

**LABORATORY REARING OF THE COCOA APHID
TOXOPTERA AURANTII (BOY) AND SCREENING COCOA
GENOTYPES FOR THEIR RESISTANCE TO THE APHID**

BY
EMMANUEL AHIA CLOTTEY
(B.Sc (Hons) Agriculture)

A thesis presented in partial fulfilment of the requirements for the de-
gree of Master of Philosophy in Entomology of the University of
Ghana.



Insect Science Programme*
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Legon

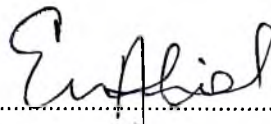
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Science (Faculty of Agriculture)



DECLARATION

I hereby declare that, except for references to works of other scholars which have been duly cited, this thesis is the result of my own original research which has neither been presented in whole or in part for the award of a degree elsewhere.



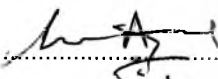
Emmanuel Ahia Clotley

(STUDENT)



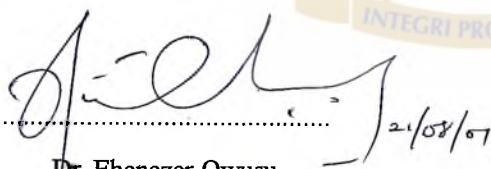
Dr. Beatrice Padi

(SUPERVISOR)



Dr. Paul K. Attah

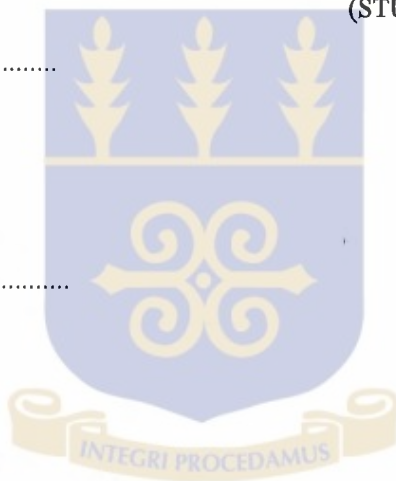
(SUPERVISOR)



21/08/07

Dr. Ebenezer Owusu

(SUPERVISOR)



ABSTRACT

A study was conducted to develop a quick and efficient laboratory mass-rearing method for the cocoa aphid *Toxoptera aurantii* (Boy) and to screen cocoa genotypes for their resistance to the aphid. In a similar experiment, two cocoa genotypes were screened to determine their level of attractiveness to the cocoa capsid *Sahlbergella singularis* Hagl and the results compared with those from the aphid study. The study formed part of an on-going programme being conducted by the Cocoa Research Institute of Ghana (CRIG) to develop more vigorous, high-yielding cocoa genotypes that are also resistant to diseases and insect pests.

To raise adequate numbers of experimental aphids, a number of leguminous and vegetable plants as well as tree crops, including cocoa (Y44), were evaluated for their suitability as rearing materials.

Two aphid susceptibility evaluation methods were developed, one involving the use of very young seedlings of open pollinated Amazon cocoa (T85/799) and Trinitario (Y44) origins, and the other involving older seedlings of 25 clonal materials and 25 pair-wise crosses. Four cocoa progenies (Na32 x T60/887, P30 x P 30, ICS39 x Y44 and T79/501 x Pa 150) of different genetic sources were also screened to determine their levels of resistance to *T. aurantii*. In addition, tender shoots of the T85/799 and Y44 were screened to determine their level of attractiveness to the cocoa capsid *Sahlbergella singularis* Hagl. using a laboratory “microtest” method and the results compared with findings from the aphid study.

None of the leguminous and vegetable plants tested were suitable for rearing *T. aurantii*. Of the tree crops tested, cocoa (Y44) emerged as the best rearing material (producing 107 aphids/seedling in two weeks), followed by the Citrus varieties Mediterranean sweet (50 aphids/seedling) and Late valencia (24 aphids/seedling), and

Coffea canephora (18 aphids/seedling). *Cola nitida* was found unsuitable.

For both the Y44 and T85/799 young seedlings, aphid multiplication rate was highest on seedlings infested one week after cotyledon opening (179 & 91 aphids/seedling, respectively) and lowest on those infested three weeks after cotyledon opening (38 & 9 aphids/seedling, respectively). There was positive correlation between the aphid infestation levels and the number of crinkled leaves produced ($r = 0.94$ for Y44; $r = 0.93$ for T85/799). Thus, the number of crinkled leaves produced after eight weeks were highest on seedlings infested one week after cotyledon opening (4 for Y 44 & 2 for T85/799) and lowest on seedlings infested three weeks after cotyledon opening (one for Y44 and zero for T85/799). The number of aphids produced after one week and the number of crinkled leaves produced after eight weeks were scaled to provide criteria for determining aphid resistance.

For the older cocoa seedlings, aphid multiplication rates differed significantly ($P < 0.01$), with the clonal materials T85/799 x Pound 25 (58 aphids/seedlings) and T60/887 x Pound 7 (52 aphids/seedlings) emerging as the most susceptible whilst P30 (5 aphids/seedling) and T63/971 (7 aphids/seedling) were the least susceptible. With the pairwise crosses, (T85/799 x Pound 7) x (T60/887 x Pound 25) (62 aphids/seedling) and IMC 85 x IMC 47 (57 aphids/seedling) were the most susceptible while Pound 10 x Pound 15 (4 aphids/seedling) and T60/887 x T63/971 (10 aphids/seedling) were the least susceptible to aphid infestation.

Results from the two evaluation methods (using very young and older seedlings) established differences in susceptibility levels among the four cocoa progenies screened. The Trinitario progeny Y44 x ICS39 was the most susceptible, followed by the Amazon progenies T79/502 x Pa150, Na32 x T60/887 and the Amelonado progeny, P30x P30, in decreasing order.

It was concluded that young cocoa seedlings of Trinitario Y44, not more than

one week after cotyledon opening, were the best of all the materials tested for rearing *T. aurantii* under insectary conditions at $24 \pm 30\text{C}$ and relative humidity range of 72-85%.

The comparable results obtained on the multiplication rate/crinkled leaf formation from the aphid study and the number of capsid lesions recorded in the laboratory microtest screening, strengthen the view by earlier workers that level of aphid resistance/ preference in cocoa types can be used as an index for determining their resistance to or preference by capsids and other sucking insects. It is anticipated that, at least, a few more of the cocoa types screened in the present study for aphid resistance/preference will be used in future studies to further confirm the view that cocoa types susceptible/resistant to *T. aurantii* are also susceptible/resistant to capsids.

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DEDICATION

This thesis is dedicated to my wife Olivia and four children, Daniel, David, Richard and Dorcas in appreciation of their love and understanding throughout the programme.

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LIST OF ABBREVIATIONS

- ANOVA — Analysis of Variance
LSD . — Least Significant Difference
CFC — Common Fund for Commodities
ICCO — International Cocoa Organization
IPGRI — International Plant Genetics Resource Institute

The following are general codes for cocoa types (genotypes).

