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INTERACTIONS BETWEEN CASSAVA AND ARTHROPOD PESTS

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ABSTRACT

Study of the interactions between plants and arthropods and especially of the resistance of plants is an essential component of integrated pest management. In the context of interactions between cassava and its three main pests in Africa (cassava green mite, variegated grasshopper and cassava mealybug), it is observed that knowledge in this field is not very advanced except for the cassava-mealybug model. The study of this began in 1987 and has revealed the following features: (i) the resistance to mealybug developed by cassava is partial and is expressed according to the three categories of resistance, i.e., non-preference, antibiosis and tolerance. The results indicate horizontal, polygenic resistance; (ii) the behavioural characteristics of the insect and the sensorial equipment of its antennae and labium (the site of olfactory and gustatory chemoreceptors) suggest that the chemistry of the surface of the plant probably plays a determinant role in the success of the plant recognition phase; (iii) cassava mealybug principally feeds on phloem sap of which the main component is sucrose and which has a low amino acids content, a high cyanide glycosides content and also contains glycosylated flavonoids including rutin. Comparison of cassava phloem sap and honeydew excreted by the insect indicates that all these compounds are ingested and metabolised; (iv) the strong positive correlation established between the degree of antibiotic resistance of various cassava genotypes and their phloem rutin contents suggests that this secondary compound contributes to plant resistance to the mealybug; (v) considerable fluctuations in the pest numbers observed each year in the field were linked with variations in phloem rutin contents and these variations are affected by cultural practices. It was concluded that: (i) research on plant-insect interactions is complex as it requires a multi-disciplinary approach involving entomologists, biochemists, plant physiologists and plant breeders; (ii) there is a need to develop such studies on the other cassava pests while deepening those on the cassava-mealybug model.

Key Words: Cassava, resistance, mealybug, *Phenacoccus manihoti*

RÉSUMÉ

L'étude des interactions plantes-insectes et en particulier de la résistance des plantes est une composante essentielle de la lutte intégrée. Dans le contexte des interactions du manioc et de ses trois principaux ravageurs en Afrique (acarien vert, criquet puant et cochenille farineuse), on contaste que l'état des connaissances dans ce domaine est globalement peu avancée à l'exception toutefois du modèle manioc-cochenille farineuse. L'étude de ce modèle a permis de préciser que: (i) la résistance du manioc développée vis à vis de la cochenille est une résistance horyzontale et polygénique;(ii) la chimie de surface du végétal joue un rôle déterminant dans le succès de la phase de reconnaissance de la plante par la cochenille; (iii) que la chochenille est un insecte phloemophage se nourissant d'une sève élaborée riche en composés secondaires (cyanure et rutine); (iv) que la rutine participe à la résistance de type antibiose de la plante; En conclusion est soulignée: (i) la complexité des recherches dans le domaine des interactions plantes

insectes car elle requiert une approche multidisciplinaire faisant intervenir des entomologistes, des biochimistes, des physiologistes végétaux et des améliorateurs des plantes; (ii) la nécessité de développer de telles études sur les autres ravageurs du manioc tout en approfondissant celles menées sur le modèle manioc-cochenille farineuse.

Mots Clés: **Manihot, résistance, Pseudococcidae, *Phenacoccus manihoti***

INTRODUCTION

One of the best ways of improving the effectiveness of biological control agents in integrated control consists of breeding varieties that are relatively unfavourable for the multiplication of their pests. This strategy makes it possible not only to limit pest reproduction but also to slow the rate of development, thus enabling entomophagous organisms to operate for a longer period of time.

Plant resistance is an essential component of integrated protection. It has proved its effectiveness in the control of a number of serious pests. Moreover, it is the ideal complement to integrated protection systems insofar as it does not form a serious constraint for farmers and can complement both biological control and pesticide spraying. Furthermore, the effects are beneficial in the long term. Painter (1951) proposed three categories of plant resistance to insects: (i) tolerance, characterised by the capacity of certain plants to withstand the effects of a pest population that would cause considerable harm to other plants. It is typically appraised in terms of plant damage (extent of damage or the physiological or economic consequences of such damage) rather than effects on the pest; (ii) antixenosis (or non-preference), characterised by factors peculiar to the plant and unfavourable for colonisation by the pest. They concern the behavioural components of interactions and especially those involved during the choice of host plant and establishment on this plant; (iii) antibiosis, characterised by plant factors that reduce the development, growth or reproduction of the insect or that cause its death. The effect is generally on a more long-term basis than antixenosis and affects population levels rather than the initial colonisation of the host.

