wounded tomato leaves stimulated the release of a proteinase inhibitor-inducing factor (PIIF) into vascular transport system of damaged plants. The feeding of larvae of *Spodoptera littoralis* on damaged tomato leaves decreased by nine fold within eight hours after damage. Within 24 h, leaves adjacent to initially damaged leaves promoted similar adverse effects on larval feeding (Edwards *et al.*, 1985).

Baker *et al.* (1991) correlated the alpha amylase inhibitor content in eastern soft wheats with development parameters of the rice weevil *Sitophilus oryzae*. They obtained a positive correlation between inhibitor content and average number of days to adult emergence.

Elden (1995) studied the effects of selected proteinase inhibitor on growth and development of alfalfa weevil, *Hypera postica*. *In vivo* studies on the effects of proteinase inhibitor on the curculionid was determined by measuring larval development (weight) and mortality. This study reported on the negative effects of specific proteinase inhibitors on insect larval foliar feeding, pupation and adult emergence when ingested with an insects' preferred host. Gomes *et al.* (1996) reported that the enzyme chitinase isolated from the cowpea seeds had negative effect on the development of *Callosobruchus maculatus*.

2.5.5 Polyphenol oxidase

The enzyme polyphenol oxidase (PPO) category refers to two enzymes laccase (p. diphenol oxygen oxido reductase) and phenolase (catechol oxidase or tyrosinase or catecholase). Both laccase and catechol oxidase oxidises phenolic substrates to quinones and tannins. These compounds are found to be toxic to extra cellular enzymes produced by the pathogens or other foreign invaders.

Maine and Kelman (1961) observed that PPO activity was much greater in infected than in healthy stem tissues and suggested that PPO activity may be involved directly or indirectly in resistance of host plants to *Pseudomonas* sp. Retig (1974) conducted studies on the role of PPO in fusarium wilt resistance in tomato and observed a very high increased activity in both roots and stems of the resistant plants after inoculation. No increase in activity was observed in susceptible plants.

Filho and Stevens (1980) used electrophoresis to detect a linkage in tomato genes expressing nematode resistance and acid phosphatase. Thus nematode

resistant cultivars are identified by the presence of an acid phosphatase electromorph and the amount of time required to identify resistance is decreased by approximately 30 per cent.

Obukowicz and Kennedy (1981) also stressed importance of PPO in resistance against *Pseudomonas solanacearum* in tobacco. Gregory *et al.* (1986) elucidated the physico chemical defense mechanism of potato glandular trichomes against several insect pests. The trichomes initially exude a sticky substance, after which sesquiterpenoids are released that disturb the insect and cause agitated movements. Subsequently, PPO and phenolic substrate react to form quinones. These events lead to insect immobilisation, cessation of feeding and ultimately death of the insect.

This enzyme can occur in both latent and active form of endogenous inhibitors which can be explained for the failure to detect the enzyme in some tissues. PPO has been reported from a variety of plant organs and tissues. Special cases of occurrence were reported in pollen grains, latex, crown gall tissue and relatively high levels in guard cells. The most convenient method for assaying the activity of catechol oxidase is to follow the initial rate of formation of quinone spectrophotometrically (Mahadevan and Sridhar, 1986).

In relation to resistance against *Pseudomonas syringae* pv. *tomato*, Bashan *et al.* (1987) observed high PPO enzyme activity in inoculated resistant cultivars than in inoculated suceptible ones.

Ganguly and Dasgupta (1988) reported lower protein and PPO in susceptible cultivar Pusa Ruby when infested with *Meloidogyne incognita*. The electrophoretic pattern for PPO in apparently resistant plants marked with non-galled roots showed three bands of PPO while two isoenzymes were obtained from galled roots. Felton *et al.* (1989) reported that the foliage and fruit of the tomato

plant contains PPO that are compartmentally separated from ortho dihydroxy phenolic substrates *in situ*. However when leaf tissue is damaged by insect feeding, the enzyme and phenolic substrates come in contact, resulting in rapid oxidation of phenolics to orthoquinones.

In potato, Karwasra and Parashar (1989) reported higher PPO activity in Kufri lalima, a potato variety resistant to bacterial soft rot compared to Kufri Badshah, a susceptible potato variety.

Schukle and Dhawale (1989) have used electrophoresis to detect differences in the foliar proteins of Hessian fly-infested wheat plants. Electrophoretic techniques are at a very early stage of utilization in the identification of plants with insect resistance.

Singh and Singh (1989) studied the content of total phenols, O-dihydroxy phenols and flavanols and the activity of peroxidase and PPO in the leaves of 2 resistant and 2 susceptible varieties of chilli pepper (*Capsicum annuum* L.) after inoculation with cucumber mosaic virus. The increase in phenols and decrease in flavanols immediately after infection was much less marked in the resistant than in the susceptible material and was associated with increased activity of the enzymes studied, leading to the formation of more quinones and other oxidation products in the resistant varieties, resulting in reduced multiplication and inactivation of the virus.

Ahmed *et al.* (1994) reported higher PPO activity in yellow vein mosaic resistant okra varieties than susceptible ones. However, Chander (1994) detected lower PPO activity in powdery mildew resistant chilli line compared to that in susceptible one.

Markose (1996) reported that PPO activity was higher in bacterial wilt resistant variety of chilli in all the plant parts at various growth stages. The enzyme activity increased upon infection, to a greater extent in the resistant genotype.

2.6 Morphological bases of resistance

The plant resistance is controlled by several morphological factors like remote factors, e.g. colour, shape, size, etc. and close range or contact factors. These include thickening of cell walls and rapid proliferation of plant tissues, solidness and other stem characteristics, trichomes, incrustation of minerals in cuticle, surface waxes and anatomical adaptations of organs.

In sorghum cultivars, Woodhead (1983) identified the major parameter contributing for resistance to stem borer as surface waxes on the leaf and stem which affected the movement of first instar larvae. Some wax components act as feeding deterrents also.

The relationship between the cell structure and biochemical characteristics in banana leaf tissue and their cold tolerance was studied by Liu *et al.* (1990). They revealed that the thickness of the cuticle cells and the thickness ratio of cuticle and palisade: mesophyll were higher but the thickness ratio of spongy tissue: mesophyll was lower in leaves of cold tolerant cultivars.

Ortiz *et al.* (1995a) observed that in natural banana germplasm resistant clones showed increased corm hardness as measured by a penetrometer in longitudinal and cross sections of outer and central corm tissues. This was suggested as a non-preference mechanism for rhizome weevil resistance. Wild banana accessions, several cooking and dessert bananas showed high levels of resistance because of corm hardness.

Ortiz *et al.* (1995b) studied the inheritance of waxiness in the pseudostem, which is composed of overlapping sheaths. They also suggested that waxiness may be involved in host plant resistance in banana to fungus and insect pests.

2.7 Olfactory responses to host plant volatiles

Ndiege *et al.* (1991) assessed the volatiles from banana (cv. Githumo) pseudostem. The volatile compounds identified included alpha-pinene, beta pinene, beta myrcene, limonene, alpha-cubebene, alpha-copaene, alpha-cedrene, beta caryophyllene and alpha humulene. Budenberg (1993) showed in a laboratory study that males and females of *C. sordidus* were attracted to freshly cut banana rhizome and pseudostem in a still air olfactometer. Females responded similarly to odours from a comparatively resistant and a susceptible cultivar of banana, when presented as either freshly cut tissue or as porapak trapped volatiles. Females were also attracted to rotten pseudostem and to volatiles collected from it. Males and females gave similar responses to both the behavioural bioassay and to collected volatiles in EAG recordings. However, they did not respond either behaviourally or electrophysiologically to a synthetic mixture of mono and sesque terpenes which made up over 9 per cent of the volatile collected from the pseudostem.

An olfactory analysis technique to investigate responses of *C. sordidus* to different odours was evaluated in the laboratory by Cerda *et al.* (1995). The tested odours were from an ethanol sample of various tissue specimens from the pseudostem and corm of *Musa acuminata*, pineapple and cocoa pods. Healthy corm and pseudostem tissues were found to be most attractive and others were ineffective. Ndiege *et al.* (1996) identified an attractant 1,8 cineole which is one of the electrophysiologically active components of the volatiles from 6 banana cultivars susceptible to the banana weevil. It was also shown that resistant cultivar did not contain 1,8 cineole. Another component beta-phellandrene which also exhibited electrophysiological activity, but did not show any attraction to the banana weevil was found only in resistant banana cultivar. The banana corms produced over 40 volatile chemicals out of which one or more is believed to attract the pest.

2.8 Epideictic compounds

Tribolium castaneum adults release ethyl quinones into the flour which regulates egg production in the females. Naylor (1961) showed that volatile secretions from both sexes repelled conspecific females from feeding and ovipositing in fresh flour. It was also discovered that immediately following oviposition into a host fruit, a female of *Rhagoletis pomonella* circles the fruit trailing her extended ovipositor on the surface, and in doing so deposits a marking pheromone that deters repeated egg laying attempts (Prokopy and Bush, 1972).

In cotton, it was found that following oviposition, females of *Anthonomus grandis* secrete a frass like substance, possibly containing a pheromone, into the oviposition cavity in the bud, which makes other females to avoid this bud for oviposition (Jenkins *et al.*, 1975).

Female cabbage loopers, *Trichoplusia ni* deposit eggs singly on the lower surface of cabbage and a variety of other host leaves. The larva feed on the leaf tissue. Renwick and Radke (1980) found that cabbage leaves treated with an aqueous solution of *T. ni* larval frass received considerably fewer eggs than did leaves treated with water alone. The evidence suggested the existence of an oviposition deterring pheromone produced by the larvae.

Howlader and Ambadkar (1995) studied oviposition deterring influence of female body wash in tobacco beetle, *Lasioderma serricorne*. Five solvents were used for the body washings.They are distilled water, insect saline, methanol, acetone and hexane. All the body washes noticeably reduced egg laying in the treated samples more than those of their controls. Hexane wash was the most effective and recorded 82 per cent deterrence. Bioassay established a clear relationship between the concentration of the hexane extract and deterrance of oviposition.

Giga (1995) studied the selection of oviposition sites by the cowpea weevils *Callosobruchus rhodesianus* and *C. maculatus*. The oviposition behaviour of these species on clean and egg-laden adzuki beans was examined in choice and no-choice experiments. Both weevil species were able to distinguish between clean and egg-laden beans. Discrimination by both species was greater against seeds with 12 eggs than with 6, regardless of which species had previously laid eggs. There was a weak indication that the presence of *C. maculatus* eggs at high densities might reduce the oviposition rate of *C. rhodesianus*.

Ruzicka (1996) reported oviposition deterring pheromone in chrysopidae (Neuroptera) and their intra and interspecific effects. Substrates contaminated with abdominal secretion of first instar larvae of *Chrysopa perla* or *C. aculeata* deterred females of these species from ovipositing. The intra and inter specific responses of the females were similar; however, the smaller species *C. aculeata* showed a stronger response over all.

Raina *et al.* (1997) reported increased pheromone production in wild tobacco budworm *Heliothis virescens* exposed to host plants and host chemicals. They compared laboratory colony females with the first generation feral females (wild F_1) of *H. virescens* and observed significantly high production of pheromones based on host plant signals obtained from cotton and tobacco, in wild population. This corresponds to the requirement of a suitable host for oviposition by the female after being mated.

2.9 Pseudostem trapping techniques

Wijesekara and Menike (1991) studied the control of *O. longicollis* on bananas in Sri Lanka. Application of carbofuran 3G at 6 g/banana pseudostem trap and benfuracarb 3G at 3 g/trap gave effective control of curculionids attracted to traps. There was no significant difference in the number of curculionids attracted to

untreated traps and traps treated with carbofuran upto 9 g/trap and benfuracarb upto 3 g/trap, but treatment with isofenphos reduced the number of beetles attracted.

Koppenhofer *et al.* (1994) reported reduction of *C. sordidus* attack with pseudostem traps. In their trials conducted in Kenya, one-week old traps made from split banana pseudostems were 1.5-1.7 fold more attractive to adults than 2-3 week old traps. In this experiment the effect of intensive use of pseudostem traps and daily collecting of trapped curculionids was estimated by mark and recapture method over a period of six weeks. Curculionid populations were reduced by 48.5 per cent after three weeks and by 62.5 per cent after six weeks. Price (1995) tested a modified pseudostem trapping technique in an area of AAB plantains infested with *C. sordidus* in Cameroon. The method demonstrated significantly increased recovery of adults after rain and significantly reduced recovery after insecticide treatments.

2.10 Control measures

The prophylactic cultural practices against the pest include the choice of a healthy site without a history of pest incidence, selection of good planting material with good sanitation in the plantation and rotation of crops for every three years (Pinto, 1928). Total destruction of badly infested plants and old plants were found to be effective in reducing the survival percentage of the pest (Hoffmann, 1932 and Kung, 1964).

Hoffmann (1932) suggested trapping of adults by cut pieces of pseudostem on which they would oviposit, but were too thin to complete its development. He also reported that the development could be completed even within a broken piece of the plant.

Removal and burning of dry leaves, leaf sheaths and dead or cut pseudostems during winter also helped to check the population. Chemically the

infestation was controlled by a drench with endosulfan (35% EC) or carbaryl (50% WP) each at 0.1 per cent strength within the leaf whorls or on the leaf sheath at monthly intervals during March-September (Isahaque, 1978).

According to Luo *et al.* (1985), Decis (Deltamethrin) is effective against the adult weevils. Trichlorfon and dichlorvos were found effective against both the adults as well as the larvae.

Reghunath *et al.* (1992) reported that insecticide spraying does not reach the border in the pseudostem and does not last on the leaf sheaths. Concentrated spraying of insecticides in leaf axils was tested. Severely infested, moderately infested and apparently healthy plants were subjected to insecticide treatment. Eight per cent of the strongly infested plants died before harvest even after application of insecticide. According to these authors early detection of *O. longicollis*, spraying of all banana plants and even those that appear to be healthy should be performed in the infested zone.

Abraham and Thomas (1995) suggested mud slurry as a base and carrier of insecticides for swabbing of banana pseudostem against *O. longicollis*. Swabbing the pseudostem of banana was done with a mud slurry prepared by pouring insecticide emulsion/suspension (15 litres) at the required concentration to moist soil (1 kg). The mixture was shown to be effective against the curculionid using 0.2 per cent carbaryl, 0.1 per cent chlorpyriphos or 0.5 per cent neem oil.

Drenching all the leaf axils, rhizome and surrounding soil and all round the entire pseudostem inserting the nozzle through the bore holes made by the larvae if any and also within the outer sheaths slightly raising the same at different spots. Insecticides used were quinalphos (0.05%) or chlorpyriphos (0.03%) or carbaryl (0.2%) (Anonymous,1996).

Mathew *et al.* (1996) studied the effectiveness of two alternate chemical insecticides, carbaryl and chlorpyriphos against the stem borer. Results indicated that swabbing chlorpyriphos (0.05%) was found to be the best treatment giving complete protection of the treated plants. This was followed by swabbing carbaryl (0.5%) in mud slurry and spraying carbaryl (0.1%) on the pseudostem filling the leaf axils.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation was undertaken in the Department of Entomology, College of Horticulture, Vellanikkara during 1997-99.

The major components of this study were as follows:

1. Preliminary field screening of *Musa* (AAB) Nendran for resistance against pseudostem weevil, *Odoiporus longicollis*
2. Laboratory rearing of *O. longicollis*
3. Evaluation of sucker and *in vitro* regenerated progenies of infested *Musa* (AAB) for resistance against *O. longicollis*
4. Identification of morphological and anatomical bases of resistance using susceptible and resistant cultivars
5. Analysis for biochemical basis of resistance

3.1 Preliminary field screening of *Musa* (AAB) Nendran for resistance against pseudostem weevil, *Odoiporus longicollis*

3.1.1 Experimental materials

Banana plants of the variety Nendran maintained by the Banana Research Station, Kerala Agricultural University, Kannara were utilised for the study. Infested fields of the susceptible variety, Nendran were selected and infested plants were scored.

Scoring for damage by *O. longicollis* was suggested by Charles *et al.* (1996). But these authors relied on the rating scale where the class intervals are not uniform and considered only the oviposition punctures for arriving at the rating scale. This rating scale based on the single parameter, ovipunctures often becomes misleading. It is known that on resistant variety there may be more number of

oviposition punctures even though the actual process of egg laying or feeding is unsuccessful (Smith, 1989).

Hence to overcome these problems a rating scale was developed considering surface area of infestation (A), size of feeding holes (B) along with the number of feeding holes/ovipunctures (n) on the pseudostem (Table 1). The rating was done based on the product of these three parameters as factor = nAB

where n = Number of feeding holes/ovipunctures

A = Surface area of infestation (m^2)
(Maximum length x breadth)

B = Size of the feeding holes (cm^2)
(Maximum length x breadth)

This facilitated the development of a new rating scale as follows

Grade	Rating	Factor
0	No infestation	-
1	Slight infestation	0.01-0.15
2	Moderate infestation	0.16-1.5
3	Heavy infestation	1.6-15.0
4	Severe infestation	> 16.0

Further, visual observations on the infestation and symptoms aided in the description of the rating scales based on damage (Table 2).

3.1.2 Biometric observations

In the field, the infested plants coming under the above grades were chosen and represented as G_1, G_2, G_3 and G_4 respectively. The following morphological parameters of the selected plants were recorded at peduncle formation stage to find out if any of these had any correlation with the degree of infestation by *O. longicollis*.

Table 1. Morphological observations of mother plants (Nendran) in the different grades

Pl. No.	No. of ovipunctures on the pseudostem (n)	Surface area of infestation (m^2) (A)	Size of feeding holes/oviposition punctures (cm^2) (B)	Factor nAB	Grade
i	7	.068075	0.15	0.07	
ii	5	.062082	0.13	0.04	
iii	10	.077095	0.15	0.11	
iv	9	.068825	0.25	0.15	
v	8	.067025	0.15	0.08	
	(1-15)	.068620	0.166	(0.01-0.15)	1
vi	23	.087085	0.50	1.00	
vii	17	.091005	0.25	0.39	
viii	22	.082615	0.50	0.91	
ix	19	.089525	0.25	0.43	
x	20	.089215	0.50	0.90	
	(16-30)	.087889	0.400	(0.16-1.5)	2
xi	31	.172825	2.50	13.39	
xii	45	.122955	1.75	9.68	
xiii	32	.188525	1.25	7.54	
xiv	37	.137153	1.75	8.88	
xv	42	.107882	1.50	6.80	
	(31-45)	.145868	1.750	(1.6-15)	3
xvi	87	.251529	5.0	109.42	
xvii	95	.275862	5.0	131.03	
xviii	85	.289297	5.0	122.95	
xix	58	.265878	5.0	77.10	
xx	71	.289925	5.0	102.92	
	>45	.274498	5.000	>16	4

Table 2. Rating scales of damage by *O. longicollis*

Damage score	Rating factor	Description of symptoms	Effect at bunching stage
0	No infestation (0)	Normal plant, No ovipunctures, No gummosis.	Normal bunches with 4-5 hands
1	Slight infestation (0.01-0.15)	Gummy exudation on the pseudostem subsequent to egg laying. Dark brown lesion developed surrounding the ovipuncture	Normal bunches with 4-5 hands
2	Moderate infestation (0.16-1.5)	Approximately equidistant feeding holes observed when outer sheaths were removed. Severe gummy exudation.	Bunches with 3-4 hands
3	Heavy infestation (1.6-15)	Splitting of the pseudostem Brownish discolouration of affected leaf sheaths often encouraging secondary infestation.	Unhealthy bunches; improper filling.
4	Severe infestation (> 16)	Pseudostem weakened and broke off ultimately due to extensive tunneling of the grubs inside.	Bunch not formed or unhealthy bunch, poor filling & earlier maturity.

(i) girth of pseudostem 1 m above ground

(ii) breadth of leaf sheath at 1 m height

(iii) width of the leaf sheath at the centre

(iv) number of sheaths overlapping the pith (compactness)

(v) height of the pseudostem up to which infestation was observed

3.2 Laboratory rearing of *O. longicollis*

Rearing studies of pseudostem weevil were undertaken in the PG laboratory, Department of Entomology.

An attempt was made to standardise methodology for mass rearing of *O. longicollis* under laboratory conditions. Among the various rearing cages tried, medium sized buckets of 32 cm height and 30 cm diameter were found to be more suitable for mass rearing and easy handling. These rearing buckets were fitted with lids having several holes of 3-5 mm size to provide air circulation. Fresh banana pseudostem pieces from Nendran plants after harvest were cut uniformly to a length of 25 cm and sprayed with 0.1 per cent formalin to prevent secondary infestation by microbes. Such prepared pseudostem pieces were arranged vertically inside the rearing buckets. Usually four large pseudostem pieces of 50 cm girth can be accommodated in a rearing bucket.

The adult weevils were collected from the field and sex determination was made based on the markings on the rostrum. Rostral punctuations were more pronounced in males than in the females. Each punctuation was placed on a slightly raised area as evidenced by the electron scanning microscopy (Jayasree, 1992). The number of such punctuations per linear unit length of the rostrum was more in males than in females, giving the rostral surface a coarse and rough appearance in the males. Absence of raised areas; small and widely spaced rostral punctuations were characteristic of the females giving the rostral surface a smooth appearance.

Ten pairs of adult weevils in the sex ratio of 1:1 were released in each rearing bucket. The old pseudostem pieces with eggs, grubs and pupa were sorted out regularly. Stem pieces used up by the grubs were removed and replenished with fresh material every week. Frequently the rearing buckets were cleaned and surface sterilized using 0.1% formalin. The water drained from the rearing buckets was collected in another bucket by providing holes at the bottom of the former. The water thus collected was emptied in alternate days to prevent rotting or decaying of pseudostems. A constant adult weevil population was maintained in the laboratory by this method, which was later used for artificial infestation of experimental plants and for conducting various other laboratory studies.

3.2.1 Duration of development of different life stages of *O. longicollis* on susceptible and resistant cultivars

A pair of male and female weevils was released into fresh pseudostem pieces at peduncle formation stages of Njalipoovan and Nendran in glass jars of 18 x 14 x 22 cm size. Water suckers of these two varieties before peduncle formation stages were also used for the study. Oviposition sites were identified by the reddish brown puncture marks. The number of eggs laid in the pseudostem of both the varieties were counted after a week's time. The optimum size of the air chamber in the pseudostem sheath which seemed to be suitable for egg laying was measured. The hatching percentage and incubation period of these eggs were also recorded.

To study the resistance mechanism viz. antixenosis and antibiosis, the grubs of different instar stages were collected and reared in both varieties. The five larval instars represented as L_1, L_2, L_3, L_4 and L_5 were separated out and reared in fresh pseudostem pieces of both varieties without removing the outer sheaths enclosing the pith. The shift of a particular larval instar to its subsequent instar stage was distinguished by the colour of the head capsules and/or with the presence of exuviae at the anal region of the grub. Generally, the grubs had pale white coloured

cephalic region immediately after moulting, which attained the normal brown colouration due to chitinization after 24 hours. The duration taken by different larval instars to complete the larval phase was observed. The mortality rate at each and every stage was also recorded. Various effects exhibited by the larval stages due to the feeding of the non-preferred variety, Njalipoovan were studied.

Apart from the above observations, pupal period, percentage of adult emergence, size of the emerged adults, fecundity of the females and adult longevity in laboratory conditions were also recorded.

3.2.2 Oviposition of *O. longicollis* reared on pseudostem of Nendran at various growth stages

The pseudostems cut from one month old to nine month old Nendran plants were kept in separate glass jars and a pair of pre-mated adult weevils were released in each of them. The number of eggs laid per female was counted. This experiment was intended to give a clear indication of the appropriate age of crop from which it becomes susceptible to infestation.

3.2.3 Epidiectic compounds

To study the existence of spacing pheromones if any, Nendran pseudostems of 25cm length were exposed for egg laying in glass jars containing one, two, three, four, five and ten pairs of pre-mated adult females. The oviposition count was taken in each case.

3.3 Evaluation of sucker and *in vitro* regenerated progenies of naturally infested *Musa* (AAB) for resistance against *O. longicollis*

The field experiments were laid out in the research fields of College of Horticulture, Kerala Agricultural University, Vellanikkara. The area is located at an altitude of 22.5 M above MSL and between 10° 32"and 76° 16"E longitude.

Vellanikkara receives warm humid tropical climate. The soil type is laterite and well deep. The meteorological data during the cropping period is presented in Appendix-I.

3.3.1 Selection of mother plants

The planting materials and explants for the study were collected from naturally field infested Musa (AAB), Nendran collection from Kannara. The infested plants were scored by 0-4 scale as mentioned earlier.