- T number — These are seedlings with pod progenies introduced from Trinidad in 1944. Various seedlings obtained from a given pod are referred to by the *pod number* and *stand number* thus:
- T85/799 — Seedlings from pod 85 from Trinidad planted at stand 799 in the introduction plot.
- ICS — Imperial College Selections
IMC — Iquitos Mixed Calabacillos
PA — Parinari (Pounds selections from the Parinari district)
NA — Nanay (Pounds selection from the Nanay peninsula)
P — Amelonado cocoa
Y — Anyinam (cocoa from Anyinam Farms)
SCI — Posnettes “Sahlbergella Control”

CHAPTER ONE

GENERAL INTRODUCTION

Cocoa has been the backbone of the Ghanaian economy virtually throughout the last century. Despite problems currently besetting the cocoa industry, the crop still plays an important role in the economy of the country. The total area under cocoa cultivation is currently estimated at 1.2 million hectares and in 1999/2000 cocoa provided 22.5% of the total export earning (Asenso-Otchere, 2001). The cocoa industry employs sizeable percentage of the country's labour force and production is steadily increasing. From a lowest level of 154,000 metric tons (m.t) in 1983/1984, production has increased gradually reaching 409,000 m.t in 1997/1998 and 420,000 m.t in 1998/1999. Cocoa production is projected to reach 500,000 m.t by the year 2004/2005 and to 700,000 m.t by the year 2009/2010 (Anon, 1999). Cocoa will, therefore, continue to play a major role in the economy of Ghana.

Cocoa capsids (Heteroptera: Miridae) were recognised as pest of cocoa in West Africa in 1910 (Dungeon, 1910) and are currently considered the most important pests of cocoa in Ghana. Yield losses due to capsid damage alone was estimated at 60,000 to 100,000 tonnes of dry cocoa beans (Stapley and Hammond, 1959) and may reach as high as 75% crop loss per annum if attacked farms are left unattended for more than three years (Anon, 1951).

The recommended method for capsid control since 1957 has been by routine spraying with conventional insecticides applied four times a year at monthly intervals from August to December, omitting November (Gibbs *et. al.* 1968, Collingwood and Marchart, 1971) There is some indication that on mature cocoa, the frequency of

insecticide application may be reduced to two per year if the tree canopy is good (ie closed) (Owusu-Manu, 1997).

Chemical control of cocoa capsids, however is plagued by several problems including the resurgence of resistance strains such as occurred with lindane in the early 1960's (Dunn, 1963, Padi, 1997), the destruction of beneficial insects such as pollinators and natural pest control agents. Moreover, the high cost of inputs such as spraying machines, chemicals and labour and the cumbersome spraying procedure (Adomako, 1990) has resulted in very low adoption rate of less than 3% (Padi *et al*, 2000a).

It has, therefore, become necessary to look for alternative control methods that are environmentally friendly and devoid of the risks and problems associated with the current practice. The breeding of varieties of cocoa that are more resistant to capsids and other sucking insects offers an opportunity for reducing insect damage and the spread of insect transmitted diseases.

The Cocoa Research Institute of Ghana has initiated a programme aimed at selecting high-yielding and vigorous cocoa types that are also resistant to the cocoa swollen shoot virus disease (CSSVD) and blackpod disease caused by *Phytophthora* species (Adu-Ampomah, 1994). Breeding for genotypes resistant to capsid attack will aid seedling establishment as well as mature tree performance in the field (Padi, 1997, and Adu-Ampomah *et al.*, 1999). It has therefore, become necessary to incorporate into the breeding programme the development of capsid tolerant or resistant cocoa types.

Screening for capsid resistance is however, hampered by unavailability of capsids since they are extremely difficult to rear and maintain in sufficient numbers under laboratory conditions (Raw, 1959; Youdeowei, 1964). Moreover, capsids occur in very low numbers in the field (Cotterell, 1926; Williams, 1954). It is however known

that resistance of cocoa to insect pests appears to be quite general and that cocoa types that are resistant or susceptible to one species of sucking insect tend to exhibit similar attributes to other sucking insects. For example, Bigger (1975) identified cocoa progenies including T85/799 x T17/359 that were susceptible to a range of sucking insects. He indicated that a cocoa variety which is resistant to *Planococcoides njalensis* may show resistance to a range of other sucking insects. The use of the cocoa aphid *Toxoptera aurantii* (Boy) as an indicator of general insect resistance in cocoa was suggested by Campbell (1990) based on the fact that aphids are more conspicuous than mealy bugs on cocoa and could be more convenient to monitor in screening programmes.

The cocoa aphid *T. aurantii* occurs in large numbers in the field (Entwistle, 1972) and could be relatively easier to rear if a suitable rearing material is identified. Thus Eskes *et. al.* (1998) suggested that resistance to aphids may be easier to evaluate than resistance to mirids and could be an indicator of capsid resistance in cocoa.

The present study was, therefore, carried out with the following objectives:

1. To identify suitable breeding material for rearing sufficient numbers of the cocoa aphid *T. aurantii* to be used in screening, for resistance as an index for capsid resistance.
2. To develop a reliable method of aphid infestation/evaluation for very young cocoa seedlings using beans from two types of open pollinated pods, Amazon (T85/799) and Trinitario (Y44) origins.
3. To develop a reliable method of aphid infestation/evaluation for older seedlings and clonal materials using young leaf flushes.
4. To screen four different cocoa progenies to determine their levels of resistance to the aphid.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Cocoa Plant

Cocoa, *Theobroma cacao* Linnaeus (Tiliales: Sterculiaceae) is the only species of the genus *Theobroma* cultivated commercially. The genus contains over twenty species all of which originated from the tropical rainforests of equatorial America (Mossu, 1992). *Theobroma cacao* is classified into two main populations, Criollo and Forastero. The third population, the Trinitario, resulted from natural crosses between Criollo and Forastero types. The Criollo type is indigenous to the north and west of the Andes, whilst the Forastero is indigenous to the Amazon basin (Mossu, 1992). The bulk of cocoa grown in West Africa is Amelonado, a variety of Forastero (Wood, 1975).

Commercial cultivation of cocoa in Ghana began with cocoa pods brought from Fernando Po by a Ghanaian blacksmith, Tetteh Quarshie in 1879 (Hammond, 1962; Hill, 1963, Manu and Tetteh, 1987). However, cocoa is believed to have been introduced earlier by the Basle and Dutch missionaries (Cardinal, 1931; Wanner 1963).

2.2 Economic importance of cocoa

Cocoa has been the backbone of the Ghanaian economy virtually throughout the last century. Ghana was the world's leading cocoa producer between 1910 and 1977 with market shares ranging from 30 to 40% of the world total production (Bateman, 1988). Until recently, cocoa contributed over 60% of the country's foreign exchange (Onosaiye, 1987). In spite of the significant gains made by other sectors of the economy in recent times, cocoa continues to be the major source of revenue for the provision

of socio-economic infrastructure in the country. The total area under cocoa cultivation is currently estimated at 1.2 million hectares and in 1999/2000 cocoa provided 22.5% of the total export earnings (Asenso-Otchere, 2001). The cocoa industry also employs a sizeable percentage of the country's labour force.

In 1965/66 production reached an all-time peak of 560,000 tonnes but this fell to a very low level of 159 tonnes in 1983/84 season resulting in Ghana losing her position to Cote d'Ivoire as the world's largest producer of cocoa (Gill and Dufus, 1989). The ravages caused by insect pests and diseases and the low producer price, among other factors, contributed to the dwindling of the industry (Anon, 1995).