It will be noted that resistance mechanisms in plants are constituent or induced features and are generally chemical.

The three main pests on cassava in Africa are cassava green mite (*Mononychellus progresivus* Doreste), cassava mealybug (*Phenacoccus manihoti* Matile-Ferrero) and variegated grasshopper (*Zonocerus variegatus* L.). The three pests have different feeding habits. However, cassava green mite and cassava mealybug can be grouped with regard to strategies for overcoming their hosts' chemical defences as they have considerable specificity for the genus *Manihot*. The first two pest species display specialist strategies while the third has broader feeding habits.

Trophic studies of interactions between cassava green mite and cassava and between variegated grasshopper and cassava are not very advanced and are only covered briefly here. Little information is currently available on the cassava-cassava green mite system. However, Byrne *et al.* (1982) showed that cassava green mite can appraise plant quality during the first few probes. Although research on the variegated grasshopper has been in progress for some years, the data are still fragmentary and sometimes contradictory. It can be noted that the insect feeds on a variety of plants and seeks in particular those known for their toxicity (Chapman *et al.*, 1986). It has been shown that older larvae cause most of the damage to cassava (Bernays *et al.*, 1977). The role of cyanide compounds is still a subject of controversy. They are reported by Bernays *et al.* (1977) to have an anti-feeding effect and by Bani (1990) to be phago-stimulants.

The state of knowledge of interactions between cassava and cassava mealybug is more advanced as study of the system began in 1987. The following questions have been answered: (i) what type of resistance does cassava have to mealybug?; (ii) what is the feeding behaviour of the mealybug (how does it identify the host plant and what does it feed on)?; (iii) what are the chemical and possible physical mechanisms of resistance of cassava to *P. manihoti*?; and (iv) what environmental factors (mainly cultural techniques) enhance resistance to the mealybug?

It will then be possible to describe the nature of cassava resistance to the mealybug and to facilitate the choice of resistant cassava varieties through varietal improvement or make it possible to establish cultural conditions that give the crop improved resistance through changed cropping practices.

CHARACTERISATION OF THE TYPE OF RESISTANCE TO *P. MANIHOTI* DEVELOPED BY CASSAVA

Multi-site varietal screening of the large range of plant material available in the Congo was undertaken to identify cassava varieties with different levels of resistance to cassava mealybug. Officials of the Congolese National Cassava Programme (Programme National Manioc Congolais) chose the genotypes to be screened using criteria of high productivity and resistance to bacterial blight (*Xanthomonas campestris* pv. *manihotis*), and to African cassava mosaic virus (ACMV).

No total resistance (complete absence of mealybugs) was observed. However, different levels of partial resistance were observed in different varieties (Tertuliano *et al.*, 1993). These levels of partial resistance were then characterised in the laboratory. Our work showed that cassava resistance falls into the three categories of Painter (1951) but with levels varying between genotypes (Le Rü and Tertuliano, 1993; Tertuliano *et al.*, 1993). The results suggest that there are many resistance mechanisms in cassava, confirming the hypothesis of Bellotti and Kawano (1980), who suggested that cassava resistance is of the horizontal type and polygenic. Incorporation of such partial resistances seemed, from an antixenotic and antibiotic standpoint, more appropriate than breeding immune varieties that is likely to impose substantial selection pressure that would enhance the appearance of resistant pest strains. It has also been shown in some simulation models of aphids on cereals that the incorporation of such partial resistance in a control programme can achieve a substantial decrease in pest densities.

FEEDING BEHAVIOUR OF *P. MANIHOTI*

How does the mealybug recognise the host plant? We considered the mealybug leaf recognition behaviour for subsequent study of the physical and/or chemical mechanisms involved in the settling phase (examination of the surface of the plant) and contributing to antixenotic resistance. The various behavioural stages were identified and the sensorial stuctures of the various organs (antennae, labium and legs) involved in recognition were described.