Twenty plants in each grade were randomly selected and five plants per grade were used for sucker planting while the plantlets from other fifteen plants formed the source for *in vitro* propagation.

3.3.2 Planting of suckers

Uniform suckers weighing about 1.5-2.0 kg were collected from selected mother plants, of ecotype 'Nendran' and planted during October 1998. The suckers were planted at a spacing of 2 m x 2 m in the pits of size 50 x 50 x 50 cm. The experimental design adopted was Randomized Block Design with five treatments including control and five replications (Fig.1a). The cultural practices were done as per the package of practices (Anonymous, 1996).

3.3.3 *In vitro* propagation

The work on *in vitro* propagation was done at the Tissue Culture Laboratory of Kerala Horticulture Development Programme, Kerala Agricultural University, Vellanikkara.

Two sets of explants, viz. apical meristem of sword suckers and apical meristem of the lateral buds of the selected mother rhizomes constituted the explants.

FIG.1. LAYOUT OF THE FIELD

N

(a) Sucker derived plants

T_3R_1	T_5R_2	T_4R_3	T_1R_4	T_2R_5
T_5R_1	T_4R_2	T_1R_3	T_2R_4	T_3R_5
T_4R_1	T_1R_2	T_2R_3	T_3R_4	T_5R_5
T_1R_1	T_2R_2	T_3R_3	T_5R_4	T_4R_5
T_2R_1	T_3R_2	T_5R_3	T_4R_4	T_1R_5

Border

(b) Tissue cultured plants

$T_{10}R_1$	T_9R_2	T_8R_3	T_7R_4	T_6R_5
T_9R_1	T_8R_2	T_7R_3	T_6R_4	$T_{10}R_5$
T_8R_1	T_7R_2	T_6R_3	$T_{10}R_4$	T_9R_5
T_7R_1	T_6R_2	$T_{10}R_3$	T_9R_4	T_8R_5
T_6R_1	$T_{10}R_2$	T_9R_3	T_8R_4	T_7R_5

Border

T_1 – Sucker derived plant of G_1

T_2 – Sucker derived plant of G_2

T_3 – Sucker derived plant of G_3

T_4 – Sucker derived plant of G_4

T_5 – Sucker derived plant of G_0

T_6 – Tissue cultured plant of G_1

T_7 – Tissue cultured plant of G_2

T_8 – Tissue cultured plant of G_3

T_9 – Tissue cultured plant of G_4

T_{10} – Tissue cultured plant of G_0

Plate - 1 Field view of the experimental plot.

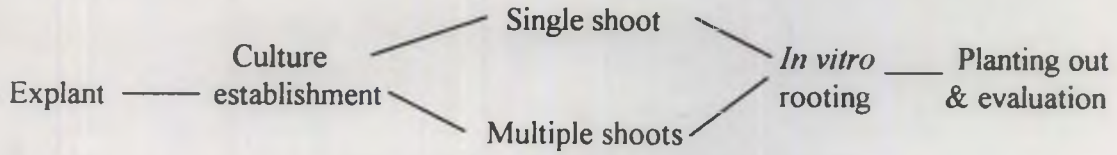
Plate - 2 Artificial infestation of the treatment plants

▶ Sleeve for release and recapture of adult weevils.

Suckers after separation from the mother rhizome were detopped and were reduced to a size, which measured about 5-8 cm in length retaining meristem and a small portion of the rhizome tissue. In the case of eye bud explants, small buds seen on the surface of mother rhizome were removed using a sharp knife without injury to the central bud.

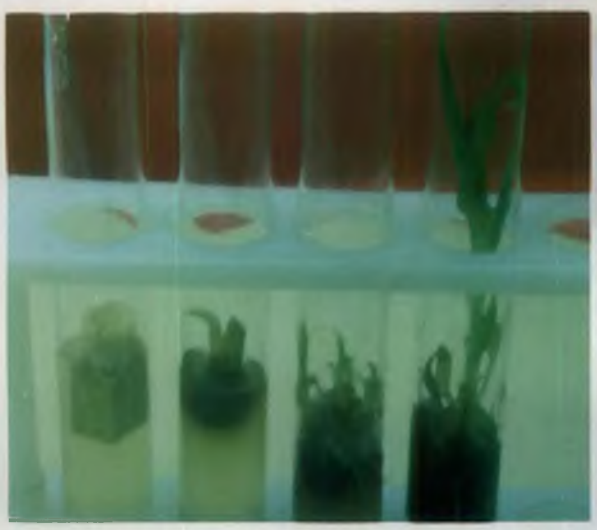
The explants after collection were immediately taken to the laboratory where they were first washed thoroughly in running tap water to remove all dirt and soil particles adhering to them. These were again washed with sterile distilled water. Further sterilization procedures were carried out under perfect aseptic conditions in a laminar airflow cabinet.

Initially explants were treated with 0.1 per cent 'Emissan' for 15 minutes and followed by 4-5 sterile water washings. The size of explants was reduced to 3 cm^3 using a sterile blade. Further treatment was done with 0.1 per cent $HgCl_2$ for ten minutes. After satisfactory sterile water washings, the size of explants was still reduced to 0.5 cm^3 exposing the meristem tissues before culturing. The media used for culturing was ½ MS media with 5 ml/litre of BAP for shooting and 3 ml/litre of NAA for rooting (Aravindakshan, 1989).



The progressive stages of *in vitro* propagation from meristem to single shoot formation are shown in Plate 3a. In order to overcome the apical dominance of shoot tips and to maintain a higher rate of axillary bud release in culture, the shoot tip explants were subjected to sub culturing by cutting apical dome longitudinally into half or quarter portion depending on the extent of proliferation. Changing the media after every 21 days accelerated the growth of the explant.

Plate-3 Meristem culture of *Musa* (AAB) Nendran.



3a.Progressive stages of meristem culture.

3b.Plantlets ready for planting out.

3c.Hardened plants in mist chamber (15 days after planting out).

3d. Plants ready for field planting (45 days after planting out).

3.3.4 Planting of tissue cultured plants

After attaining a height of about 10 cm in the test tubes (Plate 3b), the plantlets were transferred to small pots (Plate 3c) and subsequently to poly bags containing farmyard manure, sand and soilrite at the ratio of 1:1:1 (Plate 3d). These plants were kept for hardening in net house for about two months.

Such hardened, two month old tissue cultured plants produced through shoot tip culture of selected infested mother plants of ecotype 'Nendran' were planted during January 1999. The planting was carried out at a spacing of 2 m x 2 m and maintained by applying fertilizers at the rate of 840:570:1020 g of NPK per plant at 6 split doses (Aravindakshan, 1989).

The experimental design adopted was Randomized Block Design with 5 treatments including control with 5 replications (Fig.1b).

3.3.5 Artificial infestation of the experimental plants with pseudostem weevil, *O. longicollis*

For evaluating the mechanism of resistance, (antixenosis, antibiosis or tolerance) a no-choice test was conducted at field level. The weevils, which were reared with banana pseudostem as base material, were paired and used for artificial infestation. Nylon nets were wrapped around the pseudostem of five-month old plants up to 1 m height (Plate 2). A sleeve was provided for the release and recapture of weevils. Two pairs of weevils were released per each sucker-derived plant during April '99. The experiment was later repeated on tissue-cultured plants with five pairs of weevils per plant during June '99.

The following observations were recorded on the artificially infested plants to arrive at the mechanisms of resistance.

(i) basal girth of the pseudostem

(ii) girth of the pseudostem at 1 m height

(iii) height up to which infestation was observed

(iv) number of feeding holes/ovipunctures on the pseudostem

(v) surface area of infestation

(vi) size of the feeding holes

(vii) damage ratings based on scores 0-4

3.4 Identification of morphological and anatomical basis of resistance using susceptible and resistant cultivars

3.4.1 Morphological parameters of resistance

Njalipoovan was used as the resistant source and Nendran as the susceptible source. The following morphological parameters were assessed for these varieties to arrive at possible morphological bases of resistance.

(i) girth of pseudostem 1 m above ground

(ii) breadth of leaf sheath at 1 m height

(iii) width at the centre of leaf sheath

(iv) compactness

(v) size of air chamber in which eggs are laid

3.4.2 Anatomical basis of resistance

Hand sections of pseudostem sheath of both resistant (Njalipoovan) and susceptible (Nendran) varieties were taken. Since there was a distinct preference for plants nearing bunching, these observations were carried out with pseudostem of resistant and susceptible types

a) before flowering stage

b) after flowering stage

These sections were stained in aqueous saffranin. The changes in the anatomy of resistant and susceptible plants were observed under the low (40x) and

high power (100x) of the compound microscope. Photomicrographs of the sections were taken both in susceptible and resistant variety.

Micrometry studies of anatomical sections were also undertaken to observe

a) cuticle thickness

b) size of upper epidermal cell

c) compactness of upper epidermal cells

d) distance from upper epidermis to air space

e) distance from lower epidermis to air space

f) number of vascular bundles

g) size of the vascular bundles

h) size of lower epidermis

i) compactness of lower epidermal cells

3.5 Analysis for biochemical basis of resistance

The following biochemical analyses were carried out in Biochemistry laboratory, College of Horticulture, Vellanikkara.

3.5.1 Determination of moisture percentage

Pre-weighed pseudostem parts of resistant (Njalipoovan) and susceptible (Nendran) varieties were oven dried at 130±2°C to constant weight. The loss of moisture was calculated and expressed as percentage.

3.5.2 Estimation of crude fibre content

The crude fibre content of the pseudostem sheath and pith of resistant and susceptible varieties were estimated using the acid alkali digestion method (Sadasivam and Manikkam, 1996).

3.5.3 Estimation of total phenols and OD phenols

Alcoholic extracts of leaves, petiole and pseudostem sheath from plants of resistant variety Njalipoovan and susceptible Nendran were made. In Nendran, extracts from both infested and non-infested plants were prepared for phenol estimation.

To study the extent of variation of phenolic content among different stages of the plant, samples were also analysed, in susceptible variety (Nendran) at different maturity periods. During vegetative phase, one, three, five, and seven month old stages and during reproductive phase, flowering and bunching stages.

3.5.3.1 Total phenols

The colorimetric estimation of total phenols at 660 nm using folin ciocalteau reagent (Mahadevan and Sridhar, 1982) was followed. The quantity of total phenols in the sample solution was calculated from a standard curve using catechol.

3.5.3.2 Ortho dihydric phenols

The colorimetric estimation of OD phenols at 515 nm was done by Arnow's method as suggested by Mahadevan and Sridhar (1982). The quantity of OD phenols in the sample solution was calculated from a standard curve using catechol.

3.5.4 Protein estimation

The protein was estimated by the method of Lowry *et al.* (1951). The protein in the above mentioned samples were extracted with Tris buffer (pH 7) and precipitated with ten per cent Trichloro acetic acid (TCA). The quantity of protein in the sample solution was calculated from a standard curve using bovine serum albumin.

3.5.5 Polyphenol oxidase electrophoretic pattern

To study the banding pattern of isozyme polyphenol oxidase (PPO) for susceptible and resistant varieties, Polyacrylamide gel electrophoresis (PAGE) was carried out using Hoefer Mighty Small™ II gel system. Acrylamide monomers ($CH = CHCONH_2$) were polymerised with N-N methylene bisacrylamide ($CH_2(NHCONH = CH_2)_2$ bis) to obtain the gel. Fresh ammonium per sulphate was used as catalyst and N,N,N',N' tetra methylene diamine (TEMED) as chain initiator.

Polyacrylamide gel was preferred because of its chemical inertness, high resolution, and easiness in handling, transparency of the gel and easiness in preparation.

Gel preparation

The following stock solutions were prepared.

1. Monomer stock solution (30% Acry. 2.7% Bis)

Acrylamide	- 30.0 g
Bis acrylamide	- 0.8 g
Distilled water to	- 100 ml

Store at 4°C away from light

2. 4x Resolving gel buffer (1.5 M Tris-cl, pH 8.8)

Tris base	- 18.5 g
Adjust the pH to 8.8 with 1N Hcl	
Distilled water to	- 100 ml

3. Initiator (10% APS)

Ammonium per sulphate	- 0.1 g
Distilled water to (Prepared freshly)	- 1 ml

4. Destaining solution (40% Methanol, 7% Acetic acid)

Acetic acid	- 70 ml
Methanol	- 400 ml
Distilled water to	- 1000 ml

Preparation of gel column

The Hoefer Mighty Small™ II Gel system of Hoefer Pharmacia Biotech Inc, California was used. The size of the gel was 8.0 cm x 9.4 cm. The gel was prepared by using the following gel recipe.

Gel Recipe used for standardisation

	7.5%		8.5%		10%	
	10 ml	20 ml	10 ml	20 ml	10 ml	20 ml
Monomer	2.49	4.98	2.83	5.66	3.33	6.66
Resolving Buffer	2.50	5.00	2.50	5.00	2.50	5.00
Distilled water	4.94	9.88	4.60	9.20	4.10	8.20
10% APS	50 µl	100 µl	50 µl	100 µl	50 µl	100 µl
TEMED	5 µl	10 µl	5 µl	10 µl	5 µl	10 µl

Monomer

Of the above, 8.5 per cent strength gel was found suitable for obtaining clear bands. The quantity of various stocks given for 8.5 per cent gel strength were mixed serially. It was thoroughly stirred, deaerated and injected into the gel caster

with the help of gel casting syringe of Hoefer® make. The combs were placed at the top of the gel for making wells and allowed to polymerise in the caster (15-20 min). Care was taken such that the gel was devoid of gas bubbles.

Electrophoretic run

The following two buffers were prepared.

1. Electrophoresis Buffer (0.025 M Tris, pH 8.3, 0.192 M glycine)

Tris base	- 1.5125 g
Glycine	- 7.2 g
Distilled water to	- 500 ml

2. 2x Treatment Buffer (0.125 M Tris-cl, pH 6.8, 20% glycerol, 10% 2-Mercapto ethanol)

4x Tris-cl, pH 6.8	- 2.5 ml
Glycerol	- 2.0 ml
2-Mercapto ethanol	- 0.2ml
Bromophenol blue	- 0.2 mg
Distilled water to	- 10.0 ml

Divided into 1 ml aliquots, and store at -4°C.

After polymerisation, the gels were transferred to electrophoretic apparatus. The upper and lower tanks were filled with pre-chilled electrode buffer of pH 8.3. Fifteen µl of treatment buffer was mixed with 15 µl of enzyme extract in separate eppendorf's tubes. The mixture was vortexed with the help of transfer pipette of E.Merck®. The above operation was carried out at 5°C. From this mixture 15 µl was loaded to the well after removing the combs. Upper tank was connected to cathode and lower one to anode. The enzyme extracts were subjected to electrophoresis under the alkaline system of Davis (1964).

The run was carried out at room temperature for polyphenol oxidase till the tracker dye, bromophenol blue reached the anode end of the gel column. Cooling system was used to circulate cold water for maintaining room temperature as a means for heat dissipation and also to prevent the enzyme from denaturation. A current of 10 mA was maintained per plate and it took 50-75 min for completion of the run.

Sample preparation

Pseudostem sheath, petiole and leaf samples were collected during peduncle formation stage of resistant (Njalipoovan) and susceptible (Nendran) varieties. The infested and healthy plants of the susceptible variety were also analysed for comparison. The pseudostem sheath sample was taken at 1 m above the soil level. The samples were washed thoroughly with distilled water and wiped with filter paper to absorb moisture.

The following extraction buffer was used for enzyme extract preparation.

Composition of Extraction buffer

0.05 M Tris-HCl

0.1% ascorbic acid

0.1% cystein-HCl

0.002% Magnesium chloride

pH - 8.0

Stored at 4°C

Homogenization and centrifugation

This part of the experiment was carried out at 5°C. From the different proportions tried it was found that a sample: buffer ratio of 2:3 was ideal to get sufficient volume of extract in the required concentration. The samples (2 g each)

were chopped into small pieces. To this 3 ml of extraction buffer containing 17 per cent sucrose was added at the time of extraction. The sample was homogenized by grinding well with a pre-chilled mortar and pestle placed in a tray containing ice.

The slurry was centrifuged at 12,000 rpm for 20 min at 4°C in Kobato® 6900 make refrigerated centrifuge. The supernatant was used as enzyme source for polyphenol oxidase isoenzyme analysis and for assaying purpose.

Staining solution

0.1 M potassium phosphate buffer (pH 7.0)	- 200 ml
P-phenylene diamine	- 0.2 g
Catechol	- 600 mg

Equilibrated the gel for 30-60 min in the staining solution until yellow bands appear. The bands were fixed using fixing and destaining solution. Photographs were taken and zymograms were drawn. The relative electrophoretic mobility (Rm) of polyphenol oxidase isozymes was calculated as the ratio of the movement of the band to that of tracking dye.

3.5.6 Polyphenol oxidase assay

The enzyme activity of polyphenol oxidase (catechol oxidase) was determined following the method of Jennings *et al.* (1969) with slight modifications. The reaction mixture contained 0.2 ml of enzyme solution, 0.3 ml of 0.01 M catechol and 2.5 ml of 0.02 M potassium phosphate buffer of pH 6.5. The activity was recorded at 30 seconds intervals for five minutes at 495 nm. Activity was expressed in units as ΔOD per min. The enzyme solution prepared for electrophoresis was used for assaying also.

3.5.7 Enzyme activity and oxidation factor of OD phenols

The activity of the enzyme PPO depends on the levels of OD phenols. Hence, oxidation factor of OD phenols is obtained by the multiplication product of PPO activity (ΔOD/min) and amount of OD phenolic substrates (mg/g) present in the analysed samples.

Results

4. RESULTS

4.1 Preliminary field screening of *Musa* (AAB) Nendran for resistance against pseudostem weevil *Odoiporus longicollis*

The field screening allowed the development of a rating scale for the damage by pseudostem weevil. Based on the surface area of infestation (A), size of the feeding holes (B) and number of feeding holes (n), the rating scales were developed. The factor nAB was utilized for arriving at the scale. The different parameters, their multiplication product and rating scales have already been furnished in Table 1 and plants coming under these scales were utilized for further screening.

The infested banana plants of Nendran were graded based on the scoring technique explained earlier (Table 1). The graded plants from 1-4 were represented as G_1, G_2, G_3 and G_4 respectively. The number of feeding holes/ovipunctures on the entire pseudostem ranged from 1-15, 16-30, 31-45 and >45 for the grades 1-4 respectively. The surface area of infestation was found to be directly proportional to the height of the pseudostem up to which infestation was observed. The mean surface area of infestation for G_{1-4} were 0.06862, 0.087889, 0.145868 and 0.274498 m^2 respectively (Table 1). Generally the size of feeding holes varied from 0.1-5 cm^2 in all the grades.

The rating scales of damage by *O. longicollis* (Table 2) revealed that bunches were least affected in the plants scored as one and two. Unhealthy bunches, improper filling and thereby reduction in bunch weight were commonly observed among G_3 and G_4 plants. The general appearance of the infested plants also revealed that G_4 plants rarely produced bunches and even if the bunches were produced, the pseudostem broke off indicating its inability to withstand the growing weight of the bunch and/or due to heavy winds. The G_1 plants showed a comparatively greater

vigour with 4-5 hands in the bunches, even though the filling of fingers was affected at a later stage.

The symptoms observed under field conditions were gummy exudation subsequent to egg laying, feeding holes due to larval boring, brownish discolouration of the affected pseudostem portions, splitting of the pseudostem and in extreme cases lodging of the plant.

Apart from the above mentioned parameters for grading, girth of the pseudostem, breadth of the leaf sheath at 1 m height, width at the centre of pseudostem sheath, number of sheath overlapping the pith (compactness) and height of the pseudostem up to which infestation was observed were also recorded (Table 3) to study their correlation with the respective grades.

The data (Table 3) showed that the girth of pseudostem was less in the infested plants. A maximum girth of 50.3 cm was recorded among G_1 plants while the average girth of G_4 plants was 43.16 cm. The breadth of the leaf sheath at 1 m height ranged from 11-13 cm in all the grades. The width of the leaf sheath at the centre was found to range from 1.1-1.3 cm. Observations revealed that G_1 plants had greater width as compared to all the other grades while G_4 plants recorded the least width.

The compactness of the pseudostem is attributed to the number of sheaths that overlap the pith. Compactness decreased with the ascending grades and vice versa. The height of pseudostem up to which the grubs riddled during infestation ranged from 150-290 cm and was found maximum in G_4 plants (288.4 cm). G_1 plants recorded infestation up to an average height of 172.56 cm while the other grades exceeded 200 cm.

Table 3. Biometric observations of the graded mother plants

S.No.	Girth of the pseudostem at 1 m (cm)	Breadth of leaf sheath (cm)	Width of the leaf sheath at the centre (cm)	No. of sheaths overlapping the pith	Height of infestation on pseudostem (cm)
G_1R_1	49.5	12.8	1.2	8	171.8
G_1R_2	50.3	12.7	1.3	8	152.5
G_1R_3	49.8	12.5	1.2	8	170.7
G_1R_4	48.7	11.8	1.3	7	185.3
G_1R_5	47.9	12.1	1.3	8	182.5
Mean	49.24	12.38	1.26	7.8	172.56
G_1R_1	45.4	11.9	1.1	7	205.8
G_1R_2	47.2	12.3	1.2	8	195.2
G_2R_3	49.8	12.3	1.2	8	182.7
G_2R_4	47.4	12.1	1.3	8	222.6
G_2R_5	49.4	12.2	1.1	7	217.8
Mean	47.84	12.16	1.18	7.6	204.82
G_3R_1	44.8	11.5	1.2	7	187.0
G_3R_2	50.1	12.6	1.3	7	193.5
G_3R_4	49.4	12.3	1.3	8	201.8
G_3R_5	48.5	12.4	1.2	7	228.5
G_3R_5	49.3	12.6	1.3	7	215.6
Mean	48.42	12.28	1.26	7.2	205.28
G_4R_1	42.8	11.2	1.1	7	272.8
G_4R_2	43.6	11.7	1.2	7	288.4
G_4R_3	43.5	11.9	1.1	7	276.5
G_4R_4	42.7	12.2	1.1	6	267.4
G_4R_5	43.2	11.8	1.2	7	209.9
Mean	43.16	11.76	1.14	6.8	263.00

4.2 Laboratory rearing of *O. longicollis*

The methodology adopted for mass rearing of *O. longicollis* in laboratory conditions was found to be successful for maintaining a large scale population throughout the year. A continuous supply of uniformly aged test insects for exposing the experimental plants at specific pest load was made possible. The reared adult weevils were found to be competitive with wild individuals up to third generation since the former had similar fecundity (6-7 eggs/female/week) and longevity (180 days) as that of the latter.

The need for developing an artificial diet or semisynthetic diet did not arise since the natural diet of *O. longicollis*, banana pseudostem itself satisfied all the pre-requisites for a mass rearing programme.

The weevil population subsequent to third generation faced high mortality. The larval instars developed certain abnormalities like thoracic shrinking (Plate 7a), discolouration and abdominal swelling (Plate 7b). An effective means of avoiding the development of these problems was to infuse wild individuals into the laboratory colony. A high rate of cannibalism was evidenced among the different life stages during mass rearing when there was a shortage of food supply and was rectified by providing adequate supply of food material to the growing population.

The adult weevils readily mated in laboratory conditions. The pre-oviposition period ranged from 3-5 days. The isolated females after single mating deposited 3-6 eggs in an oviposition period of 1-3 days. The females generally laid their eggs inside the air chambers of the pseudostem (Plate 5). The length of the ovipositor ranged from 1-1.2 mm, which pierced through the outer epidermal layer and deposited a single egg on each air chamber. The optimum size of the air chamber generally selected for oviposition was 4 x 4 mm. However, a few eggs were also located from air chambers of size 3 x 4 mm and 4 x 5mm. The average

size of the egg is around 943.5 x 382.5 μm. The eggs had a prominent air space on the apex (Plate 5).

The incubation period of the egg ranged from 2-4 days in the most favoured host, Nendran. Generally, a gummy exudation was observed on the oviposition sites similar to the field symptom immediately after hatching of the egg. The neonate larva (L_1) slowly riddled along the air chambers longitudinally. After a day or two they pierced transversely across the outer sheath to the inner one with the help of the slightly sclerotised mandibles. This first instar showed an exclusive preference for the inner tender tissues of pseudostem sheath rather than the pith. The second instar stage (L_2) was attained in about 2-3 days. This instar also preferred feeding on pseudostem sheath, which lasted for 4-5 days. By the time the third instar stage was attained, the grub had already pierced 3-4 pseudostem sheaths that overlapped the central pith.