2.3 Cocoa varieties grown in Ghana

The bulk of Ghana's cocoa in the early years of production was the variety "Amelonado" which occupied about 80% of the total acreage. The rest were often referred to as local Trinitario. In many respects the Amelonados and Trinitarios are excellent varieties acceptable to cocoa growers and chocolate manufacturers for their flavour and other bean qualities. They, have however, major defects of slow growth in the early years, which leads to poor establishment under unfavourable conditions, and a long interval between planting and cropping and sensitivity to infection by cocoa swollen shoot virus (CSSV) (Adu-Ampomah *et al.*, 1996). In order to obtain the necessary variation needed for genetic improvement of the crop, Posnette introduced semi-wild types from the headwaters of the Amazon basin called Upper Amazon selections (Anon; 1946). As an interim measure to overcome the susceptibility to CSSV, "Mixed Amazons" were developed through multiplication of the Upper Amazon selections to replace the Amelonados and Trinitarios. Although useful, the level of resistance and tolerance in the Mixed Amazons has been inadequate.

To get superior varieties for farmers, the series II hybrids were later developed

(Thresh *et. al.*, 1988). These were the product of hybridization between selected Upper Amazon clones and selected Amelonado or local Trinitarios. The hybrids are comparable to the Mixed Amazons in many respects including response to CSSV, but they have the additional attributes of maturing early and having higher yield potential (Adu-Ampomah *et. al.*, 1996). Recently, new inter-Amazon hybrids have been released to farmers which are superior to earlier genotypes (Adomako and Adu Ampomah, 2000)

2.4 Insect pest of cocoa

Over 1,500 insect species have been recorded on cocoa but only a few are of economic importance (Entwistle, 1972). In West Africa, the most important pests are the two species of cocoa capsids, *Distantiella theobroma* (Dist) and *Sahlbergella singularis* Hagl (Heteroptera: Miridae) and the mealybug vectors of CSSVD mainly *Planococcoides njalensis* (Laing) and *Planococcus citri* (Risso). Minor pests include the leaf eating caterpillars such as *Earias biplaga*, *Anomis leona* Schaus, the psyllid *Tyora tessmani* (Aulm) and the aphid *Toxoptera aurantii* (Boy), and the pod borer, *Characoma stictigrapta* Hmps. Others are the pod feeding bugs *Bathycolia thalassina* (Heteroptera: Pentatomidae) and *Pseudothraupis devastans* (Dist) (Heteroptera: Coreidae); stem borer *Eulophonotus myrmeleon* Fldr (Lodos, 1967; Owusu-Manu, 1974; Padi *et. al.* 1998); and termites (Ackonor *et. al.*, 1993; Ackonor and Tei, 1995) which have in recent times become economically important in some parts of the country.

2.5 The cocoa mirids

Cocoa mirids (Heteroptera: Miridae) belonging to the subfamily Bryocorinae, are the most harmful insects attacking cocoa trees throughout the world except the Car-

ibbean Islands (Lavabre, 1977).

2.5.1 Mirid species

Mirids are sap-sucking insects which feed on green shoots, chupons and pods. In West Africa the species known to feed on cocoa include *Sahlbergella singularis*, *Distantiella theobroma*, *Bryocoropsis laticollis* and members of the genus *Helopeltis* (Collingwood, 1971). The most important species of mirids which cause most damage to West African cocoa is *Sahlbergella singularis* Hagl which is the dominant species in Nigeria, Cameroun, Togo, and Sierra Leone. In Ghana; *Distantiella theobroma*. (*Distant*) has been the dominant species but, in recent times, *S. singularis* has emerged as dominant species (Padi and Adu-Acheampong, 2001).

2.5.2 Life cycle of mirids

A detailed account of the biology of West African cocoa mirids is available in Cotterell (1926) and Anon (1945, 1947). The tiny white eggs are laid embedded in the cortex of pods and the bark of shoots. The eggs carry two-threadlike filaments which probably aid in breathing (Cotterell, 1943). The maximum number of eggs laid by one female is 47 for *S. singularis*, and 192 for *D. theobroma*. The eggs take 12–18 days to hatch and the nymphs which emerge begin to feed soon after hatching (Hill, 1983). The average incubation period for *S. singularis* is 17 days, and 15 days for *D. theobroma* (Taylor, 1954). There are five nymphal instars, each with a duration of 4–5 days. The average period from egg to adult for *S. singularis* is 41 days, and for *D. theobroma* 39. There is a pre-maturity period for both species of about a week, and the longevity of the adults varies from 25–30 days. The proportion of females is greater than that of males (Raw, 1959).

2.5.3 Alternative Hosts

Mirids have been found on other host plants (Box, 1944; Anon 1947). *S. singularis* has been recorded on 17 alternative hosts including *Berria amonilla*, *Sterculia foetida* and several *Cola* species (*C. nitida* and *C. acuminata*) whilst *D. theobroma* is found on three alternative hosts namely *Citrus* spp, the silk cotton tree, *Ceiba pentandra*, and the baobab tree, *Adansonia digitata* (Entwistle and Youdeowei, 1964; Entwistle, 1972; Padi *et. al.* 1996)

2.5.4 Capsid Damage

Mirid damage usually starts where there is a break in the canopy which occurs from fallen trees, removal of swollen shoot diseased trees or from poor canopy development (Williams, 1953). Canopy breaks attract mirids and may become foci for local population build-up. Thus young cocoa trees having open canopy are more vulnerable to capsid attack.

Capsids feed by inserting their mouthparts about 1.8–2.2 mm deep into the plant tissues. Toxic saliva is injected into the plant tissue after which the cell content is sucked leaving dark patches of drying tissue called feeding lesion. The feeding lesions are initially light brown but soon turn dark brown and eventually black (Goodchild, 1952). Extensive feeding on young pods (cherelles) causes them to wilt but damage to older pods is usually insignificant (Johnson, 1962). Feeding on stems causes wilting above the point of attack resulting in the death of terminal shoots. The damage ensuing from capsid attack is enhanced by the entry of parasitic fungi namely, *Calonectria rigidiuscula* Berk and Br. (Sacc) that spreads through the plant tissues causing die-back (Crowdy, 1947). This eventually results in the death of branches, breaks in canopy, poor yields, and eventually death of the cocoa tree (Aré and Gwyne-Jones, 1974). Yield losses due to capsid damage was estimated at 60,000 to 100,000

tonnes of dry cocoa beans annually (Hale, 1953; Stapley and Hammond, 1959) and may amount to as high as 75% crop loss if attacked farms are left unattended for more than three years (Anon, 1951).

2.5.5 Chemical Control

Earlier attempts at capsid control involved the use of various emulsions and dusts. Dungeon (1910) used kerosene/soap emulsion as stem paint and Cotterel (1943) tested and found nicotine sulphate effective at 0.1 percent. However, the use of nicotine sulphate was discontinued because it was found to be expensive and toxic to mammals. With the advent of motorized mistblowers in the mid 1950s, several conventional insecticides, including dichlorodiphenyl trichloroethane (DDT) and lindane (Gammalin), were introduced for capsid control (Hammond, 1957; Stapley and Hammond, 1959; Owusu-Manu, 1985).

Presently, the recommended method for capsid control involves the use of Gammalin 20 (Lindane) at 280 g a.i in 56 litres of water/ha and Uden 20 propoxur at 210g a.i in 56 litres of water/ha applied at 4-weekly intervals from August to December, omitting November (Padi, 1997). However, chemical control is plagued with several user problems and environmental hazards. These include the development of pest resistance as occurred with lindane in the early 1960's (Dunn, 1963; Marchart and Collingwood, 1969), and the destruction of beneficial natural enemies resulting in minor pests assuming major pest status as happened with *Bathycoelia thalassina* (Owusu Manu, 1974). Another problem related to the use of insecticides is the high cost of inputs such as spraying machines, chemicals and labour. Moreover, the spraying procedure is very tedious and, as pointed out by Adomako (1990), the cocoa farmer finds it difficult to support 25 kg of insecticide solution on his back during spraying. Due to these constraints, the adoption rate by farmers of the current

recommendations had been very low as evidenced by recent surveys (Donkor *et. al.*, 1991; Henderson *et. al.*, 1994; Padi *et. al.*, 2000a).