The observations show that mere movement across a leaf surface enables an insect to differentiate between plants. Morphological and ultrastructural examination of the sensilla in the three organs involved in plant recognition revealed the presence of mechanoceptors (indicating textural aspects) and chemoreceptors operating at a distance (olfactory capability) and contact chemoreceptors (taste, detection of plant surface chemistry) in the antennae and labium (Le Rü *et al.*, 1995).

All these observations lead us to make a distinction between two stages in the exploration of leaf surfaces by mealybugs: (i) a movement stage during which the plant is reconnoitred making use of the mechanical (legs), olfactory (antennae and labium) and taste (labium) functions; (ii) a period of trials during which reconnoitring mainly involves the mechanical (legs, antennae and labium) and taste (antennae and labium) functions. However, contributions by the olfactory function (antennae and labium) cannot be excluded.

The chemical and even physical (topographic) characteristics of cassava leaves play a determinant role in the success of the test phase at the surface of the plant.

What do mealybugs feed on? Like all probing and sucking insects, the cassava mealybug uses its stylets to feed in specific tissues of the plant. The route of *P. manihoti* stylets in plants was monitored by electro-penetrography. After penetrating the epidermis, the stylets mainly follow an extra-cellular route in the mesophyll before reaching the xylem and phloem cells in general (Calatayud *et al.*, 1994 b). These observations show that the mealybug is mainly a phloem-feeder.

This led to a determination of the biochemical characteristics of the primary and secondary compounds in cassava phloem sap. The amounts of secondary substances or their metabolites were also analysed in honeydew to determine when they were taken up and possibly metabolised by the mealybug (Calatayud *et al.*, 1994 a). Sucrose is the primary nutrient in cassava phloem sap. The sugar/amino acid ratio of 115 differs considerably from those observed in the phloem sap of many other plants (where it ranges from 1 to 15), suggesting that cassava has a low free amino acid content.

Cassava phloem sap also has a high content of cyanide glycosides (linamarin and lautostralin), nearly five times the level of total amino acids, traces of phenolic acids and three major glycosylated flavonoids identified as rutin and two isomers containing kaempferol. Mealybug honeydew contains cyanogenic glucosides, free cyanide and phenolic compounds. The profile of the latter reveals major differences between phloem sap and honeydew, indicating that secondary substances in cassava are translocated in cassava phloem sap and ingested and metabolised by the mealybug. The case of the cyanide compounds is interesting as our data suggest that *P. manihoti* has developed physiological mechanisms that enable it to convert toxic cyanide into toxic/non-toxic substances (Calatayud *et al.*, 1994 a). The demonstration in mealybug of linamarase activity different from that of cassava and the absence of a toxic effect of free cyanide on the insect at levels of 100 mg ml^{-1} or more confirm this interpretation and even suggest that such compounds are used as nutrients. Such utilisation of the -CN has already been reported for microorganisms and insects (Meyers and Ahmad, 1991; Modder, 1994) including the variegated grasshopper *Zonocerus variegatus.*

What are the chemical mechanisms of resistance of cassava to *P. manihoti*? Characterisation of the type of resistance developed by cassava and identification of the feeding behaviour of the mealybug was followed by a study of the chemical mechanisms involved in plant resistance. Chemical defence is the most effective strategy and the one commonly used by many plant species. Physical types of resistance are not particularly suitable for exploitation by breeders as they are difficult to transfer between genotypes.

Attention was paid first to the biochemical stubstances (amino acids, sugars and secondary compounds) that might be involved in 'antibiotic resistance' and consequently sited in phloem tissue of the plant. We, therefore, set out to discover whether the various degrees of antibiotic resistance to mealybug could be related to the levels of these substances in the phloem sap.

No correlation was established between the level of antibiotic resistance of the plants and the leaf content of primary substances (Tertuliano and Le Rü, 1992). However, examination of secondary compounds revealed a positive correlation between the degree of antibiotic resistance of the plants and their rutin content, suggesting that the latter may contribute to the resistance of cassava to mealybug. The increase in rutin contents following infestation by the mealybug also shows that it may be an induced defensive mechanism. The involvement of rutin is not surprising as the substance is known to affect insect growth, development and metabolism. In the Homoptera, it is thought to act not only on the insects themselves but also on their symbionts (Dreyer and Jones, 1981). By analogy, the possibility that the phenolics ingested by the mealybug are metabolised suggests that the products of the hydrolysis of rutin may affect the physiology of the insect, possibly by way of its symbiotic bacteria, especially as it has recently been shown that quercetin, the aglycon of rutin, is involved in the resistance of cassava to bacterial blight *Xanthomonas campestris* pv. *manihoti* (Mbaye, 1989).