The third instar (L_3) had heavily sclerotised mandibles which bored straight into the pith where it fed for about 4-6 days. The fourth instar (L_4) which lasted for 5-6 days was recorded as the most voracious feeder of pith accounting for the abrupt increase of the weight and plumpiness of the larvae that progresses towards the final instar. In certain cases if the fourth instar fails to attain a good weight it may not lead to the final instar. Instead, it would moult once or twice further before pupation. The fifth instar (L_5) generally fed for 6-7 days before going into pupation. This instar initially fed on the pith where it fed as the previous instar, and gradually migrated to the peripheral region of the pseudostem. The fibres of pseudostem sheath were cut and woven to a thick fibrous cocoon of length 2.8-3.4 cm towards the outer layers of the sheath, ensuring the easy emergence of adults. The pupal period ranged from 10-13 days. Thus the life cycle (Plate 6) gets completed in about 37-42 days.

Plate - 4 Colour morphs of *Odoiporus longicollis*

Plate - 5 Enlarged view of eggs in air chambers of pseudostem sheath

Plate - 6 Life cycle of *O. longicollis*

Plate - 7 Abnormalities of grubs due to continuous culturing

7a Thoracic shrinking of the grub

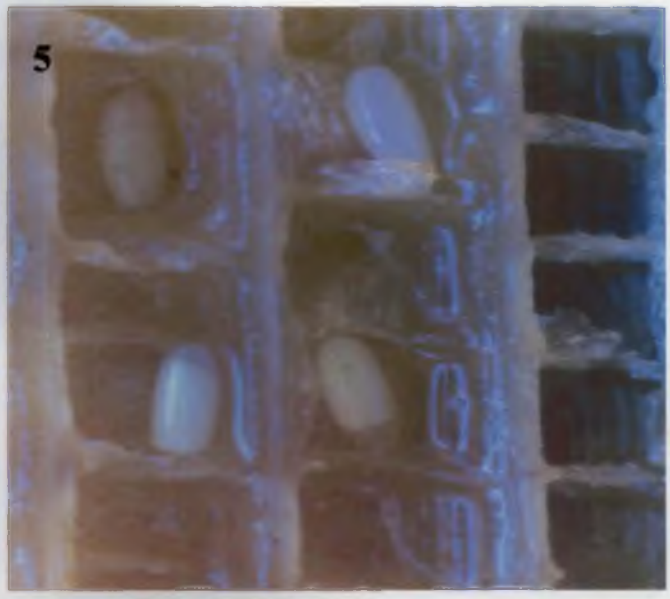
7b Abdominal swelling of the grub

Plate - 8 Antibiotic effects on the life stages reared on Njalipoovan

8a Mandible loss incurred by the third instar grub

8b Malformed pupa from pith of Njalipoovan

8c Undersized adult



→ Left mandible

The newly emerged females generally passed through a pre-mating period of 3-4 days. The longevity of the adult weevils ranged from 130-180 days, out of which the females were capable of reproducing for about 90-110 days.

There were two distinct colours for the adult weevils. Black and reddish brown coloured weevils existed in both field-collected and laboratory-reared populations (Plate 4). Observations on newly emerged adults of both these categories revealed that the differentiation of these two colours was not dependent on tanning or chitinisation process. In spite of these colour variations the weevils were found to inter-breed among themselves and hence they can be confirmed as colour morphs of the same species.

4.2.1 Duration of development of different life stages of *O. longicollis* on susceptible and resistant cultivars

The preliminary free-choice tests conducted in the laboratory revealed absence of oviposition in Njalipoovan when Nendran was provided along with it. Hence no-choice tests were designed to make comparative studies.

The results of no-choice test revealing the number of eggs deposited, per cent hatching of eggs and per cent adult emergence were presented in Table 4.

The mean number of eggs deposited by a female curculio was 6.9 per week in Nendran which was significantly higher than that of Njalipoovan (2.7 eggs/female/week). The water suckers of Nendran and Njalipoovan recorded an average of 3.1 and 2.1 eggs/female/week respectively.

The percentage hatching of eggs was remarkably high in Nendran (94.9%) as compared to 39.5 per cent recorded in Njalipoovan. Most of the eggs deposited in the air chambers of Njalipoovan got desiccated. There was a severe reduction in the percentage of hatched eggs that developed into adults in

Table 4. Oviposition, percentage hatching of eggs and adult emergence on susceptible and resistant cultivars.

Sl. No.	No. of eggs deposited		% hatching of eggs		% adult emergence from eggs	
	Nendran	Njalipoovan	Nendran	Njalipoovan	Nendran	Njalipoovan
1	10	3	90(9)	66(2)	80(8)	33(1)
2	7	5	100(7)	60(3)	86(6)	60(3)
3	5	2	100(5)	100(2)	100(5)	50(1)
4	3	1	100(3)	0(0)	100(3)	-
5	9	-	89(8)	-	89(8)	-
6	4	6	100(4)	50(3)	100(4)	50(3)
7	5	7	80(4)	86(6)	80(4)	71(5)
8	11	-	90(10)	-	90(10)	-
9	8	-	100(8)	-	88(7)	-
10	7	3	100(7)	33(1)	100(7)	33(1)
Mean	6.9	2.7	94.9	39.5	91.3	29.7

The figures in parentheses represents the actual number.

Njalipoovan. This was due to high mortality of first and second instars reared on Njalipoovan. In few situations, the third instar larvae incurred a mandible loss (Plate 8a) probably owing to the high crude fibre content of the resistant cultivar. In certain cases, the final instar which entered the pith struggled to reach the periphery for pupation. In such an extreme situation it pupated in the pith. Here the nature of the cocoon was altered from fibrous to a soft one which was often malformed (Plate 8b) such cocoons failed to support proper development of the adults leading to its death. In many cases the adults failed to emerge from the pupa. Malformed and undersized adults (Plate 8c) were also common.

The duration of development of eggs, larval instars and pupa to adult on these two varieties has been furnished in Table 5. Among the two cultivars tried as hosts, Nendran allowed the weevil to complete the life cycle from egg to adult in 38.2 days. A comparatively longer duration of 45.2 days was taken on Njalipoovan. The average incubation period was higher (5.4 days) in Njalipoovan while it was only 2.8 days in Nendran. A wide variation in pupal period was observed in Njalipoovan from 13-17 days. The pupal period in Nendran ranged from 10-13 days. The mean of total larval period was 23.8 days in Nendran while it took 25.6 days in Njalipoovan. The larval instars L_1, L_2, L_3, L_4 and L_5 recorded a duration of 2.6, 4.2, 4.8, 5.6 and 6.6 days respectively in the favourable host while it had a duration of 3.4, 5.4, 5.0. 6.0 and 5.8 days respectively in the non-preferred host.

4.2.2 Oviposition of *O. longicollis* reared on pseudostem of Nendran at various growth stages

The comparative study on the number of eggs laid on the pseudostems of Nendran at different growth stages (Fig.2) revealed a significantly higher oviposition (6.2 eggs/female) on seven month old plants. The number of eggs deposited by a female curculio was comparatively lower in three, four, five and six month old stage of the crop while absence of oviposition evidenced on the

Table 5. Duration of development (days) of *O. longicollis* on susceptible and resistant cultivars

Sl. No.	Incubation period (days)	Larval period (days)						Pupal period (days)	Egg to adult emergence (days)
		L_1	L_2	L_3	L_4	L_5	Total		
S_1	2	2	4	4	6	7	23	12	37
S_2	4	3	4	6	6	6	25	13	42
S_3	3	3	4	5	5	7	24	10	37
S_4	2	3	5	4	6	7	25	11	38
S_5	3	2	4	5	5	6	22	12	37
Mean	2.8	2.6	4.2	4.8	5.6	6.6	23.8	11.6	38.2
(Range)	(2-4)	(2-3)	(4-5)	(4-6)	(5-6)	(6-7)	(22-25)	(10-13)	(37-42)
R_1	6	3	6	6	7	5	27	14	47
R_2	5	4	4	5	5	6	24	17	46
R_3	7	3	6	5	6	6	26	14	47
R_4	5	4	6	4	6	7	27	13	45
R_5	4	3	5	5	6	5	24	13	41
Mean	5.4	3.4	5.4	5.0	6.0	5.8	25.6	14.2	45.2
(Range)	(4-7)	(3-4)	(4-6)	(4-6)	(5-7)	(5-7)	(24-27)	(13-17)	(41-47)

S – Susceptible – Nendran R – Resistant - Njalipoovan

Fig. 2. Oviposition on the pseudostem of Nendran at different growth stages in laboratory

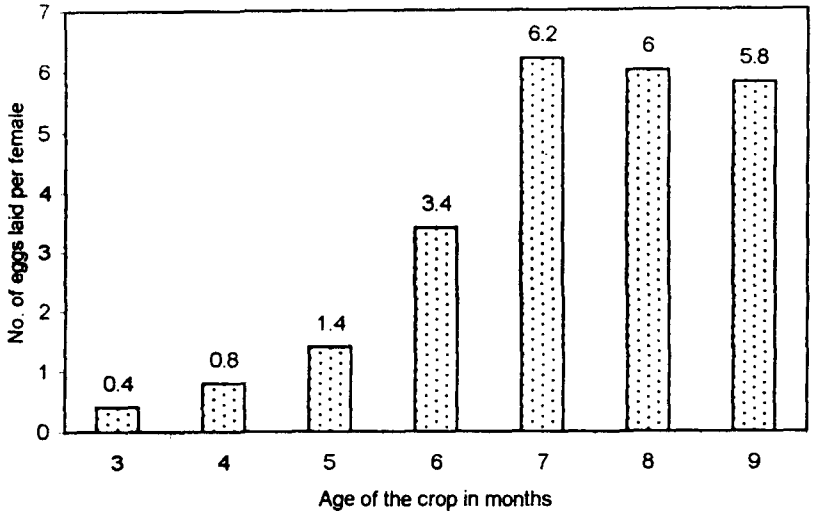


Fig. 3. Oviposition by varying number of females per pseudostem

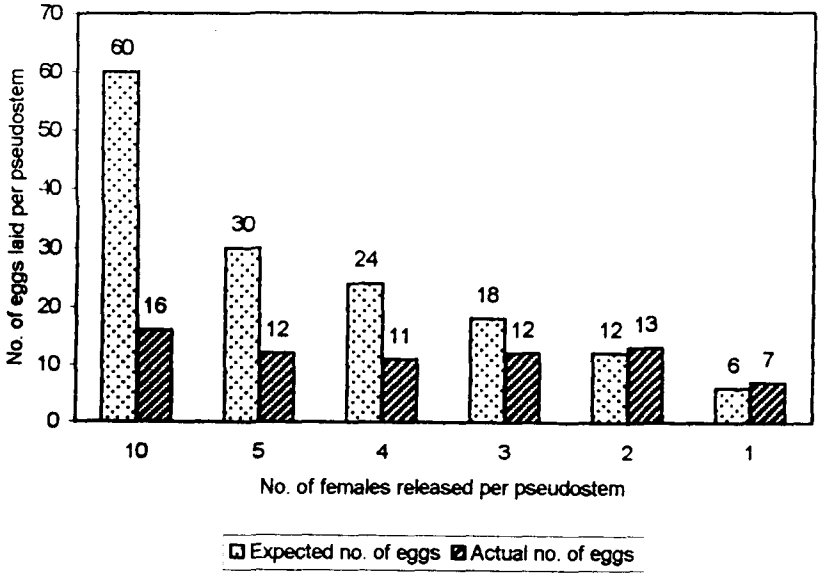


Table.6.Egg laying on pseudostems treated with various solvent extracts of oviposited pseudostem sheaths.

No. of females confined in the pseudostem	Oviposition by two females			
	water	methanol	acetone	ether
10	7	6	-	-
5	8	5	2	3
4	9	6	5	5
3	8	5	7	6
Control	10.5	7.5	6	6.5

pseudostems of one and two month old plants. Eight and nine month old pseudostems were deposited with 6.0 and 5.8 eggs/female respectively. These results gave a better understanding about the susceptible stage of the crop.

4.2.3 Epidiectic compounds

The results presented in Fig.3 depicted that the increase in the number of females did not show a corresponding increase in the number of eggs laid by them. Theoretically, the expected number of eggs for ten, five, four, three, two and one number of females were 60, 30, 24, 18, 12 and 6 respectively, considering the average number of eggs laid by a female/week as six. But the actual egg count recorded was 59.7, 60, 54.2 and 33.3 per cent lower than the expected value for ten, five, four and three females respectively. However, trials with one and two females revealed an egg count slightly higher than the expected number of eggs. These results evidently suggested the existence of a spacing pheromone which considerably reduced the number of eggs deposited as the number of weevils increased.

For further confirmation, the whole extract of the outer three pseudostem sheaths used for above mentioned experiment was prepared in water, methanol, acetone and diethyl ether. These extracts were sprayed upon fresh pseudostems and exposed to two pairs of *O. longicollis*. Absence of oviposition was evidenced in pseudostems sprayed with acetone and diethyl ether extract of pseudostem previously exposed to ten females (Table 6). In all the other cases egg laying was recorded, though less than the theoretical expectation of 12 eggs/week for two females.

4.3 Evaluation of sucker and *in vitro* regenerated progenies of naturally infested *Musa* (AAB) for resistance against *O. longicollis*

The results of the no-choice tests (artificial infestation) conducted on sucker and *in vitro* regenerated progenies were presented in Table 7, 8 and 9.

The plantlets of mother plants scored as G_1, G_2, G_3 and G_4 were represented as T_1, T_2, T_3 and T_4 respectively while the control was denoted as T_5 in sucker derived plants. In the case of tissue cultured plants, they were represented as T_6, T_7, T_8 and T_9 with T_{10} as control.

In sucker derived progenies the no-choice experiment was conducted during May-August at flower bud initiation stage of the crop with two females and two males per plant. The basal girth of the treatments did not show much difference while the girth at 1 m decreased with higher grades of infestation among treatments T_1 to T_5 (Table 7). The T_5 plants recorded least girth of 42.37 cm and maximum girth of 48.79 cm was observed in T_2 plants. The mean height up to which the grubs riddled during infestation was maximum in control (203.8 cm). The average height of infestation increased from T_1 to T_4.

Initial symptoms of oviposition was gummy exudation with a reddish brown puncture mark on the stem (Plate 9) followed by development of bore holes at approximately equidistance apart on the pseudostem due to feeding of earlier larval instars (Plate 10). Severely attacked plants snap off at the region of heavy infestation usually 1 to 1.5 m from the soil level (Plate 11).

The number of hands produced was generally, five in healthy plants. However, in T_4 and T_5 the number of hands reduced to an average of 4.4. All the treatments except the control gave considerably good yield. All the experimental plants were found invariably superior to the control plants (T_5) with bunch weight

Plate-9 Gummy exudation on pseudostem due to oviposition.

→ovipunctures

Plate-10 Bore holes of the grubs

Plate-11 A control plant showing lodging

Table 7. Biometric observations of the sucker derived experimental plants

Pl.No.	Basal girth (cm)	Girth at 1 m height	Height of infestation (cm)	No. of hands	Weight of bunch (kg)
T_1R_1	50.00	38.75	196	5	8.5
T_1R_2	60.00	52.50	-	5	7.5
T_1R_3	60.60	52.80	-	5	9.0
T_1R_4	55.00	42.50	161	4	5.5
T_1R_5	57.50	53.75	-	5	7.5
Mean	56.62	48.06	71.4	4.8	7.6
T_2R_1	62.50	50.00	239	5	8.0
T_2R_2	46.25	42.50	205	5	9.0
T_2R_3	67.50	51.25	168	5	9.0
T_2R_4	60.00	47.70	-	5	8.5
T_2R_5	60.00	52.50	192	5	7.0
Mean	59.25	48.79	160.8	5	8.3
T_3R_1	55.00	42.50	154	4	8.0
T_3R_2	53.75	41.80	167	5	5.5
T_3R_3	58.75	48.75	185	5	9.5
T_3R_4	55.00	43.75	120	6	7.5
T_3R_5	60.00	47.50	179	5	10.5
Mean	56.50	44.86	161	5	8.2
T_4R_1	56.25	42.50	187	5	5.0
T_4R_2	60.00	50.00	215	5	10.0
T_4R_3	53.75	40.00	198	4	5.5
T_4R_4	52.50	38.75	206	4	6.0
T_4R_5	60.00	52.50	-	4	7.5
Mean	56.50	44.75	161.2	4.4	6.8
T_5R_1	48.75	35.28	220	5	5.75
T_5R_2	57.50	45.00	217	4	5.0
T_5R_3	52.00	40.25	192	5	6.5
T_5R_4	57.25	43.80	205	4	5.5
T_5R_5	53.25	47.50	185	4	5.0
Mean	53.75	42.37	203.8	4.4	5.55

ranging from 5-10 kg. Among the treatments T_2 gave the highest average yield (8.3 kg) followed by T_3, T_1 and T_4.

Though all the plants were artificially infested in a no-choice test, all the treatments showed abnormal capacity to withstand lodging, due to infestation in contrast to control which lodged well before the maturity of the bunch (Plate 11).

The T_1 and T_2 plants showed moderate symptoms of attack during earlier stage of artificial infestation and were scored as G_1 and G_2 respectively (Table 8). The moderate weevil infestation harboured by the plant T_1R_1 ended up in G_2 with 32 feeding holes of 0.24 cm^2 size and 925.05 cm^2 surface area of infestation (Plate 12b). This plant produced a good bunch of 8.5 kg (Plate 12a). This clearly indicated the ability of treatment plants to exhibit greater vigour and tolerate infestation. The T_3 plants showed late infestation symptoms and hence ended up in a lesser grade G_1.

The T_4 and T_5 (control) plants succumbed to severe attack immediately after infestation. Even though T_4 and control behaved similarly during initial phases of the experiment, T_4 exhibited tolerance to a growing population of weevil grubs which was recognized with a higher yield (6.8 kg) and greater potential to stand amidst heavy winds till complete maturity of the bunch. The experimental plant T_4R_2 which incurred heavy damage among T_4 still produced a 10 kg bunch of five hands (Plate 13a). This plant had 50 feeding holes and 1350.15 cm^2 surface area of infestation (Plate 13b) and fell in G_3. T_4 plants were scored G_3 while the damage incurred by the control plants exceeded severity and were rated as G_4.

The response of tissue-cultured plants to no-choice experiments at peduncle formation stage were presented in Table 9. Ten adult curculionids in sex ratio 1:1 was used in this study during July to October.

Table 8. Response of sucker derived plants subsequent to artificial infestation

Pl.No.	No. of ovipunctures/ feeding holes (n)	Surface area of infestation m^2 (A)	Size of feeding holes (cm^2) (B)	Factor nAB	Grade
T_1R_1	32	.09251	0.24	0.7104	2
T_1R_2	0	0	0	0	0
T_1R_3	0	0	0	0	0
T_1R_4	6	.04825	0.15	0.0434	1
T_1R_5	0	0	0	0	0
				0.09074 ⇒	**1**
T_2R_1	20	.1080	0.50	1.0802	2
T_2R_2	7	.0882	0.25	0.1543	1
T_2R_3	10	.0630	0.12	0.0756	1
T_2R_4	0	0	0	0	0
T_2R_5	21	.0995	0.25	0.5221	2
				0.3664 ⇒	2
T_3R_1	4	.06000	0.50	0.1200	1
T_3R_2	8	.06808	0.25	0.1361	1
T_3R_3	5	.07203	0.12	0.0432	1
T_3R_4	2	.03605	0.12	0.0086	0
T_3R_5	4	.04822	0.25	0.0482	1
				0.0712 ⇒	**1**
T_4R_1	17	.09205	0.25	0.3912	2
T_4R_2	50	.13502	1.25	8.4380	3
T_4R_3	12	.05853	0.12	0.0842	1
T_4R_4	20	.11553	1.50	0.0842	1
T_4R_5	0	0	0	0	0
				2.475 ⇒	**3**
T_5R_1	62	.25158	5.0	77.990	4
T_5R_2	71	.28760	5.0	102.097	4
T_5R_3	38	.09925	4.5	16.972	4
T_5R_4	51	.11855	5.0	30.230	4
T_5R_5	45	.09757	3.0	13.172	3
				48.092 ⇒	**4**

Plate - 12 Response of T_1 subsequent to artificial infestation

Plate – 13 Exceptional tolerance of T_4R_2 subsequent to artificial infestation

a.Full view. b.Magnified view of infested pseudostem.

Table 9. Response of tissue cultured plants subsequent to artificial infestation, at peduncle formation stage

Pl.No.	Basal girth (cm)	Girth at 1m height (cm)	Height of infestation (cm)	No. of ovipunctures/ feeding holes (n)	Surface area of infestation m^2 (A)	Size of feeding holes (cm^2) (B)	Factor nAB	Grade
T_6R_1	45.2	32.5	0	0	0	0	0	0
T_6R_2	50.1	35.6	62.5	43	.02625	0.24	0.271	2
T_6R_3	55.2	37.5	90.3	30	.02709	0.25	0.203	2
T_6R_4	50.7	39.2	0	0	0	0	0	0
T_6R_5	55.6	42.7	0	0	0	0	0	0
							0.09482 ⇒	**1**
T_7R_1	55.7	40.2	0	0	0	0	0	0
T_7R_2	52.5	40.1	0	0	0	0	0	0
T_7R_3	60.1	42.5	0	0	0	0	0	0
T_7R_4	58.3	39.2	0	0	0	0	0	0
T_7R_5	60.0	42.5	0	0	0	0	0	0
							0 ⇒	**0**
T_8R_1	55.1	40.2	0	0	0	0	0	0
T_8R_2	47.5	35.8	172.5	52	.06555	0.70	2.386	3
T_8R_3	52.5	40.1	0	0	0	0	0	0
T_8R_4	57.5	40.3	0	0	0	0	0	0
T_8R_5	55.1	39.7	0	0	0	0	0	0
							0.4772 ⇒	**2**
T_9R_1	62.5	45.1	0	0	0	0	0	0
T_9R_2	50.1	37.5	0	0	0	0	0	0
T_9R_3	52.5	40.2	45.0	21	.02707	0.20	0.114	2
T_9R_4	52.6	37.5	82.5	63	.06605	0.80	3.329	3
T_9R_5	62.5	47.5	0	0	0	0	0	0
							0.6885 ⇒	**2**
$T_{10}R_1$	47.5	36.9	75.9	53	.06072	0.35	1.126	3
$T_{10}R_2$	50.2	37.8	0	0	0	0	0	0
$T_{10}R_3$	57.5	45.2	0	0	0	0	0	0
$T_{10}R_4$	54.8	37.5	90.2	33	.08100	0.40	1.069	3
$T_{10}R_5$	50.9	36.8	80.9	16	.09304	0.70	3.907	3
							1.221 ⇒	**2**

The treatments T_6, T_7, T_8 and T_9 were scored subsequently as G_1, G_0, G_2 and G_2 respectively. Only 32 per cent of the plants got infested in this experiment. Fifty five per cent of the control (T_{10}) plants got severe attack in contrast to most of the experimental plants which escaped infestation. The T_7 plants were not even tested for oviposition, even after artificial infestation by ten weevils (5♀+ 5♂) for about three months.

4.4 Identification of morphological and anatomical bases of resistance

4.4.1 Morphological parameters of resistance

The results on the morphological parameters of resistant and susceptible cultivars at peduncle formation stage were presented in Table 10.

The girth of the pseudostem is lesser (48.7 cm) in Njalipoovan with comparatively lesser breadth (11.59 cm) and width (1.258 cm) of the leaf sheath. The average girth of Nendran pseudostem was 51.8 cm. The mean breadth and width at the centre of the leaf sheath were 13.75 cm and 1.475 cm respectively. The number of sheaths overlapping the pith was more in Njalipoovan (8.42) than that in Nendran (6.83). The air chambers were relatively bigger in resistant variety with 3.5 mm length and 5.1 mm breadth. The average size of the cell in Njalipoovan was 17.85 mm^2 in contrast to Nendran with 14.35 mm^2.

However, the differences in the morphological parameters were not statistically significant.