2.6 Non-chemical Control Measures

The growing universal concerns against the use of conventional insecticides has created the need to look for alternative measures that are environmentally friendly and less risky to users and consumers.

According to Padi (1997), CRIG has embarked on the development of the following alternative control methods:

- (i) The use of semio-chemicals including sex pheromones.
- (ii) Biological control with natural enemies (parasitoids, predators and pathogenic fungi)
- (iii) Cultural practices involving the use of suitable shade trees, shade manipulation and the avoidance/destruction of alternative capsid host plants such as *Ceiba petandra*, *Citrus* spp, *Cola* spp, and *Adansonia digitata*.
- (iv) The use of tolerant/resistant cocoa types.

2.6.1 Biological Control

Studies in West Africa (Lodos, 1968; Entwistle 1972, Kumah 1975, 1976) have shown that developmental stages, including the egg stage of both *D. theobroma* and *S. singularis*, are affected by parasitic organisms which are either hymenopterous parasitoids or nematodes. Percentage parasitism has been generally lower in *D. theobroma* (about 4%) and higher in 4th instar nymphs of *S. singularis* (about 20–59.5%) (King 1971). In West Africa, mirid predators recorded are several species of ants including *Oecophylla longinoda* and *Macromischoides aculeatus* Mayr, salticid

spiders, reduviids, mantids, Grillidae and Pentatomidae, but most of these are facultative parasites and their predatory potential remains to be exploited.

Pathogenic fungi which attack capsids include fungi imperfecti, *Beauveria bassiana*, *Bacillus* and *Aspergillus* species (Anon, 1970, Lim Tong *et. al.* 1989, Collingwood 1971). Recent findings by Padi *et. al.* (2000 b) indicate that five *Beauveria* isolates gave promising results against *S. singularis*.

2.6.2 Shade Manipulation

Break in canopy increases light penetration and leads to emergence of young succulent chupons and mirids are attracted to such spots which become foci for local population build-up. Shade manipulation, therefore, becomes vital and should be part of an integrated approach to mirid control. Collingwood (1971) indicated that well canopied cocoa is largely self protecting against serious capsid damage because of shade and high humidity within the canopy which restricts the development of large population compared with more exposed cocoa with canopy breaks.

Owusu-Manu (1997) has demonstrated that on mature cocoa with severe capsid damage and broken canopy at least two continuous years of 4 insecticide applications per year are required to restore the canopy. Where the cocoa canopy has already closed, 2 sprays per year are adequate. However, since excessive shade favours blackpod disease and suppresses yield, it is important to properly manage it.

2.6.3 Host plant resistance in insect management

The development of resistant varieties usually takes a long period of experimentation. However, results already obtained indicate that the possibility of using plant resistance to insects deserve careful study for each major insect pest and many minor

ones (Painter, 1951). The primary objective of programmes on insect resistance in crop plants is to develop cultivars that are resistant to an insect pest while maintaining or improving their basic agronomic characteristics. Resistant varieties fall roughly into three groups namely (i) as the principal control method (ii) as an adjunct to other measures and (iii) as a safeguard against the release of more susceptible varieties than exist at the present time. Luginbill (1969) emphasised the importance of resistant varieties in pest management and the millions of dollars saved by growers. Metcalf and Luckman (1982) pointed out that the most desirable features of plant resistance, among others are:

1. *Specificity*: Plant resistance is usually specific to a pest or complex of pest organisms and seldom has direct detrimental effect on beneficial insects.
2. *Persistence*: Most resistant varieties maintain high levels of resistance for a long time despite the occasional upsurge of biotypes.
3. *Harmony with the environment*: Since no unnatural elements are used, there is no likelihood of contaminating the environment or endangering man or wild life.
4. *Ease of adoption*: Once developed, resistant varieties can easily be incorporated into normal farm operations at little or no extra cost and
5. *Compatibility*: Plant resistant is compatible with other tactics in pest management, being an ideal adjuvant when resistance alone cannot maintain a pest below the economic threshold.

2.7 Developing pest and disease tolerant /resistant varieties of cocoa

Bigger (1974) indicated that it would be preferable to avoid the necessity of spraying cocoa with insecticides by the use of resistant varieties. However, according to Eskes and Lanaud (1996) cocoa cultivation is still largely based on traditional varieties

domesticated more than 150 years ago and that less than one third consists of hybrid and clonal cultivars developed by breeding programmes initiated since the 1940's, first in Trinidad and subsequently in Ghana, Brazil and other major cocoa producing countries.

In Java, farmers planted more tolerant hybrids of Djale Roengo Criollo cross Trinitario against *Helopeltis* attack. Destroyed shoots of these hybrids are rapidly regenerated to prevent break in canopy (Hall, 1932). In West Africa SCI trees were identified among attacked trees at Asuansi in 1941 as being tolerant to capsid attack. This led to the selection of 54 cocoa clones on the basis of their resistance to capsids (Posnette 1946). Collingwood and Marchart (1971), in assessing capsid damage to the control plots of a systemic insecticide trial planted to five cocoa progenies, concluded that the enormous differences, which could be shown between progenies in canopy damage, the tree deaths, were not due to differential attack by the capsid, but to the ability of the progeny to recover from the attack. The more vigorous Amazon crosses recovered more readily than Amelonado, and the Amelonado X Amazon cross was intermediate in response. Burle (1953) has reported resistance of particular trees, to attack by *Sahlbergella singularis* and *Distantiella theobroma* in the Ivory Coast, and Coolhouse (1939) reported resistance to *Helopeltis* in Indonesia. Subsequent work in Ivory Coast showed a ten-fold difference in mean number of capsids recorded over two season between UPA 620 which had least and C409 which had most capsids. C5 and the hybrids tested were intermediate in response. A study by Bigger (1972) showed *Distantiella* to be more prevalent on series IIB hybrids and indicated that series II hybrids seemed more susceptible to attack by a range of sucking insects and their attendant ants than T85/799 x T17/359.

With regards to cocoa disease resistance, Adu Ampomah *et. al.* (1996) have indicated that due to improvement in the screening procedures coupled with large



scale introductions, some indications of sources of resistance to CSSVD have been identified especially among Pound's Upper Amazon collection. Some progress has also been made in efforts to breed for cocoa types resistant to the black pod disease caused by *Phytophthora palmivora*. T79/501 and Pa7/808 have been selected as potential resistant parents and these have been planted in the seed gardens (Abdul-Karimu *et. al.*, 1996). In the case of *P. megakarya* some forty trees showing low levels of infection have been identified. These materials are being evaluated to determine the nature of resistance.

2.8 The cocoa aphid

Seven, or possibly eight species of aphids are known to attack cocoa but, all except *Toxoptera aurantii* (Boyer de Fonscolombe), are of rather casual occurrence and have other more important and usual host plants (Entwistle, 1972). *T. aurantii* occurs in all the cocoa growing areas of the world and is quite common on cocoa in Ghana (Eastop, 1961).