How do these mechanisms vary with environmental factors (season, cultural practices)? After suggesting, in the light of laboratory experiments, that the rutin in phloem sap may be involved in antibiotic reactions of cassava to mealybug, we undertook to determine whether the substantial variations in mealybug populations observed each year in the field might be related to variations in rutin contents. Indeed, changes in mealybug populations were observed during substantial modifications to plant physiology (cessation of growth, sap surge) linked with the water stress undergone by the host plant during the dry season, suggesting a temporary decrease in resistance to mealybug.

Attention was also paid to the effects of cultural practices (organic fertiliser, mulching, NPK, etc.) on these variations as lower fluctuations in pest populations have been reported in cassava plots where the soil has a high organic content.

Although there are some differences between genotypes, it is known that the water stress undergone by cassava during the long dry season affects the synthesis of secondary leaf substances. Indeed, the defensive response of cassava (shown by an increase in leaf rutin content and probably in phloem sap rutin content during attack by the pest) varies according to season. It decreases in the dry season and is influenced by the cassava genotype (Calatayud *et al.*, 1994 c). These results confirm the hypothesis of temporary modification of cassava to mealybug resulting from the water stress suffered by the plant during the long dry season. Study of several agronomic techniques has also shown that the increase in foliar rutin during pest attacks is enhanced by mulching and application of organic fertilizer. This observation is partly explained by the decreased damage caused by mealybug when cassava is grown in soil with a high organic content (Neuenschwander *et al.*, 1994).

CONCLUSION AND PROSPECTS

Cultural control involving cropping practices and resistant varieties is one of the main components of integrated pest control. However, its application requires in-depth knowledge of plant-pest interactions. These appear to be little or very little known for cassava and its main pests in Africa. Thus, with a view to integrated management of cassava pests requiring cultural control, it would seem advisable in coming years to undertake such studies. The research performed on the cassava-mealybug model (about which there is the most information) shows the complexity and labouriousness of such work, which requires a multi-disciplinary approach involving entomologists, plant physiologists, biochemists and plant breeders. The following preliminary results have been obtained for this model: (i) the resistance developed by cassava for *P. manihoti* is partial (it can act on a broad range of pests and pathogens), multifactorial and is observed at different stages of mealybug nutrition; (ii) the feeding behaviour of the pest suggests that the chemistry of the phylloplane and of the mesophyll play a decisive role in accepting the plant (antixenosis) and that phloem chemistry is involved in antibiotic type resistance; (iii) rutin is involved in antibiotic resistance.

It is proposed to continue and amplify work on the cassava-cassava mealybug model along two main lines of research: (a) study of cassava-mealybug interactions in order to deepen analysis of the plant resistance mechanisms involved in the choice and acceptance of the host (antixenosis) and in reduction of the biotic potential of the pest (antibiosis). Special attention will be paid to studying the incidence of the physiological state of the plant on the expression of this resistance. (b) Study of the three-trophic interactions between cassava, mealybug and entomophagous agents to determine the probable repercussions of 'varietal control' (based on a modification of the chemical profile of the plant) on the various components of the biocoenosis, and in particular on beneficial agents in biological control, such as parasitoids. Indeed, the plant influences interactions between phytophagous insects and their antagonists by acting on the behaviour (localisation of the insect pests) and the development of parasitoids.

REFERENCES

Bani, G. 1990. Interactions *Zonocerus variegatus* (Orthoptére, Pyrgomorphidae) - manioc au Congo: Bioécologie d'un ravageur vecteur de la bactériose. *Thése de Doctorat de l'Université de Rennes* I, 195 pp.

Bellotti, A. and Kawano, K. 1980 Breeding approaches in cassava. In: *Breeding plants Resistant to Insects*. Maxwell, F.G. and Jennings, P.R. (Eds.), pp. 313-335. Wiley, New York, 683 pp.

Bernays, E.A., Chapman, R.F., Leather, E.M. and McCaffery, A.R. 1977. The relationship of *Zonocerus variegatus* (L.) (Acridoidea:

Pyrogomorphidae) with cassava (*Manihot esculenta*). *Bulletin of Entomological Research* 67:91-404.