4.4.2 Anatomical basis of resistance

The anatomical sections of the pseudostem sheath of the susceptible variety, Nendran and resistant variety, Njalipoovan at 'before flowering' and 'after flowering' stage revealed the following characteristics (Table 11, Plate 14). The basic anatomical structure of the pseudostem in both the varieties was the same. The

Table 10. Morphological parameters of resistant and susceptible cultivars at peduncle formation stage

Pl.No.	Girth of pseudostem (cm)		Breadth of sheath (cm)		Width at the centre (cm)		No. of sheaths		Size of air chamber (mm^2)	
	R	S	R	S	R	S	R	S	R	S
1	53.2	51.5	11.5	15.2	1.2	1.3	8	7	4x6	3x5
2	49.7	53.1	12.2	11.3	1.1	1.4	9	6	3x5	3x5
3	49.5	50.7	10.1	14.1	1.5	1.7	10	7	3x5	3x4
4	45.4	52.5	11.1	14.5	1.6	1.3	9	7	4x5	4x4
5	45.2	49.8	10.1	15.1	1.4	1.3	9	6	4x5	4x4
6	47.8	50.2	10.5	13.7	1.2	1.4	8	7	3x5	4x3
7	52.7	54.1	11.7	11.2	1.1	1.5	9	8	4x6	3x4
8	48.1	51.6	14.5	14.2	1.0	1.5	7	6	4x5	4x4
9	49.2	52.7	13.7	15.1	1.4	1.9	8	7	4x5	4x4
10	49.5	53.2	11.5	12.4	1.2	1.2	7	7	3x5	3x4
11	46.3	51.7	11.3	14.4	1.2	1.7	9	8	4x4	4x4
12	48.2	50.5	10.8	13.8	1.3	1.5	8	6	4x5	3x5
Mean	48.7	51.8	11.58	13.75	1.258	1.475	8.42	6.83	3.5x5.1 (17.85)	3.5x4.5 (14.35)

R – Resistant – Njalipoovan
S – Susceptible – Nendran

Table 11. Pseudostem sheath anatomy of resistant and susceptible cultivars

Sl.No.	Parameters	Nendran						Njalipoovan					
		Before flowering			After flowering			Before flowering			After flowering		
		Min	**Mean**	Max	Min	**Mean**	Max	Min	**Mean**	Max	Min	**Mean**	Max
1. UPPER EPIDERM													
a.	Cuticle thickness (μm)	1.090	**1.212**	1.333	0.933	**1.089**	1.245	1.212	**1.879**	2.545	1.212	**1.827**	2.442
b.	Size of upper epidermal cell (μm²) (length x breadth)	154.36 (13.25x 11.64)	**158.38**	162.39 (13.39x 12.12)	142.63 (12.74x (11.19)	**161.57**	180.53 (14.89x (12.12)	196.79 (14.85x (13.25)	**203.83**	210.87 (15.59x (13.52)	192.58 (14.87x (12.95)	**230.37**	268.16 (19.83x (13.52)
c.	No. of upper epidermal cells covered in 1 mm	63.25	**65.00**	66.75	62.50	**64.75**	67.00	46.75	**49.63**	52.50	42.75	**46.75**	50.15
2. MESODERM													
a.	Distance from upper epidermis to air space (mm)	1.112	**1.542**	1.971	0.928	**0.978**	1.028	1.171	**1.485**	1.799	1.085	**1.300**	1.515
b.	Distance from lower epidermis to air space (mm)	0.428	**0.443**	0.457	0.360	**0.399**	0.438	0.527	**0.483**	0.571	0.471	**0.491**	0.571
c.	No. of vascular bundles	10	**14**	18	9	**11**	13	7	**11.5**	16	6	**9**	12
d.	Size of the vascular bundles (mm²)	0.237	**0.576**	0.914	0.285	**0.571**	0.857	0.314	**0.614**	0.913	0.252	**0.597**	0.942
3. LOWER EPIDERM													
a.	Size of lower epidermal cell (μm)² (length x breadth)	143.44 (13.15x 10.90)	**166.97**	190.50 (14.29x 13.33)	158.92 (14.20x 11.19)	**175.80**	192.67 (14.35x (13.42)	270.82 (22.34x 12.12)	**291.49**	312.15 (24.42x 12.76)	280.52 (20.75x 13.52)	**293.19**	305.85 (24.42x 12.52)
b.	No. of lower epidermal cells covered in 1 mm	44.50	**48.00**	51.50	41.00	**49.38**	57.75	34.50	**38.33**	42.15	39.75	**40.63**	41.50

cuticle, epidermis, hypodermal region and the ground parenchymous tissue, with air columns form the basic structure.

4.4.2.1 Upper epiderm

Cuticle was found to be thinner in Nendran (1.089 µm) as compared to the resistant variety, Njalipoovan (1.827 µm) (Plate 14a, b). Among the two stages taken for comparison, the 'before flowering' stage had a relatively thicker cuticle than the 'after flowering' stage.

Epidermal cells of Njalipoovan were bigger in size as compared to that of Nendran. Just beneath the epidermis, there were a few layers of hypodermal collen chymous tissue, which was thicker in Njalipoovan with their constituent cells being small and compactly arranged (Plate 14i). In the case of Nendran the epidermal cells were relatively smaller in size and the hypodermal layers also appeared lesser in number (Plate 14j).

4.4.2.2 Mesoderm

The parenchymatous cells which formed the ground tissue were smaller in size and compactly arranged with too many starch granules in the resistant variety, Njalipoovan (Plate 14g). The vascular bundles which were arranged in a serial manner were greater in size (Table 11) and each has very well-defined sclerenchymatous bundle sheath in Njalipoovan (Plate 14c). The intercellular spaces are relatively bigger and more in number in susceptible cultivar (Plate 14h). The anatomy of Nendran pseudostem sheath revealed loosely arranged ground cells with more of air spaces. The parenchymatous cells were bigger in size. The bundle sheath of the vascular tissue was not well developed (Plate 14d). The width of the cortex was greater in Njalipoovan, excluding the central air cavity. The cross section of pseudostem also revealed a bigger air cavity in Nendran with lesser layers of ground tissues.

Plate - 14 Pseudostem sheath anatomy of *Musa* spp.

14a Upper epiderm – Njalipoovan (4 x 2. 5 X)

14b Upper epiderm – Nendran (4 x 2. 5 X)

14c Paranchymatous tissues embedding vascular bundles – Njalipoovan (10 x 2. 5 X)

14d Paranchymatous tissues embedding vascular bundles – Nendran (10 x 2. 5 X)

14e Lower epiderm – Njalipoovan (4 x 2. 5 X)

14f Lower epiderm – Nendran (4 x 2. 5 X)

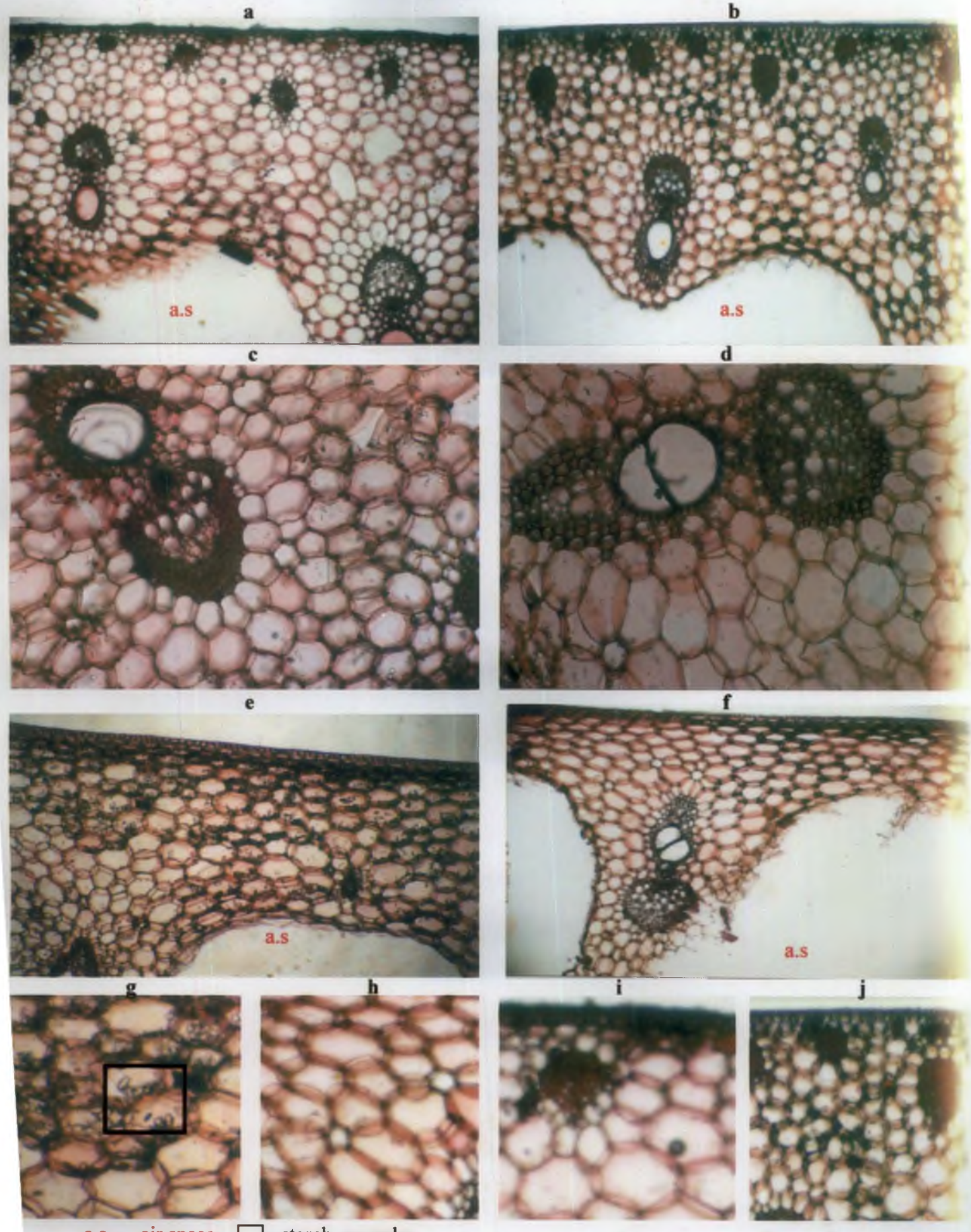
14g Mesoderm with starch granules – Njalipoovan (10 x 2. 5 X)

14h Mesoderm showing intercellular spaces – Nendran (10 x 2. 5 X)

14i Magnified view of cuticle, epidermis and hypodermal layers – Njalipoovan (10 x 2. 5 X)

14j Magnified view of cuticle, epidermis and hypodermal layers – Nendran (10 x 2. 5 X)

Plate - 14 Pseudostem sheath anatomy of *Musa* spp.



a.s - air space ☐ - starch granules

4.4.2.3 Lower Epiderm

The lower epidermal cells were relatively bigger and compactly arranged in the case of Njalipoovan with a thicker hypodermis constituting thick walled sclerenchymatous cells. Several starch granules are embedded in this region. An extra band of parenchyma of three to four layer thickness below the air cavity connecting the peripheral layers of vascular bundles was also observed in Njalipoovan (Plate 14e), while pseudostem sheath of Nendran revealed loosely packed, smaller sized epidermal cells, thinner hypodermis and lesser width of parenchymous layers beneath the air space (Plate 14f).

4.5 Analysis for biochemical basis of resistance

4.5.1 Moisture percentage

Randomization test for two independent samples, a non-parametric technique (Seigal, 1956) was the statistical analysis followed for determining the significance of moisture percentage and crude fibre content. Results were presented in Table 12.

The moisture percentage differed significantly between Nendran and Njalipoovan irrespective of the stage of plant and parts of the plant selected for analysis.

Pseudostem sheath of Nendran recorded 6.1 per cent and 5.1 per cent higher moisture content than Njalipoovan at 'before flowering' and 'after flowering' stages respectively. A significant increase in the moisture percentage was observed in 'after flowering' stage in both the varieties.

Among the plant parts analysed, central pith had greater moisture percentage. The pith of Nendran was observed to have the highest moisture per cent (97.85%) at 'after flowering' stage. The percentage of moisture in the inner

Table 12. Moisture and crude fibre content (%) in pseudostems of test entries at 'before' and 'after flowering' stages

Samples		Nendran		Njalipoovan	
		Moisture %	Crude fibre %	Moisture %	Crude fibre %
Sheath	b	95.12	53.68	89.64	57.94
Sheath	a	96.43	50.66	91.73	56.76
Centre primordia	b	95.92	34.42	90.61	49.77
Centre pith	a	97.85	37.22	96.25	49.82
Pseudostem	b	95.40	46.86	90.04	58.43
Pseudostem	a	97.30	43.91	94.08	53.70
Water sucker (Pseudostem)	b	96.41	43.74	94.40	52.96

b – before flowering
a – after flowering

primordia at 'before flowering' was 2 and 6.2 per cent lesser than the pith at 'after flowering' stage in Nendran and Njalipoovan respectively.

The total pseudostem also recorded significant difference between the stages and among the varieties. The lowest moisture per cent was observed in Njalipoovan at 'before flowering' stage (90.04%).

Water sucker of Njalipoovan had an average of 94.40 per cent moisture while water sucker of Nendran recorded 96.41 per cent. It was also evident from the Table 12 that water suckers of both the varieties had higher moisture content than the sword sucker of same age.

4.5.2 Crude fibre content

The crude fibre percentage was significantly lesser in the susceptible cultivar Nendran as compared to the resistant cultivar Njalipoovan in all the plant parts taken for analysis (Table 12).

Generally leaf sheath recorded the highest crude fibre content while pith had the lowest. In the case of leaf sheath, there was a significant reduction in the crude fibre content from 'before flowering' to 'after flowering' stage. The outer leaf sheath of Njalipoovan was found to have eight and twelve per cent higher crude fibre content than Nendran at 'before flowering' and 'after flowering' stages respectively. A remarkably higher crude fibre per cent was observed in sheath than in pith in both the varieties.

Leaf primordia at 'before flowering' stage and central pith at 'after flowering' exhibited insignificant difference in crude fibre content in Njalipoovan. But in general, the crude fibre content was much higher in both the cases than in Nendran, accounting for about 12-15 per cent difference.

The crude fibre in pseudostem at 'before flowering' and 'after flowering' stages differed significantly with a reduction of 6.7 and 8.8 per cent in Nendran and Njalipoovan respectively. An average of 23.5 per cent higher crude fibre content was recorded in Njalipoovan than in Nendran.

The water suckers of both the varieties recorded significantly lesser crude fibre than the sword suckers of same stage.

4.5.3 Estimation of total phenols and OD phenols

The results of phenol estimation were presented in the Table 13,14. The Kruskal-Wallis one-way analysis of variance by ranks (Seigal, 1956) was done to determine the significance.

4.5.3.1 Total phenols

The variation in the total phenol content of the two varieties Nendran and Njalipoovan was found to be statistically significant (Table 13). Njalipoovan leaf sheath recorded 168 per cent higher total phenol content than Nendran sheath. The total phenols of petiole samples of both the varieties were on par. The leaf samples of Njalipoovan exhibited 43 per cent higher total phenol content than Nendran leaf ($0.5 \text{ mg } g^{-1}$).

Among the infested and healthy Nendran plants, infested leaf sheath and leaf showed significant difference as compared to their healthy counterparts. In the case of petiole samples the total phenol content was not significantly varying among healthy and infested Nendran.

Table 14 represents the total phenol content of Nendran at various growth stages. The data revealed a significant difference between the stages. Among all the stages compared bunching stage recorded a higher total phenol content.

Table 13. Total phenol, OD phenol and protein content (mg g^{-1}) in test entries of banana at peduncle formation stage

Samples	Total phenol (mg g^{-1})			OD phenol (mg g^{-1})			Protein (mg g^{-1})		
	R	SH	SI	R	SH	SI	R	SH	SI
Sheath	0.255	0.095	0.111	0.081	0.033	0.039	0.781	0.524	0.211
Petiole	0.177	0.191	0.149	0.077	0.063	0.064	0.794	0.784	0.294
Leaf	0.715	0.500	0.555	0.337	0.211	0.205	1.233	1.315	1.119

R – Resistant – Njalipoovan
SH – Susceptible healthy – Nendran
SI – Susceptible infested – Nendran

Table 14. Total phenol, OD phenol and protein content (mg g^{-1}) in different parts of Nendran at progressive growth stages

Growth stages	Total phenol (mg g^{-1})			OD phenol (mg g^{-1})			Protein (mg g^{-1})		
	S	P	L	S	P	L	S	P	L
First month	0.138	0.157	0.588	0.053	0.065	0.170	0.412	0.549	1.118
Third month	0.163	0.215	0.597	0.043	0.061	0.185	0.564	0.545	1.027
Fifth month	0.083	0.156	0.555	0.036	0.076	0.179	0.803	0.837	1.054
Seventh month	0.142	0.254	0.598	0.023	0.063	0.193	0.715	0.919	1.156
At flowering	0.212	0.292	0.634	0.117	0.063	0.203	0.511	1.056	1.297
At bunching	0.266	0.343	0.664	0.049	0.067	0.205	0.411	0.672	1.191

S – Sheath; P – Petiole; L – Leaf

4.5.3.2 Ortho dihydric phenols

The content of OD phenol differed significantly in the two varieties studied (Table 13). The leaf sheath of resistant cultivar Njalipoovan recorded 145 per cent higher OD phenol than Nendran leaf sheath. The highest OD phenol content was obtained from Njalipoovan leaf (0.337 mg g^{-1}). The Njalipoovan petiole had 22 per cent higher OD phenol than Nendran petiole.

The data pertaining to OD phenol content of infested and healthy Nendran possessed insignificant difference between them. The OD phenol content of leaf sheath, petiole and leaf were on par for healthy and infested Nendran plants.

Among the plant parts, leaves registered higher OD phenol content followed by petiole and leaf sheath.

The OD phenol content was also studied at different growth stages of Nendran (Table 14). The general gradation in content is from sheath to petiole and from petiole to leaf. The highest OD phenol content in this case is again in the leaves.

4.5.4 Protein estimation

The Kruskal-Wallis one-way analysis of variance by ranks (Seigal, 1956) revealed significant differences in protein content of the two varieties Nendran and Njalipoovan (Table 13). The resistant ecotype Njalipoovan exhibited 49 per cent higher protein content than Nendran in the leaf sheath. Of the two varieties, Nendran recorded a lower protein content (0.524 mg g^{-1}) in the sheath.

The petiole samples failed to show significant difference in protein content between the varieties. Analysis on leaf samples revealed a significantly

higher protein content in Nendran (1.315 mg g^{-1}) while Njalipoovan recorded a relatively lower protein content (1.233 mg g^{-1}).

Among the infested and healthy Nendran analysed, infested plant parts showed significantly lesser protein content as compared to their healthy counter parts.

It is also evident from the analysis that leaf samples, irrespective of the varieties showed higher protein as compared to the petiole and leaf sheath.

Analysis of Nendran at different growth stages (Table 14) indicated a higher protein content in five month old pseudostem while among the reproductive stages, plants at flowering showed a maximum protein content which was relatively lower than the same in its previous growth stages.

4.5.5 Polyphenol oxidase electrophoretic pattern

4.5.5.1 Sheath samples

The resistant variety, Njalipoovan showed three isozyme bands PPO-2, PPO-6 and PPO-7 with Rm values 0.22, 0.60 and 0.63 respectively. Among the sheath samples PPO-2 (Rm = 0.22) and PPO-6 (Rm = 0.60) were commonly present (Table 15, Fig. 4, Plate 15). The fast moving band at PPO-7 region (Rm = 0.63) was found to be a characteristic band of Njalipoovan and it was absent in Nendran. The isozyme band PPO-3 (Rm = 0.32) was present in both the Nendran samples while it was absent in the resistant cultivar. Except for an isozyme band at PPO-5 region (Rm = 0.55) present in the healthy Nendran, all the other bands were similar in both healthy and infested plants of Nendran.

4.5.5.2 Petiole samples

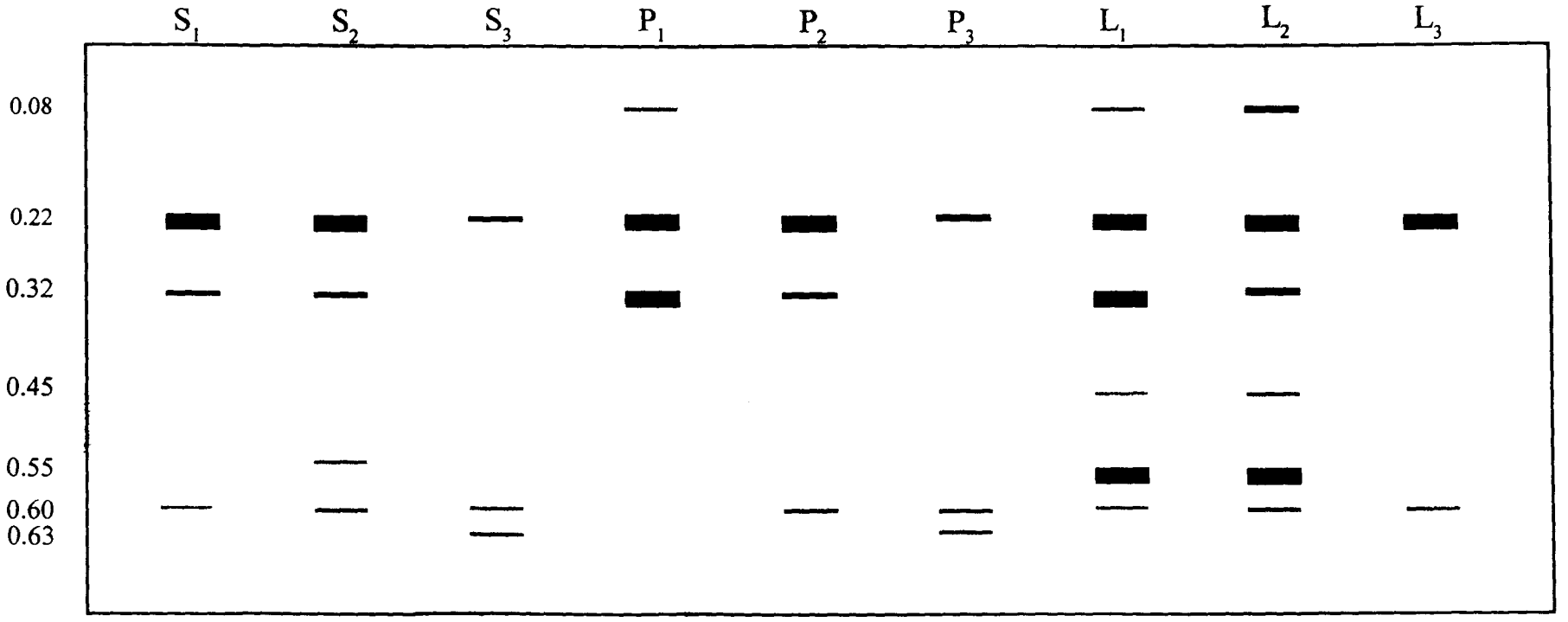
In petiole samples the isozyme band PPO-2 with Rm value 0.22 was common in all the three plants analysed. The band, however, was higher in the

Table 15. Rm value of different bands of polyphenol oxidase at peduncle formation stage of test entries

Sample		Rm							Total No. of bands
		Banding from origin							
		PPO 1	PPO 2	PPO 3	PPO 4	PPO 5	PPO 6	PPO 7	
Sheath									
	R	-	0.22	-	-	-	0.60	0.63	3
	SH	-	0.22	0.32	-	0.55	0.60	-	4
	SI	-	0.22	0.32	-	-	0.60		3
Petiole									
	R	-	0.22	-	-	-	0.60	0.63	3
	SH	-	0.22	0.32	-	-	0.60	-	3
	SI	0.08	0.22	0.32	-	-	-	-	3
Leaf									
	R	-	0.22	-	-	-	0.60	-	2
	SH	0.08	0.22	0.32	0.45	0.55	0.60	-	6
	SI	0.08	0.22	0.32	0.45	0.55	0.60	-	2

R- Resistant-Njalipoovan
SH-Susceptible healthy-Nendran
SI – Susceptible infested - Nendran

Fig. 4. Zymogram of polyphenol oxidase in test entries of banana



1 - Susceptible infested - Nendran
2 - Susceptible healthy - Nendran
3 - Resistant - Njalipoovan

S - Sheath
P - Petiole
L - Leaf

Plate – 15 Banding pattern of polyphenol oxidase in test entries of banana

1 - Susceptible infested - Nendran

2 - Susceptible healthy - Nendran

3 - Resistant - Njalipoovan

P - Petiole

S - Sheath

L - Leaf

resistant cultivar, Njalipoovan. The bands PPO-1 and PPO-3 with Rm values 0.08 and 0.32 respectively were absent in the resistant cultivar. The isoenzyme band PPO-6 (Rm = 0.60) was common in resistant and susceptible cultivar. However, this band was absent in infested Nendran. The fast moving feeble band, PPO-7 (Rm = 0.63) was absent in infested and healthy plants of susceptible cultivar. The most densely stained bands were in PPO-2 and PPO-3 regions (Fig. 4).