A brief account of the biology of *T. aurantii* on cocoa has been given in Cote d'Ivoire by Alibert (1951) and in the British West Indies by Kirkpatrick (1955). Firempong (1975) worked on the biology of the aphid in Ghana. Apterar individuals are small, oval, brown-black or black with black-and-white banded antennae and black siphunculi and cauda. Alate individuals have a dark brown to black abdomen. On cocoa, the aphid is found on young flush leaves of apical shoots, on chupons, flower stalks and cherelles. Its seasonal occurrence is closely tied up with the flushing cycle of cocoa.

According to Firempong (1975), *T. aurantii* takes 6 days to become adult on cocoa seedlings and the temperature range most suited to its life function is 20°C – 25°C; with 22°C as the optimum temperature. Populations of the aphid comprise

only females, reproduction being entirely parthenogenetic and the young produced viviparously. Broughton and Harris (1971) analysed the sound produced by *T. aurantii* which is the only aphid with audible stridulation. It attacks flower clusters causing abortion of the flowers and damages young leaves (Ingram, 1964; Gowdey, 1913). He considered the pest important enough to mention it annually in his report in Uganda. Saunders (1979) reported that heavily attacked leaves become cupped or rolled and flower development may be arrested. It's pest status is most apparent in the nursery where it may severely retard the growth of growing plants if not controlled. Firempong (1975) indicated that severe attack on young cocoa causes crinkling in leaves. In addition to leaf crinkling; premature leaf fall, flower wilt, withering of young stems and etiolation of affected plants may occur (Alibert, 1951). In Peru it has been established as a pest of Cocoa (Wille, 1944) but in Ghana and other W. African cocoa growing countries, *T. aurantii* has always remained a pest of minor importance on cocoa.

· Due to their small size, parthenogenetic reproduction, high capacity for multiplication and world-wide distribution, aphids are ideal for studying many of the topical issues in ecology and plant breeding (Dixon, 1985; Maxwell and Jennings, 1980).

CHAPTER THREE

STUDY AREA

3.1 Locality

The study was conducted at the Cocoa Research Institute of Ghana (C.R.I.G.) formally known as the West Africa Cocoa Research Institute(WACRI)

CRIG is located at New Tafo-Akim (Latitude 6° 17'N and Longitude 0° 22'E) about 40 km (24 miles) from Koforidua, the capital of Eastern Region, and 107 km (67 miles) away from Accra at an altitude of 220 metres above sea level (Fig.1).

New Tafo is located southwest of the Mampong Scarp on the Togo hills within the equatorial climatic Zone (Udo, 1978). Keay (1959), giving it a broad classification, puts it in the moist forest zone at low and medium altitude whilst Taylor (1952) locates it within the celtis – Triplochiton sub-division of the moist semi-deciduous forest.

3.2 The Climate

The rainfall pattern shows two wet seasons (March to July and September to November) with double maxima occurring in May/June and October (Church, 1957; Boateng, 1960). There are two peaks for the mean monthly temperature occurring from February to May and November to December and the minima occurring in July/August.

The average monthly relative humidity has a single peak occurring from July to October. The average monthly rainfall for the year 2000 was 127.0 mm, higher than that of the past ten years which averaged 119.3 mm (Fig 2a). Daily maximum and

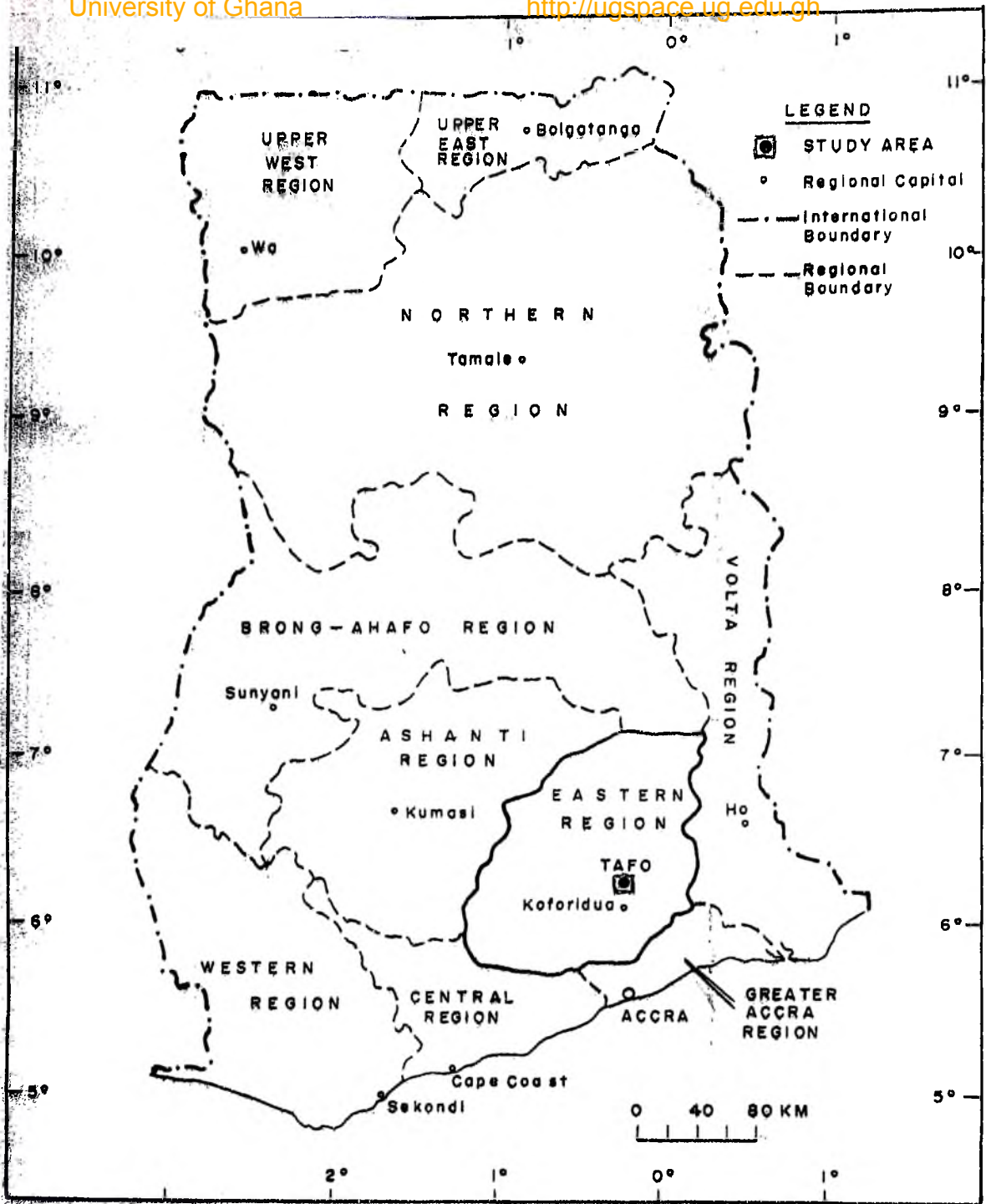


Fig. 1 Map of Ghana showing position of the study area (Tafo)

minimum temperature, ranged between 18.9 and 34.7°C in the year 2000 compared to 19.5 and 34.7°C, the average for the past ten years (Appendix 1). The relative humidity averaged 74.9% and 75.8% for the year 2000 and the past ten years, respectively (Fig. 2b).

The harmattan weather which begins from November to mid-February is characterized by dry and hazy conditions, with relative humidity sometimes as low as 40% and with corresponding low night and morning temperatures.