Byrne, D.H., Guerrero, J.M., Bellotti, A.C. and Gracen, V.E. 1982. Behaviour and development of *Mononychellus tanajoa* (Acari: Tetranychidae) on resistant and susceptible cultivars of cassava. *Journal of Economic Entomology* 75:924-927.

Calatayud, P.A., Rahbe, Y., Delobel, B., Khuong-Huu, F., Tertuliano, M. and Le Rü, B. 1994a. Influence of secondary compounds in the phloem sap of cassava on expression of antibiosis towards the mealybug *Phenacoccus manihoti* (Homoptera, Pseudococcidae). *Entomologia Experimentalis et Applicata* 72: 47-57.

Calatayud, P.A., Rahbe, Y., Tjallingii, W.F., Tertuliano, M. and Le Rü, B. 1994b. Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants. *Entomologia Experimentalis et Applicata* 72:219-232.

Calatayud, P.A., Tertuliano, M. and Le Rü, B. 1994c. Seasonal changes in secondary compounds in the phloem sap of cassava in relation to plant genotype and infestation by *Phenacoccus manihoti* (Homoptera: Pseudococcidae). *Bulletin of Entomological Research* 84:453-659.

Chapman, R.F., Page, W.W. and McCaffery, A.R. 1986. Bionomics of the variegated grasshopper (*Zonocerus variegatus*) in west and central Africa. *Annual Review of Entomology* 31:479-509.

Dreyer, D.L. and Jones, K.C. 1981. Feeding deterrency of flavonoids and related phenolics towards *Schizaphis graminum* and *Myzus persicae* aphid feeding deterrents in wheat. *Phytochemistry* 20:2489-2493.

Le Rü, B. and Tertuliano, M. 1993. Tolerance of different host-plants to the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Homoptera: Pseudococcidae). *International Journal of Pest Management* 39:379-384.

Le Rü, B., Renard, S., Allo, M.R., Le Lannic, J. and Rolland, J.P. 1995. Ultrastructure of sensory receptors of the labium of the cassava mealybug. *Entomologia Experimentalis et Applicata* 77:31-36.

Mbaye, N. 1989. Etude des mécanismes de défense conférant au manioc le résistance aux *Xanthomonas*. *Mémoire Scientifique, Université Catholique de Louvain*. 120 pp.

Meyers, D.M. and Ahmad, S. 1991. Link between 1-3 cyanoalanine synthase activity and differential cyanide sensitivity. *Phytochemistry* 1075:195-197.

Modder, W.W.D. 1994. Control of the variegated grasshopper *Zonocerus variegatus* (L.) on cassava. *African Crop Science Journal* 2:391-406.

Neuenschwander, P. 1994. Control of the cassava mealybug in Africa: lessons from a biological control project. *African Crop Science Journal* 2:369-383.

Neuenschwander, P., Hammond, W.N.O., Gutierrez, A.P., Cudjoe, A.R., Baumgaertner, J.U., Regev, U. and Adjakloe, R. 1989. Impact assessment of the biological control of the cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) by the introduced parasitoid *Epidinocarsis lopezi* (De Santis) (Hymenoptera: Encyrtidae). *Bulletin of Entomological Research* 79:579-594.

Painter, R.H. 1951. *Insect resistance in crops plants*. The University Press of Kansas, Lawrence. 520 pp.

Tertuliano, M. and Le Rü, B. 1992. Résistance de différentes plantes-hôtes et cultivars de manioc a la cochenille du manioc *Phenacoccus manihoti*. Influence de la teneur de la séve en acides aminés et en sucres. *Entomologia Experimentalis et Applicata* 64:1-9.

Tertuliano, M., Dossou-Gbete, S. and Le Rü, B. 1993. Antixenotic and antibiotic components of resistance to the cassava mealybug, *Phenacoccus manihoti* (Hom., Pseudococcidae), in various host-plants. *Insect Science and Its Application* 5-6:657-665.

Tertuliano, M., Calatayud, P.A. and Le Rü, B. Seasonal changes in the secondary compounds content of cassava phloem sap in relation to plant growth and infestation by the mealybug, *Phenacocus manihoti*. Influence of fertilisation. *Agriculture, Ecosystems and Environment*. (in press).