4.5.5.3 Leaf samples

Among the three parts considered for analysis,leaf samples showed more number of bands in susceptible Nendran. The fresh unfurled leaves of infested and healthy Nendran gave similar banding pattern. But it is seen that, when Nendran is infested, the band PPO-1 became lighter, at the same time band PPO-3 became denser. The isoenzyme bands PPO-1 (Rm =0.08), PPO-3 (Rm = 0.32), PPO-4 (Rm = 0.45) and PPO-5 (Rm = 0.55) were absent in Njalipoovan. In all the samples the slow moving band at PPO-6 region (Rm = 0.60) was found to be feeble.

4.5.6 Polyphenol oxidase assay

Activity studies of polyphenol oxidase in the test entries (Table 16) revealed a greater activity in resistant variety, Njalipoovan in sheath (0.015 Δ OD/min) and petiole (0.019 Δ OD/min). A remarkably lesser activity of PPO (0.006 Δ OD/min) was observed in pseudostem sheath of infested plants. However, the PPO activity at petiole and leaf of healthy and infested Nendran were on par. The leaf samples of resistant cultivar had lower PPO activity than healthy and infested plant samples of Nendran.

The results of PPO assay studies in Nendran at progressive growth stages were presented in the Table 17. Assaying of sheath samples revealed a gradual

Table 16. Polyphenol oxidase activity (OD value) in test entries of banana at peduncle formation stage

Plant parts	Time (minutes)															Δ OD per minute		
	1			2			3			4			5					
	R	SH	SI	R	SH	SI	R	SH	SI	R	SH	SI	R	SH	SI	R	SH	SI
Sheath	0.021	0.027	0.019	0.048	0.030	0.020	0.054	0.042	0.025	0.063	0.054	0.029	0.075	0.065	0.029	0.015	0.013	0.006
Petiole	0.008	0.004	0.003	0.047	0.030	0.028	0.068	0.038	0.032	0.079	0.044	0.045	0.094	0.053	0.051	0.019	0.011	0.010
Leaf	0.194	0.009	0.012	0.215	0.374	0.282	0.233	0.0384	0.295	0.254	0.393	0.301	0.293	0.402	0.360	0.059	0.0804	0.072

R – Resistant – Njalipoovan
SH – Susceptible healthy – Nendran
SI – Susceptible infested – Nendran

Table 17. Polyphenol oxidase activity (OD value) in different parts of Nendran at progressive growth stages

Growth stages	Time (minutes)															Δ OD per minute		
	1			2			3			4			5					
	S	P	L	S	P	L	S	P	L	S	P	L	S	P	L	S	P	L
First month	0.008	0.021	0.026	0.032	0.037	0.420	0.047	0.042	0.551	0.047	0.055	0.064	0.047	0.093	0.647	0.009	0.019	0.129
Third month	0.005	0.015	0.022	0.074	0.041	0.281	0.093	0.050	0.486	0.112	0.061	0.560	0.112	0.088	0.605	0.022	0.018	0.121
Fifth month	0.041	0.026	0.131	0.056	0.047	0.224	0.083	0.067	0.297	0.112	0.073	0.336	0.114	0.099	0.362	0.023	0.020	0.073
Seventh month	0.013	0.025	0.010	0.016	0.039	0.165	0.017	0.048	0.240	0.017	0.062	0.288	0.017	0.086	0.310	0.003	0.017	0.062
At flowering	0.005	0.007	0.002	0.015	0.025	0.235	0.016	0.037	0.0321	0.016	0.047	0.364	0.016	0.059	0.392	0.003	0.012	0.078
At bunching	0.001	0.005	0.009	0.023	0.036	0.375	0.023	0.051	0.384	0.023	0.069	0.393	0.023	0.081	0.403	0.004	0.016	0.081

S – Sheath; P – Petiole; L – Leaf

decrease in PPO activity as the age of the plant progressed. The enzyme activity was very meagre from seventh month onwards.

In the case of petiole samples difference in PPO activity was not predominant within the various growth stages.

In the leaf samples, the enzyme activity was minimum (0.062 Δ OD/min) during seventh month while the highest activity was recorded in one month old plants (0.129 Δ OD/min).

Among the three plant parts taken for analysis leaves had the highest PPO activity followed by petiole and sheath.

4.5.7 Enzyme activity and oxidation factor of OD phenols

The enzyme activity and its respective oxidation factor of OD phenols were found to be highest in resistant variety, Njalipoovan irrespective of the plant part taken for analysis (Table 18). Among the healthy and infested plants of Nendran, healthy plants showed relatively higher enzyme activity, and thereby a greater oxidation factor of the substrate.

The results of enzyme activity and oxidation factor of OD phenols at progressive growth stages of Nendran were represented in the Table 19. Among various growth stages taken for analysis seventh month old Nendran recorded lowest oxidation factor while the enzyme activity and its oxidation rate were higher in younger stages.

Table 18. Enzyme activity and oxidation factor of OD phenols in test entries of banana at peduncle formation stage

Test entries	Sheath			Petiole			Leaf		
	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}
Resistant	0.015	0.081	1.215	0.019	0.077	1.463	0.059	0.337	19.88
Susceptible healthy	0.013	0.033	0.429	0.011	0.063	0.693	0.080	0.211	16.88
Susceptible infested	0.006	0.039	0.234	0.010	0.064	0.640	0.072	0.205	14.76

Resistant – Njalipoovan
Susceptible – Nendran

Table 19. Enzyme activity and oxidation factor of OD phenols in different plant parts of Nendran at progressive growth stages

Growth stages	Sheath			Petiole			Leaf		
	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}	PPO activity Δ OD/min	OD phenols mg/g	Oxidation factor x 10^{-3}
First month	0.009	0.053	0.477	0.019	0.005	1.235	0.129	0.170	21.93
Third month	0.022	0.043	0.946	0.018	0.061	1.098	0.121	0.185	22.39
Fifth month	0.023	0.036	0.828	0.020	0.076	1.520	0.073	0.179	13.07
Seventh month	0.003	0.023	0.069	0.017	0.063	1.071	0.062	0.193	11.97
At flowering	0.003	0.117	0.351	0.012	0.063	0.756	0.078	0.203	15.83
At bunching	0.004	0.049	0.196	0.016	0.067	1.072	0.081	0.205	16.61

Discussion

5. DISCUSSION

5.1 Preliminary field screening of *Musa* (AAB) Nendran for resistance against pseudostem weevil *Odoiporus longicollis*

The only rating scale that was available at the commencement of the study was Charles *et al.* (1996). This was found to be insufficient to correctly grade the plants because the sole parameter used was the number of ovipunctures on the pseudostem. The adults of the pseudostem weevil can make fewer ovipunctures on a susceptible variety and fill all of them with eggs. Majority of these eggs would hatch out and cause substantial damage. It is also possible that in a resistant variety, there may be more ovipunctures where the insect tests the host with its ovipositor for a good egg laying site, but shifts to another site without oviposition. More number of ovipunctures on a resistant variety had previously been reported on brown plant hopper (Smith, 1989). The ovipunctures from which the eggs had hatched would usually be widened by the grub which eats sides of the air space before making its path towards the pith. An increased surface area of infestation also manifests increased feeding of grubs on susceptible variety. Since all the above three parameters add to the intensity of attack the factor nAB as explained earlier was chosen to indicate the rating scale.

Visual observation of plants belonging to G_1 and G_2 had revealed that they could still produce some yield and the intensity of attack increased in the plants which fell in G_3 and G_4. Here the plants failed to produce bunches or succumbed to wind damage. The blockage of translocation through the pith and pseudostem in G_3 and G_4 is directly responsible for this. In G_1 and G_2 the damage to the translocating system is not appreciable enough so that the plant is able to give some yield.

The factors adding to the vigour of the plant such as girth of pseudostem, breadth, width and related characters (Table 3) indicated that girth is directly

affected by the degree of infestation. There is also some effect on the width of the leaf sheath and number of sheaths overlapping the pith. Higher gradings of damage showed a corresponding increase in the height up to which infestation was observed. All these indicate a slow decline of the vigour of the plant due to damage to the food translocation system and consequent shrinkage of the plant.

5.2 Laboratory rearing of *O. longicollis*

The pseudostem weevil, *O. longicollis* can be mass reared successfully with the pseudostem of banana as base material. However, a rapid decline in the population and greater mortality of the grubs were evidenced from the third generation onwards. Berenbaum (1986) also reported decreased genetic diversity of test insect population which were continuously reproduced under artificial conditions. To avoid these problems, quality control measures must be made a part of the rearing programme to ensure that the behaviour and metabolism of the laboratory insects is similar to that of wild individuals. In order to prevent such problems in mass reared insects, wild individuals collected from the field was infused with the laboratory colony from the third generation onwards.

Earlier studies suggested that the artificial diet should be ensured of a close resemblance to the nutritional and allelochemical composition of the host plant. In the present study, though the cut pseudostem of banana formed the base material for mass rearing, there was a gradual deterioration of the plant part after it has been removed from the surviving stems. Hence, a change in the food material is essential every three days to ensure healthy lab reared population.

Greater number of curculionids, reared in inadequate food material often resulted in cannibalistic behaviour of the dominant life stages. The adult weevils fed on egg, larval instars and pupa while larval instars L_3 and L_4 attacked other immature stages during food scarcity. Other dominant forms like L_3, L_4 and adults

frequently preyed upon the fifth instar being sluggish. Hence regular sorting out of the population based on different life stages is advocated. Supply of adequate food material becomes a pre-requisite to prevent intra-specific competition.

Predators like earwigs and ants preyed upon egg, larval and pupal stages. The adults were reported to be infected by *Metarhizium anisopliae* in certain situations (Anitha *et al.*, 1998). Initially, the lab population got infected. However, in the present study, good sanitation measures like treatment of rearing equipments with 5 per cent formaldehyde and removal of sluggish or infected grubs and adults immediately from the culture prevented such casualties among the population.

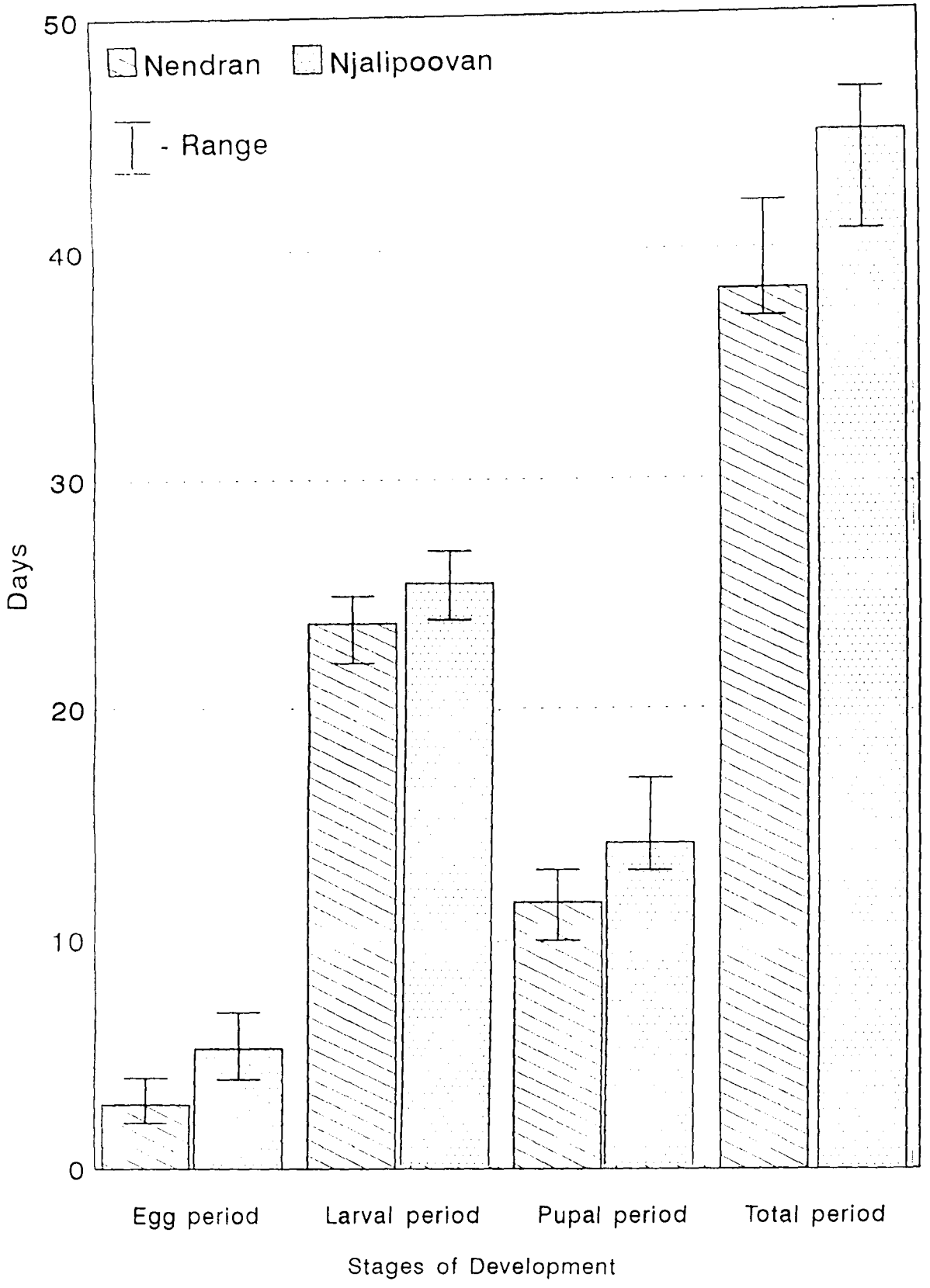
5.2.1 Duration of development of different life stages of *O. longicollis* on susceptible and resistant cultivars

Preliminary free-choice tests and field surveys revealed that *Musa* spp. (AB), Njalipoovan as a non- preferred variety for *O. longicollis*. Therefore, to study the resistance mechanism exhibited by Njalipoovan, a no-choice test was designed.

The adult weevils were exposed to the pseudostem of resistant variety alone and were forced to oviposit on them. The reduction in the number of eggs from an average of 6.9 to 2.7 in Njalipoovan (Table 4) indicates an antixenosis mechanism operating in Njalipoovan. The conditions available in Njalipoovan has further reduced the percentage hatching in Njalipoovan to 39.5 per cent while it was as high as 94.9 per cent on Nendran. The acute antibiosis offered by Njalipoovan is manifested in percentage adult emergence of only 29.7 per cent in contrast to 91.3 per cent in Nendran. The only visual symptom that could be recorded was the loss of mandible (Plate 8a). Similar results were reported by Panda *et al.* (1975) and Ukwungwu and Odebiyi (1985) on *Chilo suppressalis* in resistant rice cultivars.

Duration studies on susceptible and resistant varieties (Fig. 5) revealed extended larval period (25.6 days) in Njalipoovan as compared to Nendran (23.8

Fig. 5. Duration of development of *O. longicollis* on susceptible and resistant cultivars



days). Here also all the first larval instars (L_1) did not succeed in growing up to L_2 stage. A similar situation existed with L_2 growing to L_3, L_3 growing to L_4 and L_4 growing to L_5. In order to study the duration of instars on Njalipoovan, when the immature stages died during the no-choice test, a correspondingly aged larva was collected from the laboratory culture on Nendran and allowed to complete the stadium. The completion of life cycle from egg to adult took 45.2 days in Njalipoovan in contrast to 38.2 days in Nendran. This gives ample evidence to the existence of antibiosis in Njalipoovan. Ranjith (1988) also reported that host plants can induce changes in the life cycle of *Bemisia tabaci* by affecting the duration of development. A reduction in the feeding was also seen in L_4 and consequently these grubs, which fed on the non-preferred Njalipoovan, produced malformed and under sized adults (Plate 8c). This information can be used while deciding varieties for homesteads and alternate varieties in endemic areas, where it would be advisable to use hosts on which a longer time is taken by the weevil to complete its life cycle. This would reduce the number of broods per year and hence may cause lower levels of infestation on the crops.

5.2.2 Oviposition of *O. longicollis* reared on pseudostem of Nendran at various growth stages

The specific preference of adult females of *O. longicollis* to banana at the flowering stage has been reported by Jayasree (1992). It was necessary to find out the exact growth stage from which oviposition is preferred. For this the females were made to oviposit on pseudostem of banana from first month up to ninth month in a no-choice situation. The results (Fig. 2) have clearly indicated that maximum oviposition (6.2 eggs/female) was obtained from seventh month onwards. This makes it possible to evolve a management strategy against this weevil from the seventh month onwards. The current recommendation is against this weevil is drenching quinalphos (0.05%) or chlorpyriphos (0.03%) or carbaryl (0.2%) on the

leaf axils, rhizome and all around the entire pseudostem, inserting the nozzle through the bore holes made by the larvae (Anonymous, 1996).

The present results help us to restrict insecticidal application from seventh month only. This would reduce the insecticidal load in the banana ecosystem. Results of oviposition on water suckers show that there exists a greater preference for water suckers. This finding pinpoints to the necessity of removal of water suckers and unproductive lanky plants from the banana plantation to prevent the adult weevils from ovipositing on them and building up residual colony.

5.2.3 Epidiectic compounds

It is already known that some of the pheromones deposited by ovipositing females act as aggregation pheromones (Prokopy, 1981). The very same pheromone at much higher concentrations or a slightly different pheromone, produced after a heavy egg laying, acts as an oviposition deterring pheromone or epidiectic hormone. The existence of such a phenomenon in banana with *O. longicollis* was tested with varying adult female numbers from one to ten.

The results (Fig. 3) clearly indicate that weevil numbers above two per pseudostem piece of 25 cm is deterrent to conspecific females. For ten females the maximum number of eggs obtained was only 16. Further experiments on this line, in field conditions are required to understand the level at which oviposition deterrency occurs. To utilise the information obtained so far, extracts of pseudostem on which ten, five, four and three females oviposited were utilised. The pseudostems containing the epidiectic hormone from those where ten females oviposited have shown the deterrency effect (Table 6). It is also clear that this fraction is soluble in either acetone or ether. The acetone and ether extracts did not allow egg laying while control treatments involving just acetone or ether without

extract allowed normal oviposition. Identification of epidiectic hormones and further experimentation is the next line of work proposed in this regard.

5.3 Evaluation of sucker and *in vitro* regenerated progenies of naturally infested *Musa* (AAB) for resistance against *O. longicollis*

The girth of the plants at the base was not showing much significant differences because the insect does not prefer basal region for attack. But at 1 m height which has a direct correlation with normal site of infestation, shows appreciable reduction in T_3 to T_5 (Table 7). This is an indication of non-preference and antibiosis exhibited by the grubs of weevil consequent to feeding on T_1 and T_2. It is also clear that T_1 plants derived from a mildly infested mother plant (G_1) were not preferred by the grubs as indicated by the reduced height of infestation (71.4 cm). The number of hands was generally five in treatments up to T_3 and got reduced in the further grades. The number of hands in banana is pre-fixed from the flower initiation itself. However, the number of hands that develop into fruits is dependent upon the nutrition, other favourable factors and also the biotic resistance including the insect attack. Hence the reduction in the number of hands contributing to the yield in T_4 and T_5 in this experiment is directly attributed to the damage caused by the grubs. This is further substantiated by the weight of the bunches which shows a progressive reduction in T_4 and T_5. However, T_4 plants exhibited greater tolerance than control. The plants having a lower history of infestation of the mother plant (T_1 to T_3) gave appreciably higher yields (Table 7).

It was also attempted to classify the sucker-derived plants consequent to artificial infestation to study the probability of induced resistance (Table 8). It is seen that plants from T_1 ended up as G_1 itself while T_2 ended up as G_2. T_3 however, was only G_1. The general response of the initial three treatments with individual plants falling under grade 0, 1 or 2 establishes the existence of induced resistance. It should be assumed that consequent to insect infestation there is a triggering off of

enzymatic reactions involving plant resistance. This may not be a quantitative production of allelochemicals against the insects because the biomass of the suckers in comparison to mother plants is substantially low. Hence the possibility of induced allelochemicals being transferred to the suckers in sufficient quantities, to exert antibiotic effects on the grubs that are to infest the sucker plants about seven months from them is removed. Thus it can only be the production of enzyme systems akin to conditioned reflexes which triggers off the production of antibiotic chemicals in the sucker plants when it is artifically infested towards the flower initiation stage. Zangari and Rutledge (1996) also explained the constitutive defense and their inducibility in reproductive parts while explaining optimal defense theory.

No-choice tests involving the pseudostem weevil *O. longicollis* were conducted on tissue-cultured plants with 5 females and 5 males per plant. The results (Table 9) show that in spite of a five fold increase in the adult weevil population than the sucker derived plants, the tissue cultured plants showed very great resistance against the pseudostem weevil. In the tissue-cultured plants which were regenerated from moderately resistant plants (G_2), there was no infestation at all. These plants (T_7) were not even tested for oviposition by the adult weevils which were confined on these plants for more than ninety days. In the case of plants regenerated from G_1 grade, the infestation was scored as G_1. A similar trend was also seen in the plants regenerated from G_3 and G_5 which ended up in G_2. A general observation on the level of infestation by *O. longicollis* is that the period from August to October is not favourable for the build up of grubs and weevils. However, the complete lack of infestation on G_2 regenerated plants (T_7) shows the existence of triggering off reactions in this case also. These experiments with *in vitro* regenerated progenies offer the potentiality for mass multiplication for such in borne resistance for commercial release. However, these experiments with tissue-cultured plants will have to be repeated during May to August which is the most favourable season.

5.4 Identification of morphological and anatomical bases of resistance

5.4.1 Morphological parameters of resistance

The morphological parameters of resistant and susceptible cultivars were taken at peduncle formation stage (Table 10). The breadth of leaf sheath and width at the centre of Nendran was more when compared to Njalipoovan and hence contributed to increase in girth of the pseudostem. But the resistant variety, Njalipoovan has more number of sheaths with bigger sized air chambers in the sheath. A smaller width and breadth of the sheath coupled with more number of sheaths in the resistant variety would increase the number of cuticular layers and cell walls the grub has to traverse before entering the central pith. Since the younger instars L_1 and L_2 have to feed on the pseudostem sheaths, the extra effort for feeding manifests itself in reduced survival of these instars which in turn accounts for the resistance of Njalipoovan. The morphological parameters are also greatly dependent on the quantum of manures and fertilizers applied. Hence these parameters cannot be relied as sole factors influencing resistance among cultivars.

5.4.2 Anatomical basis of resistance

5.4.2.1 Upper epiderm

The thick cuticle on epidermal cells of Njalipoovan gives it certain degree of resistance. The cuticle thickness is found to decrease towards 'after flowering' stage in the case of Nendran. Such a variation does not exist in Njalipoovan. The size of the cells increases during 'after flowering' in both the cases. It would be expected that when the size of cells increases the thickness of the cuticle might go down. However, the perusal of the data (Table 11, Plate 14) shows that reduction in cuticle thickness is not commensurate with increase of cell size. It is only in Nendran that the thickness of cuticle goes down drastically. Hence, Nendran becomes more susceptible towards this stage. Chemically, cuticle is composed of plant waxes constituting esters formed by the linkage of a long-chain fatty acids and

an aliphatic alcohol. This cuticular wax layer plays an important role in the resistance to pest as the sense organs on the insect tarsi, mouth parts and ovipositor receive negative chemical and tactile stimuli from the plant surface. The results are in agreement with Lupton (1967) in raspberry against beetles and aphids. The cross section of the pseudostem sheath of Njalipoovan had four to five layers of thick collenchymatous hypodermis contributing a physical barrier against oviposition. Similar results were reported in rice against stem borer (Israel, 1967) and BPH (Peraiah and Roy, 1979b). It can also be inferred that sclerenchymatous tissues which are comparatively thicker walled than parenchymatous tissues, offer resistance to larval boring inside the pseudostem sheath.

5.4.2.2 Mesoderm

The ground tissue which constituted of compactly arranged parenchymatous cells (Plate 14c) and less number of intercellular spaces in resistant cultivar deter or limit the oviposition of female curculionid. A thick cortex layer (1.485 at 'before flowering' stage, 1.300 mm at 'after flowering' stage) in Njalipoovan (Plate 14a,i) including collenchymatous hypodermis also imposes a mechanical barrier for feeding and egg laying. Similar factors contributed resistance in sugarcane against *Scirpophaga nivella* (Chang and Shih, 1959) and stem borers in rice (Patanakamjorn and Pathak, 1967), sugarcane (Martin *et al.*, 1975) and wheat (Wallace *et al.*, 1974). The mechanical tissues especially the thick sclerenchymatous bundle sheath embedded in the comparatively thicker cortex region seemed to be a desirable character in resistant cultivar. Even though the number of vascular bundles is more in Nendran it has only a smaller size. The bigger and well-defined vascular bundles in Njalipoovan compensate for the lower numbers. Lesser layers of ground tissues and greater air space favoured more oviposition in Nendran.