Tafo experiences four basic seasons with two repetitions (Gibbs and Leston, 1970). The 'dry sunny season' in November to mid-February is followed by two 'Wet-sunny season' in late February to late May and late October to mid-November. Two 'wet dull seasons' occur between June/July and late August to Mid-October. The dry dull season' occur between the period mid-July to late September. The limits of the above seasons are not precise since great yearly variations in both rainfall and sunshine do occur.

3.3 The Insectary

All study activities were carried out in the insectary of the Entomology Division of CRIG located a few metres behind the block which houses the offices and laboratories of the Division. Temperature and relative humidity at the insectary during the study period fluctuated between $24 \pm 3^\circ\text{C}$ and 72–85%, respectively.

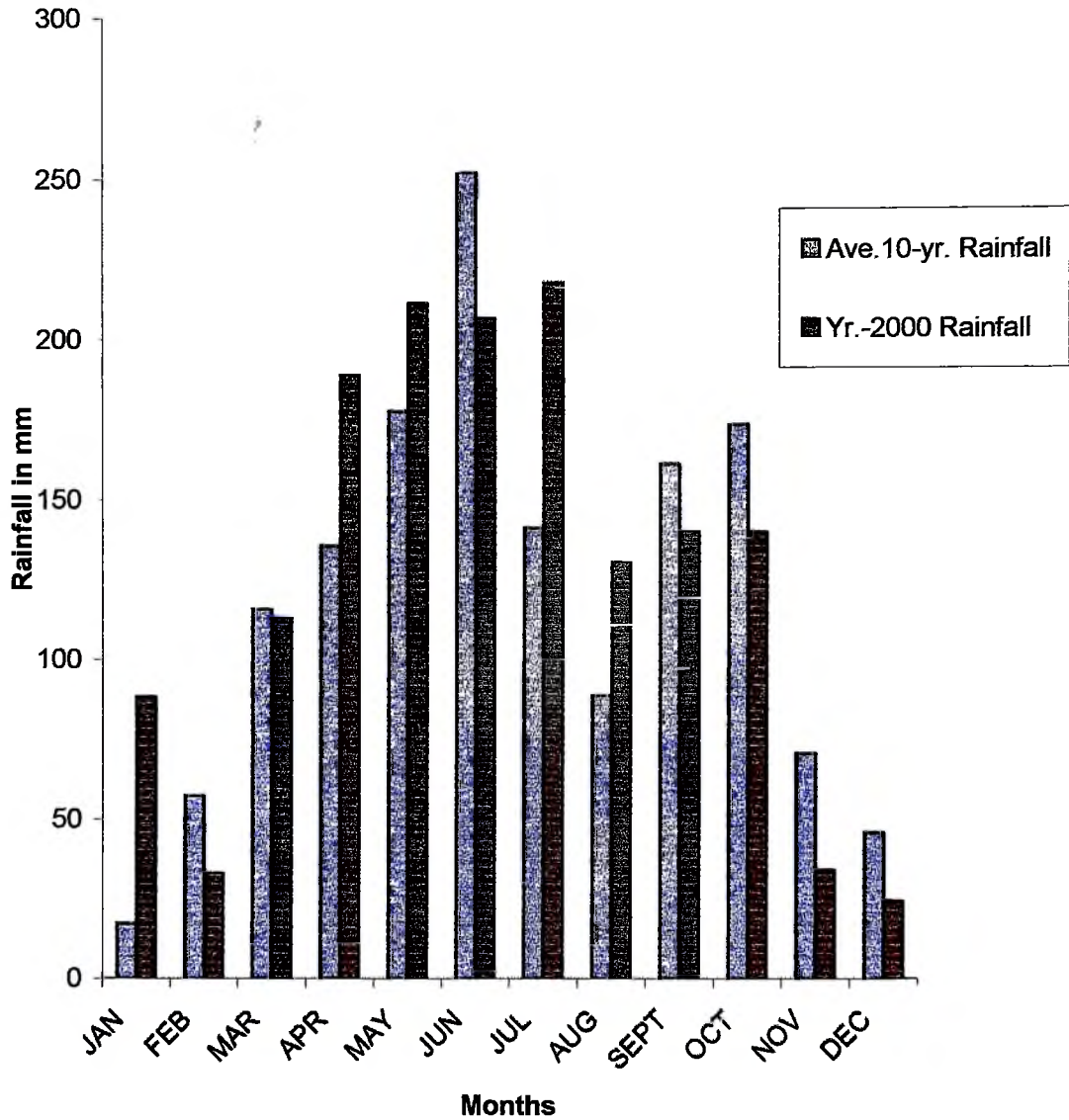


Fig2a: Mean monthly rainfall for ten years 1990-1999 and the year 2000

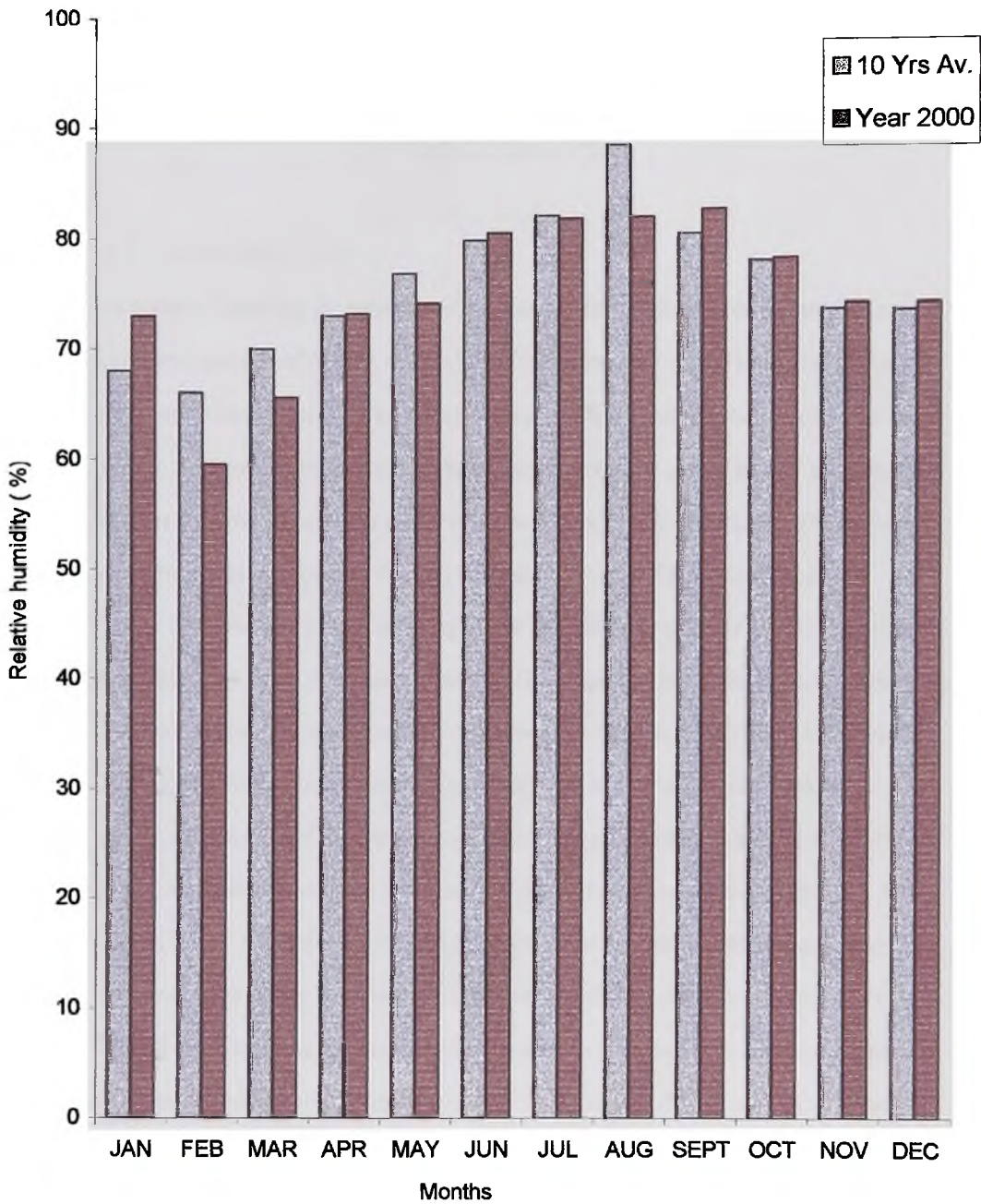


Fig.2b: Mean monthly relative humidity for ten years(1990-1999) and the year 2000 for Tafo