5.4.2.3 Lower epiderm

The compactly arranged bigger sized lower epidermal cells in Njalipoovan offer resistance to larval boring inside the pseudostem. The additional parenchymal layers (Plate 14e) below the air cavity operate as a mechanical barrier adding to the failure of the newly hatched larvae to penetrate the inner pith. This is more important in the case of first instar grubs which has to cross the lower epidermis and further into the inner layers for food.

It is also seen that there is a preponderance of starch grains in parenchymatous tissue of Njalipoovan (Plate 14g). Such grains are substantially low in Nendran. It has to be assumed that the storage of food is done in the form of starch in Njalipoovan and probably as soluble mono and disaccharides in Nendran. For breaking up starch, the insect would require considerable quantities of amylase and further break down with disaccharases. Hence, easy availability of soluble sugars in Nendran would make it a preferred target site for attack. It can also be proposed that these simple sugars act as attractants for *O. longicollis*.

5.5 Analysis for biochemical basis of resistance

5.5.1 Moisture percentage

The moisture percentage in Nendran was significantly higher than that in Njalipoovan irrespective of age and parts of the plant selected for analysis (Table.12). The higher moisture percentage in Nendran indicated its succulent nature and hence greater susceptibility. Similar results were obtained by Ghosh (1960) in rice against stem borer.

There was a significant increase in the moisture percentage in the 'after flowering stage' in both the varieties (Fig 6). Studies conducted earlier, revealed that the incidence of pseudostem weevil usually coincided with the flower bud initiation (Visalakshi *et al.*, 1989). So a positive correlation between susceptibility

Fig. 6. Moisture percentage in resistant and susceptible sources

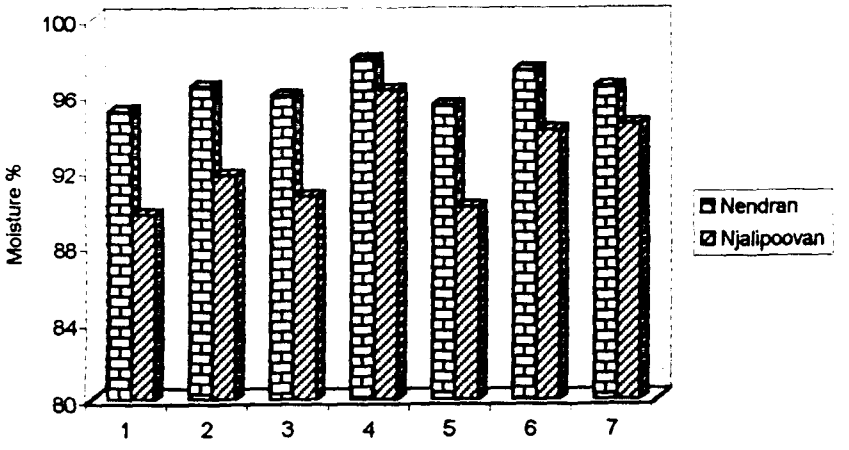
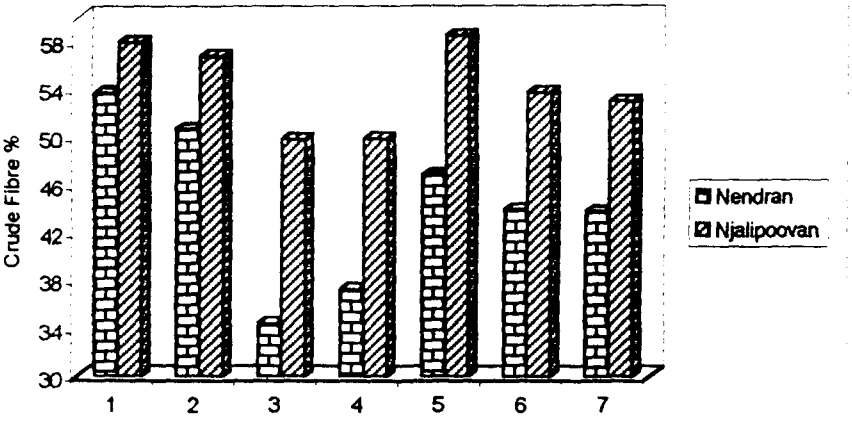


Fig. 7. Crude Fibre content in resistant and susceptible sources



1-Sheath before flowering
2-Sheath after flowering
3-Central primordia before flowering
4-Central pith after flowering
5-Pseudostem before flowering
6-Pseudostem after flowering
7-Water sucker (Pseudostem)

Resistant - Njalipoovan
Susceptible - Nendran

and increase in moisture percentage can be made. Moreover, the central pith formation is completed only after flower bud initiation stage. The pith formation is of prime importance to the curculionid as it becomes the target site of feeding for third and fourth instars. It can also be suggested that subsequent to maturity of the plant, pseudostem emanates certain cues to the weevil for host selection and oviposition.

Even though the moisture content of Njalipoovan pith (96.25%) was comparable with Nendran pith (97.85%), the primary barrier was set by the pseudostem sheath enclosing the pith which has recorded 5.1 per cent lower moisture content than the favourable host Nendran. A significantly lower moisture content in pseudostem sheath of Njalipoovan can be accounted for the desiccation of the eggs. The eggs of *O. longicollis* are generally laid inside the air chambers of the pseudostem sheath. The moisture content of pseudostem sheath will invariably have a greater influence on the relative humidity existing inside the enclosed air chamber. The hatching percentage of eggs are determined by various microclimatic factors including the relative humidity. The lesser moisture percentage recorded in the resistant cultivar, results in decreased egg hatching percentage.

The water suckers of both the cultivars revealed a higher moisture percentage than the sword suckers of the same age. This implied that the water suckers are more prone to damage by pseudostem weevil even before their maturity. They may also form the source of infestation in healthy banana plots. Hence it is not advisable to retain water suckers.

The information on moisture content is of practical utility in the management of the pest in endemic areas. It may be possible to artificially create moisture stress in the plants at the flower initiation stage by regulating irrigation. The level of irrigation can be adjusted so as not to induce a yield reduction, but at

the same time to induce moisture stress at least in the outer sheaths, so that the pseudostem becomes less preferred for oviposition.

5.5.2 Crude fibre content

The crude fibre content was significantly lower in the susceptible cultivar than the resistant cultivar. Generally, the percentage of crude fibre can be negatively correlated to susceptibility. Njalipoovan had greater crude fibre content in pseudostem sheath (Fig. 7) which would probably act as a preventive barrier against oviposition.

The sheath of resistant cultivar recorded the highest crude fibre percentage of 57.94 per cent indicating the difficulty imposed on the earlier larval instars of the weevil. The first and second larval instars are adapted to feed on the tender tissues of the pseudostem sheath. These immature stages cannot resist higher crude fibre ratio in their diet. The results of no-choice test in Njalipoovan have clearly indicated the antibiotic effects on the immature stage L_1 to L_5. The higher content of crude fibre causes additional pressure on the mandible which gets worn out. Probably higher crude fibre would have caused lesser food digestibility and lower nutriments. The indices of digestion like approximate digestibility (AD), growth rate (GR) and efficiency of conversion of digested food (ECD) were reported to be affected by non-preferred host (Ranjith, 1981). Such reasons lead to higher mortality of neonate larva in the resistant cultivar. Similar non-preference mechanisms were earlier reported in the case of stem borer resistance in the rice varieties (Subbarao and Perraju, 1976).

In general a considerable reduction of crude fibre content was observed from 'before flowering' to 'after flowering' stage in both the varieties (Fig. 7). This decrease in crude fibre is presumably due to interconversion of carbohydrates during

the maturity of the plant. This again explains the reason for the plant being more preferred by *O. longicollis* after flower bud initiation stage.

The results also gave an indication that the moisture percentage and crude fibre content were inversely proportional to each other. However, this was not true in the case of Njalipoovan central pith, which recorded higher moisture percentage and higher crude fibre content as compared to its leaf primordia during 'before flowering' stage. This clearly revealed the resistance mechanism exhibited by Njalipoovan involving higher crude fibre even though the percentage moisture was higher. The physiological changes during maturity in Njalipoovan would add to the resistance mechanism.

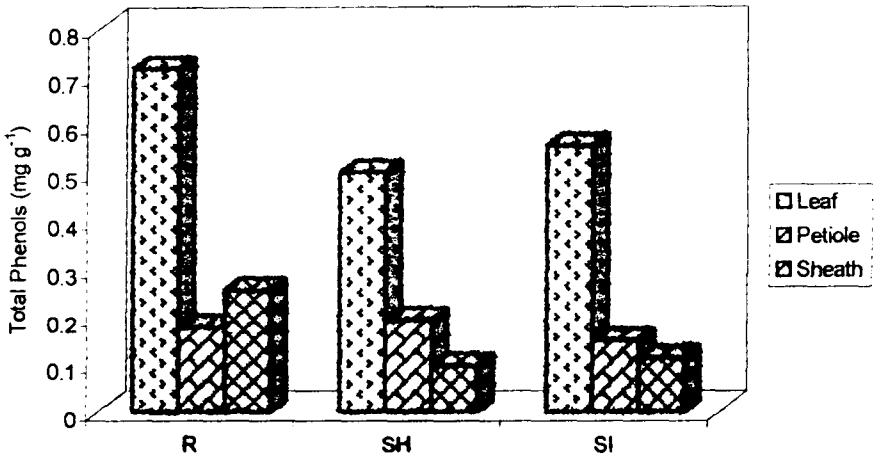
Water suckers possessed lesser crude fibre than the sword suckers of the same age. This evidently indicated that they serve as conducive hosts for the pest even before sufficient maturity. Hence it is advisable to destroy the water suckers regularly in areas prone to weevil attack.

5.5.3 Estimation of total phenols and OD phenols

5.5.3.1 Total phenols

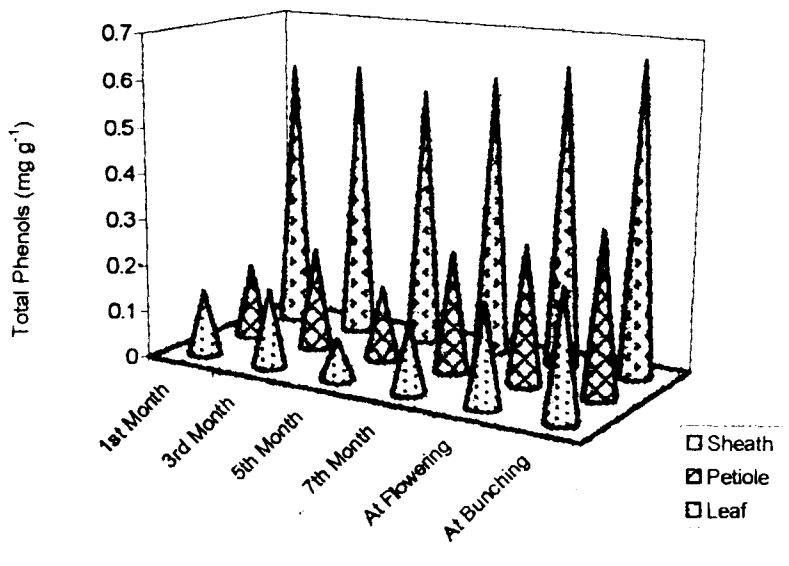
Phenolics were known to play an important role in resistance. A significantly higher total phenol content in Njalipoovan (Fig. 8) can be implicated as a factor of resistance. The higher content is mostly due to increased quantity in the leaf sheath and the leaves. Leaf is probably the source and sink of most of the metabolic products including phenols. Phenols are generally reported to cause deterrence to feeding and oviposition (Reese, 1981 and Dreyer *et al.*, 1981). Higher phenolics were correlated positively to resistance in sorghum against locust (Woodhead, 1982) and cotton against whiteflies (Ranjith, 1988 and Butter *et al.*, 1992).

Fig. 8. Total phenol content in test entries of banana at peduncle formation stage



R - Resistant-Njalipoovan
SH - Susceptible healthy-Nendran
SI - Susceptible infested-Nendran

Fig. 9. Total phenols in different parts of Nendran at progressive growth stages



A higher total phenol content is seen towards the bunching stage (Fig. 9). But this stage is also attacked to a substantial level by the weevils. This may probably be due to a lower replenishment of phenolics from the leaves which acts as a source and sink for the phenolics. The total phenolics in the leaf is always lower in the case of Nendran when compared to Njalipoovan. Also phenols being a general inhibitor may not be responsible as the sole factor contributing to resistance.

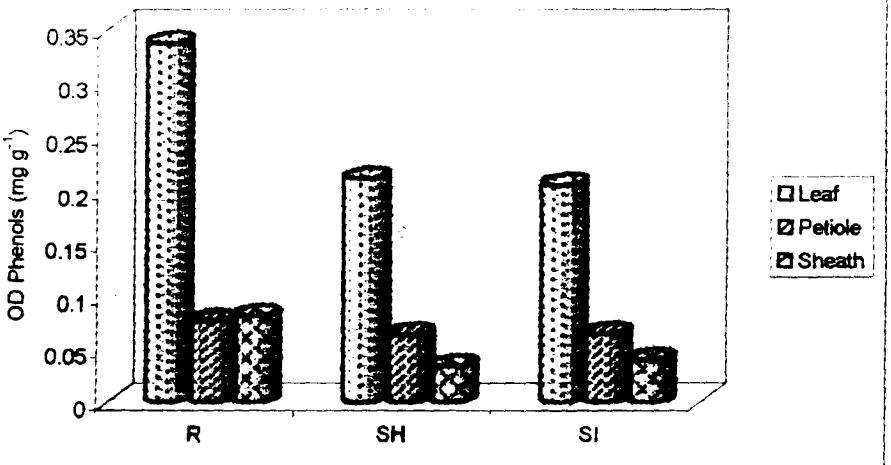
Table 13 also revealed that infested Nendran had slightly higher phenolics in the leaf and sheath as compared to their healthy counterparts. Similar observations have been reported in tomato (Farooqi *et al.*, 1980) and in brinjal (Ganguly and Dasgupta, 1983) infected by *Meloidogyne incognita*. This suggests that wounding of tissues consequent to infestation, induced the plants to liberate phenolics for self defence, but was not sufficient enough to prevent the damage. Such triggering reaction *in vivo* appears to elicit a general plant response that is often beneficial to the plant but detrimental to the insect. Generally in susceptible plants the pest overcomes the host reaction and invades while in resistant plants operation of self-defense mechanisms takes an upper hand over the pest.

5.5.3.2 Ortho dihydric phenols

In the case of OD phenols, Njalipoovan registered more than twice the quantity estimated in Nendran indicating a chemical defense barrier exhibited against *O. longicollis* (Table 13, Fig. 10). Mahadevan and Sridhar (1986) referred that OD phenols were important in resistance reactions as they oxidise to liberate toxic quinones subsequent to infestation. The results are in agreement with the findings of Leszcynski *et al.* (1985) who associated OD phenols quantitatively with feeding deterrence to the aphid in resistant wheat cultivars.

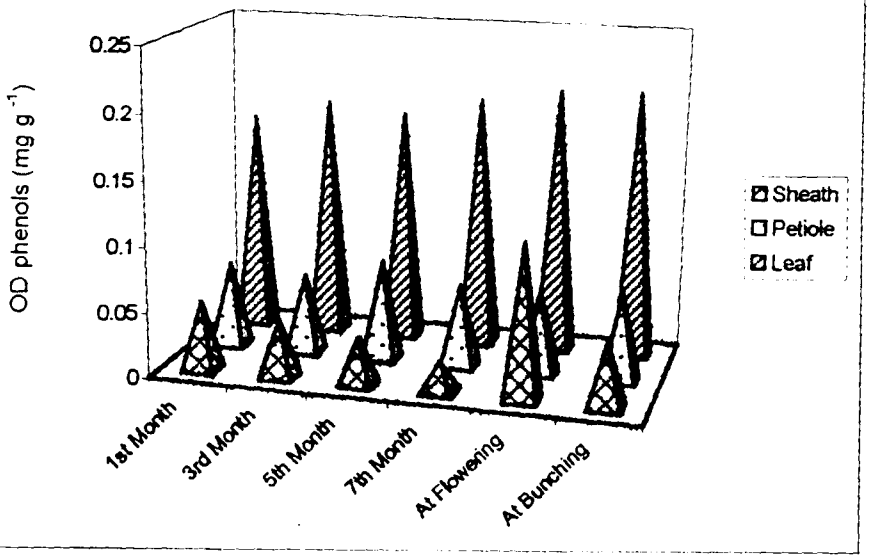
In respect to OD phenols of infested and healthy Nendran there was not much difference. This indicates that not much of OD phenols were converted to

Fig. 10. OD Phenol content in test entries of banana at peduncle formation stage



R - Resistant-Njalipoovan
SH - Susceptible healthy-Nendran
SI - Susceptible infested-Nendran

Fig. 11. OD phenols in different parts of Nendran at progressive growth stages



toxic quinones in Nendran. This points to a situation where it may be assumed that current OD phenol level in infested and healthy Nendran is the base minimum level of phenolic content in banana.

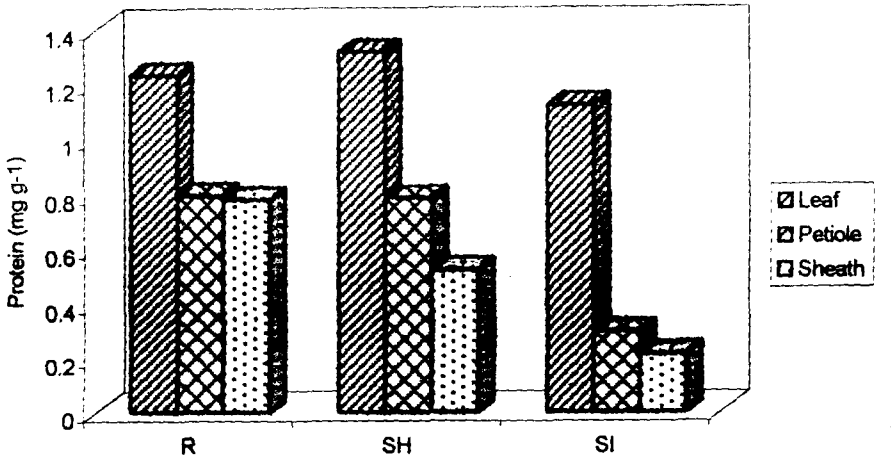
Fig. 11 reveals increasing OD phenol content towards the progressing growth stages in leaf. However, this stage of crop is found to be frequently attacked by the pseudostem weevil. A greater replenishment of OD phenols from the leaf to the sheath is seen during flowering stage. The oxidation of OD phenols is however dependent on the enzymatic activity at that stage. Since there is a lesser protein and PPO activity during the flowering stage, the presence of OD phenols, though greater among all the stages becomes insignificant in attributing resistance to the plant.

5.5.4 Protein estimation at the time of activity study

Results of quantitative estimation showed a 49 per cent higher protein activity in Njalipoovan than Nendran in pseudostem sheath (Table 13, Fig. 12). However analysis of leaf samples recorded a higher protein content in Nendran. It is evident from these results that at the target site of attack, pseudostem had higher protein activity in resistant source which may induce a greater vigour in the plants during the course of infestation due to enzymatic reactions. The higher protein content may be an indication of higher enzymatic activity which would trigger off induction of chemical defenses to prevent the attack and further damage by the weevil. Such allelochemical interactions are also targeted for killing of eggs, death of neonate larvae and other reactions involving antibiosis.

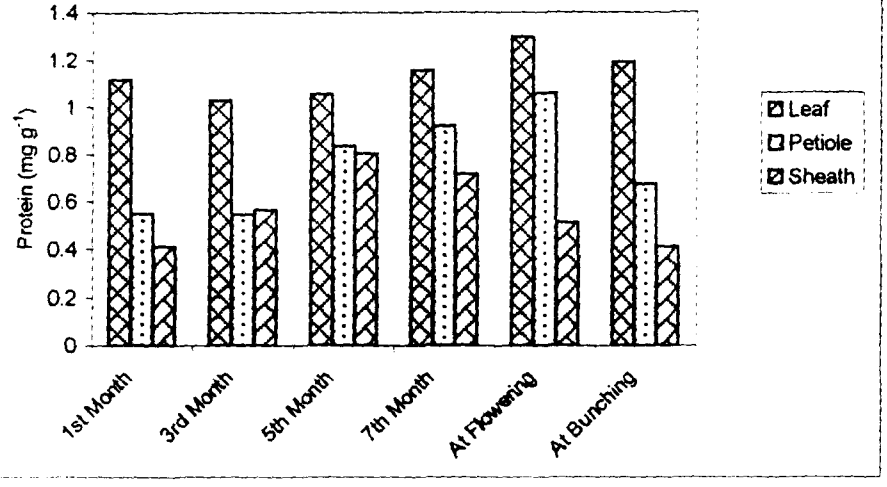
Among the infested and healthy Nendran analysed, infested plants showed significantly lesser protein as compared to their healthy counterparts. This probably indicates the weakening of the plant both physiologically and nutritionally subsequent to infestation. The leaf being the factory of production of photosynthates, a general increase in protein content is expected. The present results agree with this.

Fig. 12. Protein content in test entries of banana at peduncle formation stage



R - Resistant-Njalipoovan
SH - Susceptible healthy-Nendran
SI - Susceptible infested-Nendran

Fig. 13. Protein content in different parts of Nendran at progressive growth stages



However, most of the protein accounting for higher enzymatic activity gets transported to the sheath and inter converted to other biomolecules in resistant Njalipoovan, while Nendran does not show such a capacity. This trend of progressive reduction from leaf to petiole and petiole to sheath is shown by all samples. However, consequent to infestation, the relative amounts of protein goes down drastically in the petiole and sheath, suggesting that the enzymatic reactions that occurred in the susceptible samples could not contain the attack by the weevil grubs. Probably the metabolites produced by Nendran were not potent enough to conquer the attack by the grubs.

5.5.5 Polyphenol oxidase electrophoretic pattern

Isozyme analysis by electrophoresis is an effective method to detect genetic differences among individuals. Among the organic molecules, isozymes are very useful aids to compare genotypes, though they act as a supplementary tool along with morphological, genetic or other biochemical methods. The pattern of enzyme bands is an expression of the particular enzyme system assayed and its mode of inheritance. Certain additional bands or shifts in migration may arise from post-translational modification of enzymes.

In the present study the test entries were the resistant Njalipoovan, susceptible healthy, Nendran and susceptible infested, Nendran (Table 15, Plate 15). The zymogram (Fig. 4) reveals that PPO-2 (Rm = 0.22) is common in all the plant parts analysed. The band PPO-6 (Rm = 0.60) is also present in all except the susceptible infested petiole. There is an additional band at PPO-1 region (Rm = 0.08) in infested Nendran petiole and leaf. The band PPO-3 (Rm = 0.32) is found to be characteristic of only Nendran while PPO-4 (Rm = 0.45) is the characteristic band of Nendran leaf.

The band PPO-7 (Rm = 0.63) is fast moving, light and transitory, seen only in resistant variety Njalipoovan. Banding pattern thus indicates that PPO-7 is the band which might be contributing to resistance. The light and transitory nature of this band explains the faster reactions catalysed by it PPO-7 is also absent in the leaf. All these lend credence to the possibility of PPO-7 being the fast moving and transient band specifically intended against pseudostem weevil in the leaf sheath and petiole.

Resistance mechanisms involving PPO have also been studied by Ganguly and Dasgupta (1988) in tomato against the nematode *Meloidogyne incognita and* in chillies (Markose, 1996) and in tomato (Bose, 1999) against bacterial wilt.

5.5.6 Polyphenol oxidase assay

The reduction of PPO activity in sheath subsequent to infestation is due to the involvement of the enzyme in host reactions to weevil attack. In general a greater activity of PPO is seen in the sheath and petiole of resistant variety. There is a decrease in the PPO activity in the infested sheath than in healthy sheath of Nendran. This probably may be because the PPOs have been used up in building up resistance mechanisms.

The trend lines of PPO activity in sheath, petiole and leaf (Fig. 17, 18, 19) in progressive growth stages gave inconclusive results to interpret with respect to resistance. Hence, putting forth an oxidation factor as explained below made further explanations on the enzyme activity.

5.5.7 Enzyme activity and oxidation factor of OD phenols

Results of PPO activity and OD phenols of the different plant parts gave an indication that neither of these factors alone may be in a position to explain the

Fig. 14. Polyphenol oxidase activity in Sheath samples at peduncle formation stage

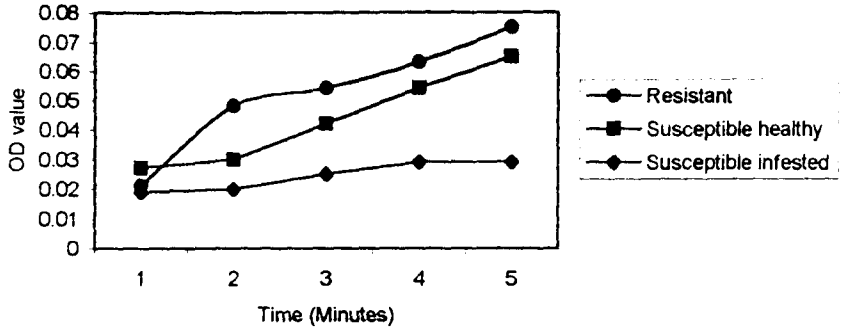


Fig. 15. Polyphenol oxidase activity in Petiole samples at peduncle formation stage

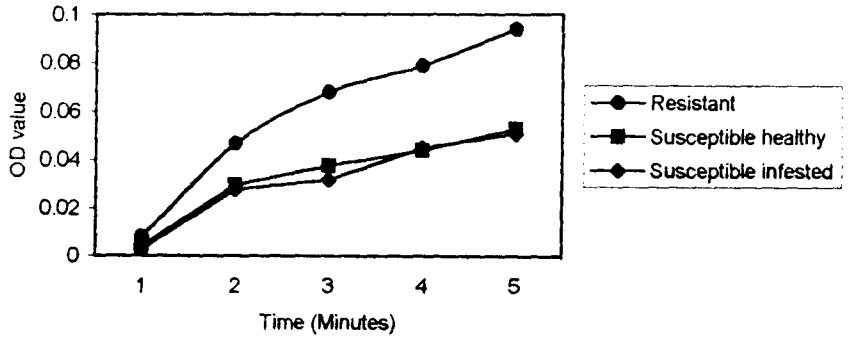


Fig. 16. Polyphenol oxidase activity in Leaf samples at peduncle formation stage

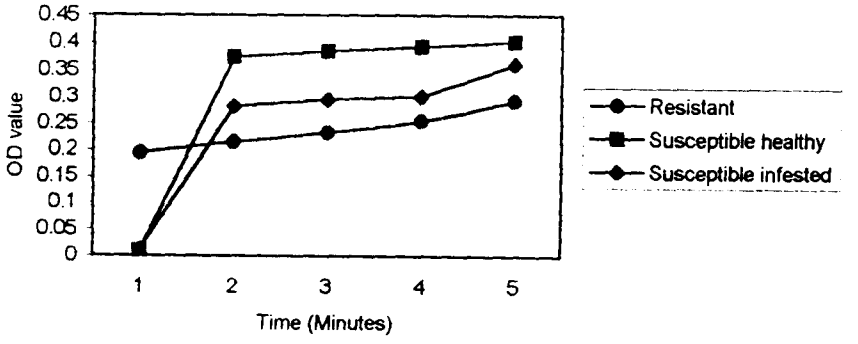


Fig. 17. Polyphenol oxidase activity in Sheath samples of Nendran at progressive growth stages.

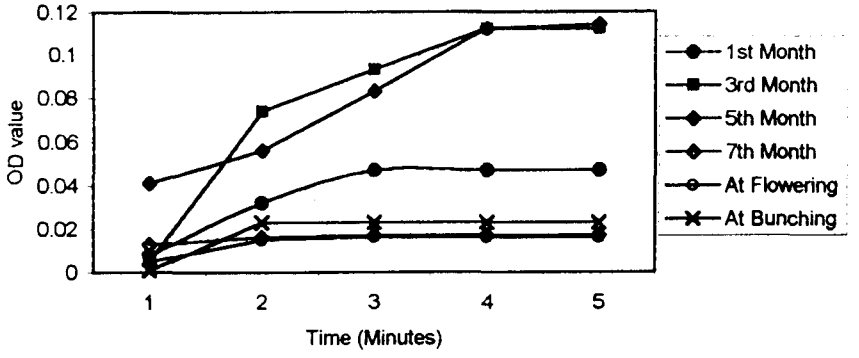


Fig. 18. Polyphenol oxidase activity in Petiole samples of Nendran at progressive growth stages.

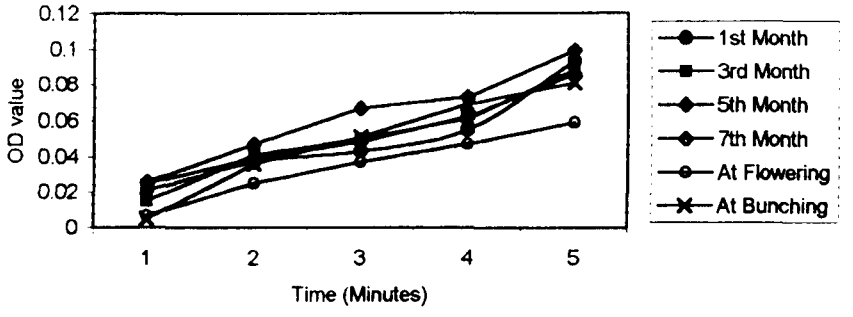
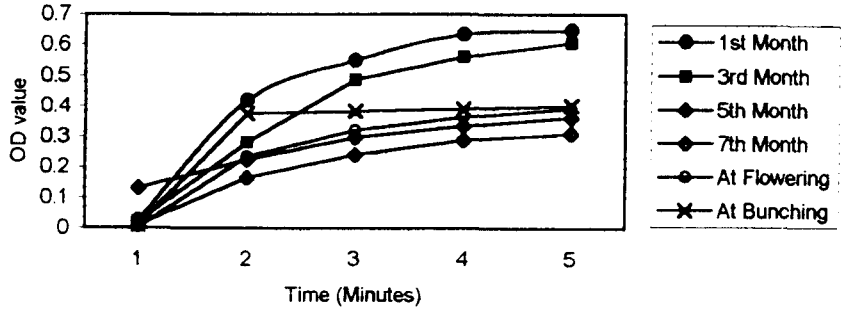


Fig. 19. Polyphenol oxidase activity in Leaf samples of Nendran at progressive growth stages.



induction of resistance. In the plant system, it is already known that ortho dihydroxy phenolic substrates are converted to quinones and tannins consequent to oxidation by PPO (Mahadevan and Sridhar, 1986). These oxidised products are further responsible for inducing the resistance. Hence, to find out the probable induction of resistance an oxidation factor which is the product of PPO activity and OD phenols was attempted. The results (Table 18) clearly indicate the supremacy of resistant variety, Njalipoovan in this regard in all the plant parts tested.

The enzyme activity and oxidation factor of Nendran at progressive growth stages (Table 19) indicate that there is a general reduction in the oxidation factor from the fifth month onwards. Towards the seventh month this reduction is very prominent and further decreases as the age progresses. The decrease in the preparedness of the plant for enzymatic conversion of phenols to quinones consequent to reduction in the capability for oxidation makes it an easy target site for oviposition of adult weevils and further development of immature stages. This probably is one of the other factors that contribute to increased susceptibility of Nendran from the flower initiation stage.

Summary

6. SUMMARY

The pseudostem weevil *Odoiporus longicollis* Olivier has recently become a devastating pest on banana recently in South India. The commercial cultivar Nendran is highly susceptible to the weevil attack. Since, incidence of this pest coincides with the inception of peduncle, heavy load of insecticides cannot be advocated to manage the pest. Hence host plant resistance to *O. longicollis* in Nendran becomes very essential.

Field screening against *O. longicollis* was conducted in Banana Research Station, Kannara. In order to correctly grade the plants the infestation rating scale originally proposed by Charles *et al.* (1996) after suitable modification was used. Thus plants were scored based on three parameters i.e., number of ovipunctures/feeding holes (n), surface area of infestation (m^2) (A) and size of feeding holes (cm^2) (B) which add to the intensity of attack. The factor nAB was chosen to indicate a new rating scale. The factors 0.01-0.15, 0.16-1.5, 1.6-15 and >16 indicate the grades 1, 2, 3 and 4 respectively.

The plantlets of the graded mother plants were raised from suckers and meristem culture, which formed the treatments of the field experiment. The treatment plants were artificially infested during flower bud initiation stage. The results clearly indicated that the plants having a lower history of infestation of mother plants (G_1-G_3) gave appreciably higher yields compared to the control. This suggested the possible existence of induced resistance involving triggering off of enzymatic reactions. Besides these lower grades, the plantlets of G_4 exhibited better tolerance than control by exerting greater potential to stand amidst heavy winds till complete maturity of the bunch. The *in vitro* regenerated plants performed much better than the sucker derived treatment plants. This offers potential for mass multiplication of such in borne resistance for commercial release. The results obtained so far opens up the possibility of induced resistance that may be genetically inherited to the plantlets from its mother as an evolutionary character.

The pseudostem weevil was successfully mass reared with the pseudostem of banana as base material. The decreased genetic diversity among populations after third generation was prevented by infusing wild individuals with lab-reared adults. The incubation, larval and pupal period ranged from 2-4, 22-25 and 10-13 days in Nendran with the total life cycle completion in 37-42 days. Njalipoovan was found to be less preferred by *O. longicollis* for oviposition itself. Even if the eggs were laid,survival percentage of the earlier instars were very low. A longer duration of life cycle (41-47 days) was induced by the resistant host. This suggests that the variety if used along with commercial varieties would reduce the number of generations per year and hence would decrease the cumulative effect of the pest in endemic areas.

A significantly lower moisture and higher crude fibre content in Njalipoovan poses a mechanical barrier for oviposition in pseudostem sheath and also accounted for the desiccation of the eggs. The results of no-choice test in Njalipoovan have clearly indicated the antibiotic effects on the immature stages L_1 to L_5. The neonate larva, which has exclusively adapted to feed on the tender tissues of the pseudostem sheath cannot resist higher crude fibre ratio in their diet.

The information on moisture content is of practical utility in the management of the pest in endemic areas. It may be possible to artificially create moisture stress in the plant attaining flower bud initiation stage by regulating irrigation. The level of irrigation has to be adjusted so as not to induce a yield reduction but at the same time to induce moisture stress at least in the outer sheaths to prevent oviposition.

The water suckers possessed lesser crude fibre and greater moisture content than the sword suckers of the same age. This evidently indicated that they serve as conducive hosts for the pest even before sufficient maturity. Hence, it is advisable to destroy the unproductive water suckers and the lanky plants regularly in

areas prone to stem weevil attack. The cut stems of the harvested banana plants also encourage building up of a residual weevil population. Good field sanitation and regular treatment of cut stems with insecticides would also alleviate the pest.

Exclusive studies with respect to oviposition at various growth stages show a distinct preference exhibited by *O. longicollis* to pseudostem sheath from the seventh month stage or flower bud initiation stage. This information helps to reduce the insecticidal load in the banana ecosystem by advocating pesticides from seventh month only.

Preliminary experiments on epidiectic hormones revealed the oviposition deterrency exhibited by the pseudostems already deposited with eggs. This pinpoints the possibility of epidiectic hormones being secreted by egg laying females. Further, confirmatory studies in field conditions and identification and isolation of these hormones are the next line of research proposed in this regard.

Morphological bases of resistance were also identified. The moisture percentage and crude fibre content were found to be the prime factors influencing resistance. The higher moisture and lower crude fibre content in Nendran indicated the succulent nature and hence greater susceptibility. The pith formation is of prime importance to the curculionid as it becomes the target site of feeding for third and fourth instars. Subsequent to the maturity of the plant, pseudostem emanates certain cues to the weevil for host selection and oviposition.

The anatomical studies revealed a thick cuticle on epidermal cells of Njalipoovan with four to five layers of thick collenchymatous hypodermis contributing a physical barrier against oviposition. The sclerenchymatous tissues in Njalipoovan, which are comparatively thicker, walled than the parenchymatous tissues in Nendran offered resistance to larval boring inside the pseudostem sheath. The ground tissue that constituted of compactly arranged parenchymous cells and

lesser intercellular spaces in the resistant cultivar Njalipoovan deterred the oviposition of female curculionids to a great extent. The closely packed bigger sized lower epidermal cells along with additional parenchymatous layers below the air cavity operates as a mechanical barrier causing the failure of younger instars to penetrate the inner pith. There is a preponderance of starch grains in parenchymatous tissue of Njalipoovan, suggesting them as the storage form of food. Digestion of starch requires considerable quantities of amylase. However, such grains are substantially low in Nendran, indicating that food is stored on soluble sugars itself. Hence, easy availability of soluble sugars in Nendran makes it a most susceptible variety to attack by *O. longicollis*.

Biochemical bases of resistance were also identified. Higher content of phenolics showed positive correlation with resistance. However, the role of ortho dihydric phenolic substrates in resistance is greatly dependent on the enzymatic activity of polyphenol oxidase to form toxic metabolites like quinones, lignin and tannins. Hence, the oxidation factor of OD phenols clearly indicated dominance of the resistant variety, Njalipoovan. The accelerated activity in Njalipoovan is endowed with greater potentiality to activate polyphenols aggressively effecting production of toxic compounds.

References

REFERENCES

Abraham, C.C. and Thomas, J. 1995. Mud slurry as a base and carrier of insecticides for swabbing of banana pseudostem against the weevil *Odoiporus longicollis* oliver. *Insect. environ.* **1**(2):14

* Ahloowalia, B. 1995. *In vitro* mutagenesis for the improvement of vegetatively propagated plants. *Proc. Induced Mutations and Molecular Techniques for Crop Improvement.* International Atomic Energy, Agency, Vienna, Austria pp.531-541

Ahmed, N., Thakur, M.R., Bajaj, K.L. and Cheema, S.S. 1994. Biochemical basis of resistance to yellow vein mosaic virus in okra. *Pl. Disease Res.* **9**:20-25

Ananthakrishnan, T.N. 1990. Chemical ecology in biological control. *Curr. Sci.* **59**:1319-1322

Anitha, N., Nair, G.M. and Mathai, S. 1998. *Metarhizium anisopliae* (Met.) Sorokin as a Biocontrol agent of *Odoiporus longicollis* (Oliv.) (Coleoptera:Curculionidae). *Insect Environ.* **4**(3):96-97

Anon. 1996. *Package of Practices Recommendations - Crops 1996.* Directorate of Extension, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, p. 179

Aravindakshan, K. 1989. Studies on *in vitro* propagation of diploid banana hybrids and their parents. Ph.D. (Hort.) thesis, Tamil Nadu Agricultural University, Coimbatore p. 100

Baker, J.E., Woo, S.M., Throne, J.E. and Finney, P.L. 1991. Correlation of alpha-amylase inhibitor content in eastern soft wheats with development parameters of the rice weevil (Coleoptera:curculionidae) *Environ. Ent.* **20**(1):53-60

Baldwin, I.T. and Schultz, J.C. 1983. Rapid changes in tree leaf chemistry induced by damage: Evidence for communication between plant. *Science* **221**:277-279

Bashan, Y., Okon, Y. and Hemis, Y. 1987. Peroxidase, poly phenol oxidase and phenols in relation to resistance against *Pseudomonas syringae* pv *tomato* in tomato plants. *Can. J. Bot.* **65**:366-372

Beer, Z.C. and De-Beer, Z.C. 1992. Screening technique against panama disease. *Bull. Inst. Trop. Subtropical Crops* **241**:6-7

* Berenbaum, M. 1986. Post ingestive effects of phytochemicals on insects: On paracelsus and plant products. *Insect_Plant Interactions.* (Ed. Miller, J.R. and Miller, T.A.). Springer, New York, pp.121-153

Binks, R.H., Greenham, J.R., Luis, J.G. and Gowen, S.R. 1997. A phytoalexin from roots of *Musa acuminata* var. Pisang sipulu. *Phytochemistry* **45**(1):47-49

Bose, S.C. 1999. Screening and biochemical characterization of tomato genotypes for resistance to bacterial wilt. M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p.50

Budenberg, W.J., Ndiege, I.O., Karago, F.W. and Hansson, B.S. 1993. Behavioural and electrophysiological responses of the banana weevil, *Cosmopolites sordidus. J. Chem. ecol.* **19**(2):267-277

Butter, N.S., Yir, B.K., Kaur, G., Singh, T.H. and Reheia, R.K. 1992. Biochemical basis of resistance to whitefly, *Bemisia tabaci* Genn. (Aleyrodidae:Hemiptera) in cotton. *Trop. Agric.* **69**(2):119-122

Cerda, H., Lopez, A., Fernandez, G., Sanchez, P. and Jaffe, K. 1995. Analysis of banana weevil olfactory responses to stimuli released from different plants. *Fruits* Paris **50**(5):323-331

Chander, M.S. 1994. Biochemical properties associated with resistance to powdery mildew in chillies. *Pl. Disease Res.* **9**:103-104

* Chang, H. and Shih, C.Y. 1959. A study on the leaf mid-rib structure of sugarcane as related with resistance to the top borer (*Scirpophaga nivella* F.) Taiwan Sugar Exp. Sta. Rep. **19**:53-56

Charles, J.S.K., Thomas, M.J., Menon, R., Premalatha, T. and Pillai, S.J. 1996. Field susceptibility of banana to pseudostem borer *Odoiporus longicollis* oliver. *Musarama* **10**(1):32

Croughan, S.S. and Quisenberry, S.S. 1989. Enhancement of fall armyworm (Lepidoptera:Noctuidae) resistance in bermuda grass through cell culture. *J. econ. Ent.* **82**(1):236-238

* Davies, B.J. 1964. Disc electrophoresis II, Method and application to human serum protein. *Ann. N.Y. Acad.Sci.* **121**:404-427

* Davies, E. and Schuster, A. 1981. Inter cellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal. *Proc. Nat. Acad. Sci.*, U.S.A. **78**:2422-2426

Dethier, V.G. 1954. Evolution of feeding preferences in phytophagous insects. *Evolution* **8**:33-54

Dhaliwal, G.S. and Bhathal, J.S. 1992. Perspectives of allelochemicals in pest management. *National Symposium on Allelopathy in Agroecosystems*, Hisar, Feb.12-14

Dhaliwal, G.S. and Dilawari, V.K. 1993. *Advances in Host Plant Resistance to Insects.* Kalyani Publishers, New Delhi, p.443

Dreyer, D.L., Reese, J.C. and Jones, K.C. 1981. Aphid feeding deterrents in sorghum. Bioassay, Isolation and characterization. *J. Chem. Ecol.* **7**: 273-283

Duffey, S.S. and Felton, G.W. 1989. Plant enzymes in resistance to insects. *ACS Symposium Series* **389**:289-313

Dutt, N. and Maiti, B.B. 1973. Bionomics of the banana pseudostem weevil *Odoiporus longicollis* Oliv. (Coleoptera:Curculionidae). *Indian J. Ent.* **32**:20-30

Edward, J.C., Tripathi, S.C. and Singh, K.P. 1973. Observations on a tip over disease of banana in Allahabad. *Curr.Sci.* **42**(19):699-697

* Edwards, P.J. and Wratten, S.D. 1983. Wound induced defenses in plants and their consequences for patterns of insect grazing. *Oecologia* **59**:88-93

Edwards, P.J., Wratten, S.D. and Cox, H. 1985. Wound-induced changes in the acceptability of tomato to larvae of *Spodoptera littoralis*: A laboratory bioassay. *Eco. Ent.* **10**:1555-1558

Elden, T.C. 1995. Selected proteinase inhibitor effects on alfalfa weevil (Coleoptera:Curculionidae) growth and development. *J. Econ. Ent.* **88**:1586-1590

Farooqi, T.N.A., Ganguly, A.K. and Dasgupta, D.R. 1980. Some chemical changes in tomato plants infected with root knot nematode, *Meloidogyne incognita*. *Indian J. Ent.* **42**:226-232

Felton, G.W., Donato, K., Vecchio, R.J. and Duffey, S.S. 1989. Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *J. Chem. Ecol.* **15**:2667-2694

* Filho, M.H. and Stevens, M.A. 1980. Tomato breeding for nematode resistance : Survey of resistant varieties for horticultural characteristics and genotype of acid. *Acta Hortic.* **100**:383-393

Fletcher, T.B. 1914. Report of the Imperial Entomologist. *Rept. Agric. Res. Inst. Coll. Pusa.* Calcutta p.62-75

* Fowler, M.W. 1983. Commercial application and economic aspects of mass plant cell culture. *Plant Biotechnology* (Ed. Mantell, S.H. and Smith, H.) Cambridge University Press, New York, p.3-37

Frogatt, J.L. 1928. The banana weevil borer in Java with notes on other crop pests. *Queesland Agric. J.* **30**:530-591

Galliard, T. 1978. Lipolytic and lipoxygenase enzymes in plants and their action in wounded tissues. *Biochemistry of Wounded plant tissues*. de Gruyter, New York, pp.156-202

Ganguly, A.K. and Dasgupta, D.R. 1983. Chemical changes in brinjal plants induced by root knot nematode, *Meloidogyne incognita. Indian J. Ent.* **45**:45-47

Ganguly, S. and Dasgupta, D.R. 1988. Polyphenol oxidase from 'apparently healthy' roots of tomato variety Pusa Ruby : infected by *Meloidogyne incognita. Indian J. Nematol.* **18**:154-155

Ghosh, B.N. 1960. A note on the resistance of boro paddy to stem borer infestation. *Sci. Cult.* **25**(9):547-548

Giga, D.P. 1995. Selection of oviposition sites by the cowpea weevils *Callosobruchus rhodesianus* and *C. maculatus. Insect Sci. application* **16**(2):145-149

* Gomes, V.M., Oliveria, A.E.A. and Xavier, F.J. 1996. A chitinase and a beta-1, 3-glucanase isolated from the seeds of cowpea, inhibit the growth of fungi and insect pests of the seed. *J. Sci. Fd Agric.* **72**(1):86-90

* Gregory, P., Tingey, W.M., Ave, D.A. and Bouthyette, P.Y. 1986. Potato glandular trichomes : A phytochemical defense mechanism against insects. *Natural Resistance of Plants to Pests : Roles of Allelochemicals.* (Ed. Green, M.B. and Hedin, P.A.). ACS Symp. Ser. 296, Am. Chem. Soc., Washington, D.C. pp.160-167

Green, T.R. and Ryan, C.A. 1972. Wound-induced proteinase inhibitor in plant leaves: A possible defense against insects. *Science* **175**:776-777

* Guerra, D.T. 1981. Natural and *Heliothis zea* (Boddie)-induced levels of specific phenolic compounds in *Gossypium hirsutum*. L.M.S. thesis, Univ. Arkansas

Harborne, J.B. 1982. *Introduction in Ecological Biochemistry*. Academic Press, New York, p.278

Hart, S.V., Kogan, M. and Paxton, J.D. 1983. Effect of soybean phytoalexins on the herbivorous insects: Mexican bean beetle and soybean looper. *J. Chem. Ecol.* **9**:657-672

Hedin, P.A. 1986. Developing research trends in the chemistry of plant resistance to pests. *Natural Resistance of Plants to Pests*. (Ed. Green, M.B. and Hedin, P.A.) ACS Symp. Ser. 296, *Am. Chem. Soc.*, Washington, D.C. pp.2-15

Hoffmann, W.E. 1932. Observations on a weevil injurious to Banana. *Hongkong Nat.* **4**(1):48-54

Horber, E. 1980. Types and classification of resistance. *Breeding Plants Resistant to Insects*. (Ed. Maxwell, F.G. and Jennings, P.R.) John Wiley and Sons, Inc., New York, pp. 15-21

* Hori, K. and Atalay, R. 1980. Biochemical changes in the tissue of Chinese cabbage injured by bug *Lygus disponsi*. *Appl. Ent. Zool.* **15**:234-241

Howlader, A.J. and Ambadkar, P.M. 1995. Oviposition deterring influence of female body wash in tobacco beetle, *Lasioderma serricorne* (F.) (Coleoptera:anobiidae). *J. stored products Res.* **31**(1):91-95

Isahaque, N.M.M. 1978. A note on the incidence of *Odoiporus longicollus* Oliver on banana in Assam. *Pesticides* **12**(6):22-24

Isenhour, D.J., Duncan, R.R., Miller, D.R., Wasrom, R.M., Hanning, G.E., Wiseman, B.R. and Nabros, M.W. 1991. Resistance to leaf feeding by the fall armyworm (Lepidoptera:Noctuidae) in tissue culture derived sorghum. *J. econ. Ent.* **84**(2):680-684