CHAPTER FOUR

DEVELOPMENT OF LABORATORY REARING METHOD FOR
THE COCOA APHID

4.1 INTRODUCTION

Success in breeding for resistance to insect requires efficient rearing methods. The principal sources of insects for studies of plant resistance are field populations and laboratory (or glasshouse) colonies (Tingey, 1986). Insects can be reared in the laboratory, both on artificial media or host plants. Artificial media lead to more refined studies for basic research but according to Maxwell and Jennings (1980) naturally occurring hosts are better selectors for insect biotypes if they should occur.

In the laboratory, insect rearing can be done from single pair matings or by mass rearing. When using mass rearing methods homogenous populations can be obtained if plants having specific genes for resistance are used as host plants. Maxwell and Jennings (1980) reported that homogenous populations can be used to determine the genetics and nature of resistance in the plant. The possibility of using non-target host plants for rearing insects for resistance studies has been reported (Jermy *et. al.* 1968, Smith, 1978). According to Panda (1979) due to techniques in rearing large numbers of insect pests in the laboratory, it has been possible to produce insect infestations needed for evaluating the pest resistance qualities of many crops. It has also made it feasible to achieve reliable test results at different stages of plant growth by screening a large number of lines in a short period and in all seasons.

This chapter reports on investigation into the possibility of using selected vegetables, legumes and tree crops in rearing the cocoa aphid. The seven different

legumes and four vegetables selected are known to be alternative host plants for aphids, though not specifically for *Toxoptera aurantii* (Boy). The six perennials tested are known to be alternative host plants for the cocoa aphid. In all experiments cocoa seedlings were used as control. Also investigated was how long the aphid could stay without food (starvation test) and the preference of the aphid to the different test crops.

4.2 MATERIALS AND METHODS

4.2.1 Rearing of the aphid on leguminous and vegetable plants.

The legumes used were cowpea (*Vigna unguiculata*), broad bean (*Vicia faba*), pigeon pea (*Cajanus cajan*), groundnut (*Arachis hypogaea*), green gram (*Phaseolus aureus*), *Glyricidia sepium* and winged bean (*Psophocarpus tetragonolobus*). The seedlings were raised in black polythene bags (measuring 18 cm by 25 cm) in the insectary.

Four seedlings of each legume and cocoa (Y44) were infested 3, 5, 7 and 10 days after cotyledon openings with *T. aurantii* collected from cocoa plots at the Cocoa Research Institute. Five (5) adult apterous aphids were introduced onto each seedling using a soft camel hair brush and kept in wooden/wire mesh cages measuring (70 x 45 x 45 cm) at the insectary (Plate 1). A hinged door permitted easy access to the seedlings for watering and assessment of aphid numbers. Temperature and relative humidity at the insectary during the study period fluctuated between $24 \pm 3^\circ\text{C}$ and 72–85% respectively.

The vegetables tested included tomato (*Lycopersicon esculentum*), eggplant (*Solanum melongena*), sweet pepper (*Capsicum frutescence*) and okro (*Hibiscus esculentus*). The seedlings were infested 3, 5, 7 and 10 days after germination using the same method described above for the legumes. Each treatment was



Plate 1. Cages used in aphid rearing.

replicated four times in a Completely Randomised Design. Following preliminary investigations which showed that the aphids could survive for ten hours without food (Fig 3, Appendix 2) the two rearing experiments described above were repeated using aphids starved for eight hours. This was to investigate whether starvation would encourage the aphids to feed on any of the crops tested. The seedlings were inspected daily with the aid of a hand lens for a period of two weeks to observe aphid reproduction and multiplication. The number of aphids found on each plant was recorded daily.

4.2.2 Rearing of the aphid on tree crops

The tree crops used included three varieties of *Citrus* namely Late valencia, Washington navel and Mediterranean sweet, *Coffea canephora*, *Cola nitida* and cocoa (*Theobroma cacao*). Eight weeks old seedlings were used. There were four seedlings in a treatment, with each treatment replicated four times in a Completely Randomised Design. Each seedling was infested with ten aphids and the aphids allowed to multiply. All the seedlings were kept in cages at the insectary. The number of aphids were counted daily for two weeks.

4.2.3 Feeding preference test.

4.2.3.1 Leaf disc test on feeding preference

The feeding response of the aphid was determined in the laboratory by using leaf disc (1.7 cm diameter) punched with a cork borer from fresh leaves of each of the leguminous and vegetable plants. The vegetables and legumes used were the same as those used in experiments 1 and 2. . Cocoa (Y44) was used as control.

The leaf discs were placed on moist filter paper in a petri-dish (Plate 2). Each dish was infested with five adult apterous aphids starved for eight hours and left at

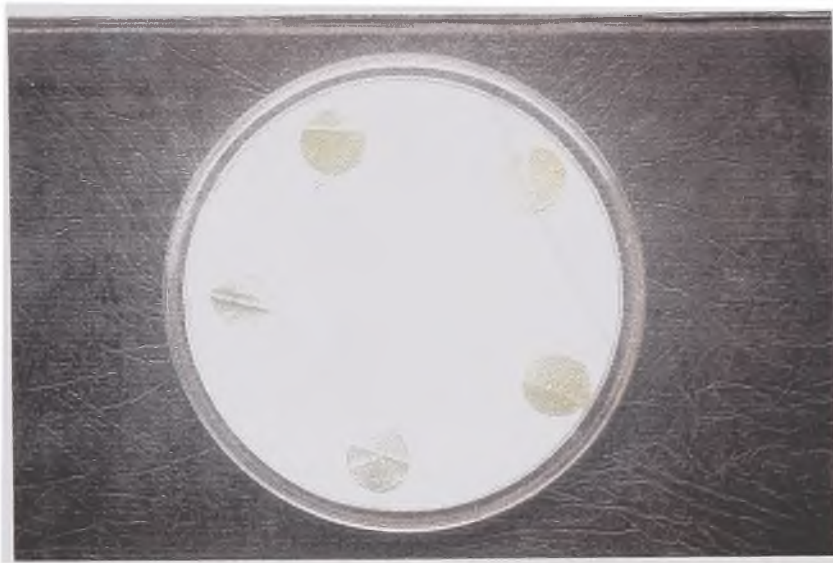


Plate 2. Arrangement of leaf discs in preference test.