* Israel, P. 1967. Varietal resistance to rice stem borers in India. *The Major Insect Pests of the Rice Plant*, International Rice Research Institute, John Hopkins Press, Baltimore, pp.391-403

Jayasree, T.V. 1992. Biology of banana pseudostem weevil *Odoiporus longicollis* oliv. (Coleoptera:Curculionidae). M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, p.72

Jenkins, J.N., Parott, W.L. and Jones, J.W. 1975. Boll weevil oviposition behaviour:Multiple punctured squares. *Environ. Ent.* **4**:861-867

Jennings, P.H., Brannaman, B.L. and Zscheile, F.P. 1969. Peroxidase and polyphenol oxidase activity associated with *Helminthosporium* leaf spot of maize. *Phytopathology* **59**:963-967

* Kalazich, J.C. 1989. Introgression of the trichoma characteristics of *Solanum berthaultii* (Howkes) into *S. tuberosum* (L.). Ph.D. thesis, Cornell University, New York

Karban, R. and Carey, J.R. 1984. Induced resistance of cotton seedlings to mites. *Science* **225**:53-54

Karwasra, S.S. and Parashar, R.D. 1989. Polyphenol oxidase and peroxidase activities in relation to resistance in potato against bacterial soft rot. *Veg. Sci.* **16**:205-207

Klun, J.A. and Robinson, J.F. 1969. Concentration of two 1,4-benzoxazinones in dent corn at various stages of development of the plant and its relation to resistance of the host plant to the European corn borer. *J. Econ. Ent.* **62**:214-220

Kogan, M. and Paxton, J. 1983. Natural inducers of plant resistance to insects. *Plant Resistance to Insects*. (Ed. Hedin, P.A.). ACS Symp. Ser. 208, *Am. Chem. Soc.*, Washington, pp.154-171

* Kogan, M. and Ortman, E.F. 1978. Antixenosis - a new term proposed to define Painter's 'Non-preference' modality of resistance. *Bull. ent. Soc. Am.* **24**:175-176

Kogan, M. 1982. Plant resistance in pest management. *Introduction to Insect Pest Management*. (Ed. Metcole, R.L. and Luckmann, W.) John Wiley and Sons, Inc., New York, pp.83-134

* Kogan and Schroeder. 1989. Cited by Smith, C.M. *Plant Resistance to Insects:A Fundamental Approach*. John Wiley & Sons, New York, p.142

Koppenhofer, A.M., Reddy, K.V.S., Sikora, R.A. and Seshu, R.K.V. 1994. Reduction of banana weevil populations with pseudostem traps. *Int. J. Pest Management* **40**(4):300-304

* Kreuzaler, F., Ragg, H., Fautz, E., Kuhn, D.N. and Hablbrock, K. 1983. UV-induction of chalcone synthase M-RNA in cell suspension cultures of *Petroselinum hortense*. *Proc. Nat. Acad. Sci.*, USA. **81**:1102-1106

* Kuhn, D.N., Chappell, J., Boudet, A. and Hahlbrock, K. 1984. Induction of phenyl alanine ammonia lyase and 4-coumarate:Co A ligase mRNAs in cultured plant cells by UV light of fungal elicitor. *Proc. Nat. Acad. Sci.*, USA. **81**:1102-1106

Kung, K.S. 1955. The banana stem borer weevil *Odoiporus longicollis* Oliv. in Taiwan. *J. Agric. Taiwan.* **4**:80-113

Kung, K.S. 1964. Ecological studies on banana stem borer weevil *Odoiporus longicollis* Oliv. *J. Agric. Taiwan* **11**:137-160

Lentini, Z., Earle, E.D. and Plaisted, R.L. 1990. Insect resistant plants with improved horticultural traits from interspecific potato hybrids grown *in vitro*. *Theor. appl. Genet.* **80:**95-104

Leszczynski, B., Warchol, J. and Niraz, S. 1985. The influence of phenolic compounds on the preference of winter wheat cultivars by cereal aphids. *Insect Sci. Appl.* **6**:157-158

Liu, X.H., Wang, H.H., Chai, J.M. and Wui, X.G. 1990. The relationship between the cell structure and biochemical characteristics in banana leaf tissue and their cold tolerance. *J. Fujian Agric. Coll.* **19**(2):181-185

Lowry, O.H., Rosebrough, N.J., Fan, A.L. and Randall, R.J. 1951. Protein measurement with the folin-phenol reagent. *J. Biol. Chem.* **193**:265-275

Luis, J.G., Quinones, W., Echeverri, F., Grillo, T.A., Kishi, M.P., Garcia, G.F., Torres, F. and Cardona, G. 1996. Musanolones: Four 9-phenyl phenalenones from rhizomes of *Musa acuminata*. *Phytochemistry* **41**(3):753-757

Luo, L.Y., Luo, Q.C., Yao, X. and Liu, Z.L. 1985. Weevils injurious to banana in Guizhou (China) and their biological features. *Insect Knowledge* **22**(6):265-267

* Lupton, F.G.H. 1967. The use of resistant varieties in crop protection. *World Rev. Pest-Control* **6**:47-58

Mahadevan, A. and Sridhar, R. 1986. *Methods in Physiological Plant Pathology.* Sivakami Publications, Madras, p.328

Maine, E.C. and Kelman, A. 1961. The influence of reducing substance on resistance to bacterial wilt in tobacco. *Phytopathology* **51**:491-492

Markose, B.L. 1996. Genetic and biochemical basis of resistance to bacterial wilt in chilli. Ph.D. thesis, Kerala Agricultural University, Thrissur, p.90-110

Martin, G.A., Richard, C.A. and Hensley, S.D. 1975. Host resistance to *Diatraea saccharalis* (F.):Relationship of sugarcane node hardness to larval damage. *Environ. Ent.* **4**:687-688

Mathew, M.P., Thomas, J., Paul, T.S., Sivaraman, S., Nair, S.R. and Ibrahim, K.K. 1996. Chemical control of pseudostem borer of Banana *Odoiporus longicollis* Oliver Symp. on Technological Advancement in Banana/Plantain Production and Processing. India – International, Kerala Agricultural University, Thrissur, August 20-24 Abstract of papers. p.31

Matsumoto, K. and Yamaguchi, H. 1990. Selection of aluminium tolerant variants from irradiated protocorm-like bodies in banana. *Trop. Agric.* **67(3)**:229-232

McSwiney, J. 1920. Report of the Agricultural Department, Assam for the year ending 31st March 1920, Shillong. pp.6-7

Mehlenbacher, S.A., Plaisted, R.L. and Tingly, W.M. 1983. Inheritance of glandular trichomes in crosses with *Solanum berthoultii*. *Am. Potato J.* **60**:699-708

* Morpurgo, R., Lopato, S.V., Afza, R. and Novak, F.J. 1994. Selection parameters for resistance to *Fusarium oxysporum* f.sp. *Cubense* race 1 and race 4 on diploid banana. *Euphytica* **75**(2):121-129

Naylor, A.F. 1961. Dispersal in the red flour beetle *Tribolium castaneum*. *Ecology* 42:231-237

Ndiege, I.O., Budenberg, W.J., Otieno, D.O., Ahmed, H. and Hassanali, A. 1996. 1,8-cineole: an attractant for the banana weevil, *Cosmopolites sordidus*. *Phytochemistry* **42**(2):369-371

Ndiege, I.O., Budenberg, W.J., Lwande, W. and Hassanali, A. 1991. Volatile components of banana pseudostem of a cultivar susceptible to the banana weevil. *Phytochemistry* **30**(12):3929-3930

Norris, D.M. and Kogan, M. 1980. Biochemical and morphological bases of resistance. *Breeding Plants Resistant to Insects.* (Ed. Maxwell, F.G. and Jennings, P.R.). John Wiley & Sons, New York, pp.24-60

Obukowicz, M. and Kennedy, G. 1981. Phenolic ultra cytochemistry of tobacco cells undergoing the hyper sensitive reaction to *Pseudomonas solanacearum. Physiol. Pl. Pathol.* **18**:339-344

Ortiz, R., Vuylsteke, D., Dumpe, B. and Ferris, R.S.B. 1995a. Banana weevil resistance and corm hardness in Musa germplasm. *Euphytica* **86**(2):95-102

Ortiz, R., Vuylsteke, D. and Ogburia, N.M. 1995b. Inheritance of pseudostem waxiness in banana and plantain (Musa spp.). *J. heridity* **86**(4):297-299

Painter, R.H. 1951. *Insect Resistance in Crop Plants*. University press of Kansas, Lawrence. p.521

Panda, N., Pradhan, B., Samalo, A.P. and Rao, P.S.P. 1975. Note on the relationship of some biochemical factors with the resistance in rice varieties to yellow rice borer. *Indian J. Agric. Sci.* **45**:499-501

* Patanakamjorn, S. and Pathak, M.D. 1967. Varietal resistance of rice to Asiatic rice borer, *Chilo suppressalis* (Lepidoptera:Crambidae), and its association with various plant characters. *Ann. Ent. Soc. Am.* **60**:287-292

Pathak, M.D. and Dhaliwal,G.S. 1986. Insect Control. *Global Aspects of Food Production.* (Ed. Swaminathan, M.S. and Sinha, S.K.). Tycooly International, Oxford, England, pp.357-385

Peraiah, A. and Roy, J.K. 1979a. Studies on biochemical nature of gall midge resistance in rice. *Oryza* **16**(1):149-150

Peraiah, A. and Roy, J.K. 1979b. Anatomical characters of rice stems in relation to brown plant hopper resistance. *Oryza* **16**(1):73-74

Perry, D.A. and Pitman, G.B. 1983. Generic and environmental influence in host resistance to herbivory. Douglas-fir and the western spruce bud worm. *Z. angew. Ent.* **96**:217-228

Pinto, M.P.D. 1928. The weevil pests of plantains (*Musa sapientum*), *Odoiporus longicollis* Oliv. *Trop. Agriculturist* **90**(4):216-224

* Price, N.S. 1995. The use of a modified pseudostem trapping technique for assessing the efficacy of insecticides against the banana-borer weevil. *Fruits.* Paris **50**(1):23-26

* Prokopy, R.J. and Bush, G.L. 1972. Mating behaviour in *Rhagoletis pomonella* III. Male aggregation in response to an arrestant. *Can Ent.* **104**:275-283

Prokopy, R.J. 1981. Epideictic pheromones that influence spacing patterns of phytophagous insects. *Semiochemicals their Role in Pest Control.* (Ed. Nordlund, D.A., Jones, R.L. and Lewis, W.J.). John Wiley and Sons, New York, pp.181-213

Raina, A.K., Jackson, D.M. and Ray, F.S. 1997. Increased pheromone production in wild tobacco bud worm Lepidoptera:Noctuidae) exposed to host plants and host chemicals, *Environ. Ent.* **26**(1):101-105

Ranjith, A.M. 1981. Studies on the consumption, digestion and utilization of food plants by *Pericallia ricini* (Arctiidae:Lepidoptera). M.Sc.(Ag.) thesis, Kerala Agricultural University, Thrissur, p.50

Ranjith, A.M. 1988. Studies on host plant resistance in cotton to the whitefly *Bemesia tabaci* Gennadius (Aleyrodidae:Homoptera) Ph.D.(Ag.) thesis, Tamil Nadu Agricultural University, Coimbatore, p.176

* Reese,J.C.1981.Insect dietetics: Complexities of plant-insect interactions. *Current Topics in Insect Endocrinology and Nutrition.* (Ed.Bhaskaran, G., Friedman, S. and Rodriguez, J.G.) Plenum, New York,pp.317-335

Reghunath, P., Visalakshi, A., Thomas, B.M. and Mohandas, N. 1992. Insecticidal management of the pseudostem borer *Odoiporus longicollis* oliver (Coleoptera:Curculionidae) in banana. *Entomon.* **17**(2):113-115

Renwick, T.A.A. and Radke, C.D. 1980. An oviposition deterrent associated with frass from feeding larvae of the cabbage looper, *Trichoplusia ni. Environ. Ent.* 9:318-320

*Retig, N. 1974. Changes in peroxidase and polyphenol oxidase associated with natural and induced resistance of tomato to Fusarium wilt. *Physiol. Pl. Pathol.* **4**:145-150

Rhoades, D.F. 1983. Herbivore population dynamics and plant chemistry. ***Variable Plants and Herbivores in Natural and Managed Systems.*** (Ed. Denno, R.F. and McClure, M.S.) Academic Press, New York, pp.3-55

Rhodes, J.M. and Wooltorton, L.S.C. 1978. The biochemistry of phenolic compounds in wounded plant storage tissues. *Biochemistry of Wounded Plant Tissues* (Ed. Kahl, G.) de Gruyter, New York, pp.243-286

Ruzicka, Z. 1996. Oviposition deterring pheromone in Chrysopidae (Neuroptera) Intra and inter-specific effects. *Eur . J. Ent.* **93**(2):161-166

Sadasivam,S. and Manickam,A.1996.*Biochemical Methods for Agricultural Sciences.* Wiley Eastern Ltd., New Delhi,p.21

* Sarah, J.L., Blavignac, F., Sabatmi, C. and Boisseau, M. 1992. A laboratory method for screening banana germplasm for resistance to nematodes. *Fruits Paris* **47**(5):559-564

* Schukle and Dhawale. 1989. Cited by Smith, C.M. *Plant Resistance to insects: A Fundamental Approach.* John Wiley and Sons, New York, p.141

Sherif, A.K. and Thomas. M.J. 1988. Pseudostem borer threat to banana. *The Hindu* (1988) May 11. p.24

Shukla, G.S. and Kumar, K. 1969. A new record of *Odoiporus longicollis* Oliv. (Coleoptera:Curculionidae) from Uttar Pradesh. *Sci. Cult.* **35**:481-482

Siegel, S. 1956. *Non-parametric Statistics for the Behavioural Sciences.* McGraw – Hill Kogakusha, Ltd. London, p.312

* Singh, M.J. and Singh, J. 1989. Mechanisms of resistance to cucumber mosaic virus in chilli pepper (*Capsicum annuum* L.). Role of phenols and phenologes. EUCARPIA VIIth meeting on genetics and breeding on capsicum and egg plant, Yogoslavia, 193-203

Smith, C.M. 1988. Effects of mechanical damage to plants on insect populations. *Plant Stress-Insect Interactions.* (Ed. Heinrichs, E.A.), John Wiley and Sons, New York, pp.321-340

Smith, C.M. 1989. *Plant Resistance to Insects: A Fundamental Approach.* John Wiley and Sons, New York p.286

Smith, M.K., Hamill, S.D., Langdon, P.W. and Pegg, K.G. 1993. Mutation breeding programme produces a plant with potential fusarium wilt (race 4) resistant cavendish variety. *Mutation breeding Newsl.* **40**:4-5

Speyer, E.R. 1918. Report on the work of the Entomological division. *Ceylon Adm. Rept.*, Feb.1918

Subharao, D.V. and Perraju, A. 1976. Resistance in some rice strains to first instar larvae of *Tryporyza incertulus* (Walker) in relation to plant nutrients and anatomical structure of the plants. *Int. Rice Res. Newsl.* **15**(4):16-17

Tingey, W.M. 1981. The environmental control of insects using plant resistance. *Handbook of Pest Management in Agriculture*. (Ed. Pimentel, D.). CRC Press, Boca Raton, Florida, pp.175-197

Ukwungwu, M.N. and Odebiyi, J.A. 1985. Incidence of *Chilo zacconius* Bleszynski on some rice varieties in relation to plant characters. *Insect Sci. Appl.* 6:653-656

Velusamy, R. and Heinrichs, E.A. 1986. Tolerance in crop plants to insect pests. *Insect Sci. Appl.* 7(6):689-696

* Verschaffelt, E. 1910. The cause determining the selection of food in some herbivorous insects. *Proc. Royal Acad. Amsterdam* **13**:536-542

Visalakshi, A., Nair, G.M., Beevi, S.N. and Amma, A.M.K. 1989. Occurrence of *Odoiporus longicollis* Oliver (Coleoptera:Curculionidae) as a pest of banana in Kerala. *Entomon.* **14**(3):367-368

Wallace, L.E., McNeal, F.H. and Berg, M.A. 1974. Resistance to both *Oulema melanopus* and *Cephus cinctus* in pubescent-leaved and solid stemmed wheat selections. *J. Econ. Ent.* **67**:105-110

* White, W.H. and Irvine, J.E. 1987. Evaluation of variation in resistance to sugarcane borer (Lepidoptera:Pyralidae) in a population of sugarcane derived from tissue culture. *J. Econ. Ent.* **80**:182-184

Whittaker, R.H. and Feeny, P.P. 1971. Allelochemicals: Chemical interactions between species. *Science* **171**:757-770

Wijesekara, G.A.W. and Menike, J.I. 1991. Management of banana root and pseudostem weevils. (*Cosmopolites sordidus* and *Odoiporus longicollis*) Coleoptera:Curculionidae. *Asia and Pacific Pl. Prot. Common. Q. Newsl.* **34**(2):10-15

Woodhead, S. 1982. P-Hydroxybenzaldehyde in the surface wax of sorghum: its importance in seedling resistance to acridids. *Ent. Exp. Appl.* **31**:296-302

* Woodhead, S. 1983. Surface chemistry of *Sorghum bicolor* and its importance in feeding by *Locusta migratoria Physiol. Ent.* **8**:345-352

Zangari, A.R. and Rutledge, C.E. 1996. The probability of attack and patterns of constitutive and induced defense:A test of optimal defense theory. *Am. Nat. J.* **147(4)**:599-608

* Originals not seen

APPENDIX-I

Meteorological data during the cropping period from August 1998-September 1999

Months	Temperature °C		Rainfall (mm)	Rainy days	Relative humidity(%)		Mean sunshine hours	Wind speed Km/h
	Maximum	Minimum			1	2		
1998								
August	29.8	23.9	433.6	18	95	77	3.6	2.5
September	30.2	23.3	571.3	24	96	78	4.1	2.0
October	28.0	22.8	452.8	18	94	76	4.8	1.7
November	31.5	23.1	109.4	9	92	64	7.2	1.8
December	30.1	22.9	33.0	4	79	58	6.6	5.7
1999								
January	32.4	21.5	0.0	0	76	40	9.3	-
February	34.5	23.3	22.8	1	77	35	9.1	-
March	35.5	24.5	0.0	0	88	48	8.8	-
April	33.4	25.6	39.0	4	88	58	10.3	-
May	30.7	24.7	430.5	18	92	72	4.9	-
June	29.4	23.0	500.2	28	94	75	5.0	-
July	28.4	23.0	823.3	28	96	82	2.4	-
August	29.8	22.9	260.1	12	94	73	5.5	-
September	31.6	23.4	28.4	3	89	63	7.1	2.1

RESISTANCE MECHANISMS AGAINST THE PSEUDOSTEM WEEVIL *Odoiporus longicollis* OLIVIER (COLEOPTERA : CURCULIONIDAE) IN BANANA

By

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ABSTRACT OF THE THESIS

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ABSTRACT

The present investigation on resistance mechanisms against pseudostem weevil *Odoiporus longicollis* Olivier (Coleoptera:Curculionidae) in banana was undertaken in the Department of Entomology, College of Horticulture, Vellanikkara during 1997-99. In insect-plant interactions, it is always the survival of the fittest. There is also a constant enhancement in the physiological preparedness both by the insect and the host plant to gain the upper hand. It is felt that even though the insect conquers the resistance of the host plant in majority of cases, there may be a threshold of insect attack, which would induce plant defenses to deter the attack. It was also possible that some of these plant defenses may be triggered off by enzymes produced in the plant system consequent to the attack. These incitory enzymes or chemicals may be transferred to the offspring, which would make them resistant to attack, by the weevil. Hence, major objectives of the study were to evaluate sucker and *in vitro* regenerated progenies of infested Nendran (AAB) for resistance against *O. longicollis*, to evolve methods for screening resistance under artificial conditions, laboratory rearing of *O. longicollis* and identification of morphological, biochemical and anatomical bases of resistance using Nendran as susceptible and Njalipoovan as the resistant source.

Preliminary field screening of *Musa* (AAB) Nendran for resistance against pseudostem weevil was done based on the number of ovipunctures/feeding holes on the pseudostem, surface area of infestation and the size of feeding holes. All of these contributed to the intensity of the attack. The multiplication product of these factors led to the development of a new 0-4 rating scale, for scoring the damage by *O. longicollis* in the field.

No-choice tests conducted on sucker and *in vitro* regenerated progenies of such graded mother plants revealed a better performance by plants having lower history of infestation. This suggested the existence of induced resistance involving

triggering off of enzymatic reactions. Besides these lower grades, the plantlets of G_4 exhibited better tolerance than control by exerting greater potential to stand amidst heavy winds till complete maturity of the bunch. The *in vitro* regenerated plants performed much better than the sucker derived treatment plants. This offers potential for mass multiplication of such in borne resistance for commercial release. The results obtained so far opens up the possibility of induced resistance that may be genetically inherited to the plantlets from its mother as an evolutionary character. Such triggering mechanism operating *in vivo* offers greater potential to select resistant clones.

The methodology for screening banana for resistance to *O. longicollis* by artificial infestation in field situations was standardized. Four adult weevils in the sex ratio 1:1 (2♀ and 2♂) was found to be optimum to create moderate levels of infestation during the most favourable season (May-August).

Mass rearing of *O. longicollis* in laboratory conditions using natural diet was quite successful for maintaining a large-scale population throughout the year. A continuous supply of uniformly aged test insects for exposing the experimental plants at specific pest load was made possible by this.

Studies involving the duration of development of the weevil revealed acute antibiosis in Njalipoovan manifested by reduced egg laying prolonged duration of life cycle, reduced hatching of eggs (39.5%) and reduced adult emergence from eggs (29.7%) in contrast to 94.9 per cent hatching of eggs and 91.3 per cent adult emergence in Nendran.

A no-choice test was designed to study the appropriate age of Nendran from which it becomes susceptible to infestation. Results indicated a distinct preference for oviposition by the adults on pseudostem of banana from seventh month onwards. This information helps to reduce the insecticidal load in the banana

ecosystem by advocating pesticides from seventh month only. The existence of oviposition deterrency when more females were confined to Nendran pseudostem pieces revealed the deposition of spacing pheromones by conspecific females after egg laying. It was seen that the acetone and ether extracts of pseudostem pieces where ten females had laid their eggs, when sprayed on fresh pseudostem, deterred the oviposition by other females. Isolation and identification of such epidectic compounds will help in their utilisation in the field to prevent egg laying on banana pseudostem.

The morphological, anatomical and biochemical bases of resistance were also identified. The morphological parameters associated with resistance were smaller width and breadth of the sheath coupled with more number of sheaths in the resistant variety. These increased the number of cuticular layers and cell walls the grub had to traverse before entering the central pith. The pith formation is of prime importance to the curculionid as it becomes the target site of feeding for third and fourth instars. There are certain cues emanating from the pseudostem subsequent to maturity, which aids the adult weevils in host selection and oviposition.

The lesser moisture and higher crude fibre content of Njalipoovan pseudostem exhibited greater influence on resistance by posing a mechanical barrier to egg laying and feeding of neonate larva. The information on moisture content is of practical utility in the management of the pest in endemic areas. It may be possible to artificially create moisture stress in the plant attaining flower bud initiation stage by regulating irrigation. The level of irrigation has to be adjusted so as not to induce a yield reduction but at the same time to induce moisture stress at least in the outer sheaths to prevent oviposition. The water suckers served as more conducive hosts for the pest even before sufficient maturity and this highlighted the need for removal of water suckers from endemic fields.

The anatomical sections of pseudostem sheath clearly revealed a thick cuticle on epidermal cells with four to five layers of dense collenchymatous hypodermis imposing a physical barrier against oviposition in the resistant cultivar. Further, the ground tissues which constituted of compactly arranged parenchymatous cells with several starch grains offered feeding deterrency. The closely packed bigger sized lower epidermal cells along with additional parenchymal layers below the air cavity operated as a mechanical barrier which caused the failure of the neonate larva to penetrate inner tissues.

Higher content of phenolics generally showed a positive correlation with resistance. However, the role of ortho dihydric phenolic substrates in resistance is greatly dependent on the enzymatic activity of polyphenol oxidase to form toxic metabolites like quinones and tannins. Hence, the oxidation factor of OD phenols clearly indicated the dominance of the resistant variety, Njalipoovan. The accelerated activity in Njalipoovan is endowed with greater potentiality to activate polyphenol aggressively effecting more production of lignin, quinone and tannins, in the event of egg laying or feeding by the pseudostem weevil.