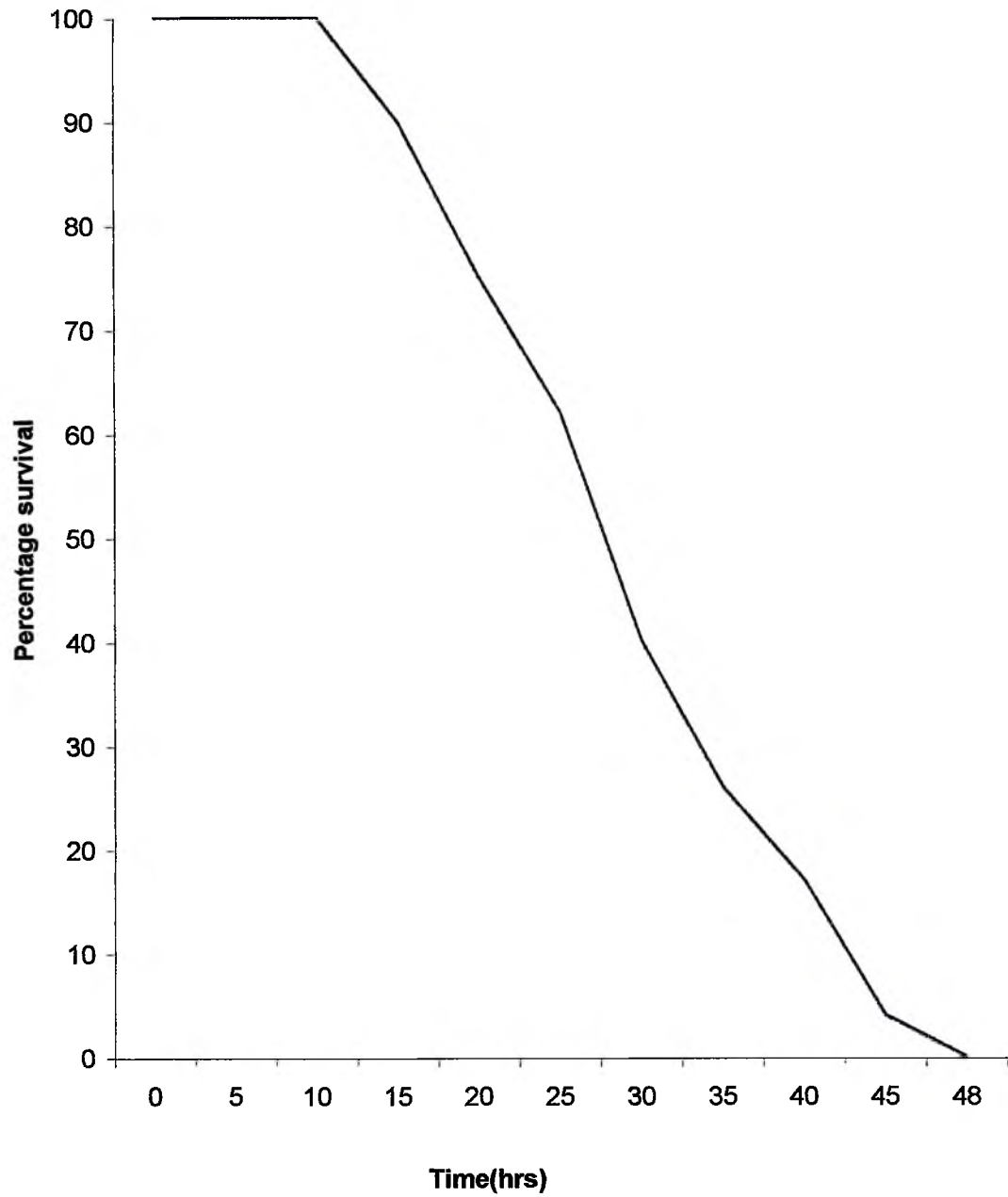


Fig.3:Survival of aphids under starvation with time.

room temperature in the laboratory. There were four replicates. The aphids were observed closely to monitor their movement pattern, preferences and where they finally settled or died.

4.2.3.2 Investigation under field conditions to determine aphid survival on leguminous and vegetable plants

Young seedlings of the different test legumes and vegetables were arranged around the base of cocoa trees close to heavily aphid infested basal chupons (vegetative shoots arising from the base of the main stem) and observed daily for two weeks for signs of aphid infestation.

In another experiment, young seedlings of the test crops were artificially heavily infested with cocoa aphids of all developmental stages using soft camel hair brush and placed under cocoa trees just behind the insectary. The seedlings were observed daily for two weeks.

4.2.4 Statistical analysis

For all the experiments, where aphids survived and multiplied, aphid counts were transformed using logarithm transformation ($\log x$) to meet analysis of variance (ANOVA) assumptions of normality. Analysis of variance was carried on the transformed data using MINITAB and differences in means separated with the Least Significant Differences (LSD) test.

4.3 RESULTS

4.3.1 Survival of aphids on the leguminous and vegetable plants

The performance of the cocoa aphid *T. aurantii* on the seven different leguminous and four vegetable plants is presented in Tables 1 and 2, respectively.

Table 1
Daily survival of aphids on seedlings of different leguminous plants infested 3, 5, 7, and 10 days after cotyledon opening

Legume	Day of infestation	Number of aphids on day :						
		0	1	2	3	4	5	6
Cowpea	3	5	3	1	0	0	0	0
	5	5	3	1	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	2	0	0	0	0	0
Broad bean	3	5	4	2	2	0	0	0
	5	5	3	2	0	0	0	0
	7	5	3	1	0	0	0	0
	10	5	2	1	0	0	0	0
Groundnut	3	5	5	4	2	1	1	0
	5	5	4	3	1	0	0	0
	7	5	3	1	0	0	0	0
	10	5	2	1	0	0	0	0
Green gram	3	5	2	1	0	0	0	0
	5	5	2	0	0	0	0	0
	7	5	1	0	0	0	0	0
	10	5	1	0	0	0	0	0
Pigeon pea	3	5	3	2	0	0	0	0
	5	5	3	1	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	0	0	0	0	0	0
Glyricidia sp	3	5	3	1	0	0	0	0
	5	5	3	1	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	0	0	0	0	0	0
Winged bean	3	5	2	1	0	0	0	0
	5	5	2	0	0	0	0	0
	7	5	1	0	0	0	0	0
	10	5	1	0	0	0	0	0

Table 2

Daily survival of aphids on seedlings of different vegetable plants infested 3, 5, 7, and 10 days after germination

Vegetable	Day of infestation	Number of aphids on day :				
		0	1	2	3	4
Tomato	3	5	4	2	2	0
	5	5	3	1	0	0
	7	5	2	1	0	0
	10	5	2	0	0	0
Sweet pepper	3	5	2	1	0	0
	5	5	2	0	0	0
	7	5	1	0	0	0
	10	5	0	0	0	0
Okro	3	5	3	2	1	0
	5	5	3	2	0	0
	7	5	2	0	0	0
	10	5	2	0	0	0
Garden eggs	3	5	3	1	0	0
	5	5	2	1	0	0
	7	5	2	0	0	0
	10	5	0	0	0	0

With the exception of groundnut all the five aphids on the legumes died three days after infestation and none of them reproduced. On the groundnut, one aphid survived till the 5th day. Similar results were observed on the vegetable plants with all the aphids dying before the 4th day (Table 2). The aphids survived longer on the seedlings

infested three days after cotyledon opening or germination, of the legumes and vegetables respectively. On seedlings infested ten days after cotyledon opening or germination the aphids survived for only one or two days.

Similar results were obtained when the aphids were starved for eight hours before transferring them onto the test plants (Appendices 3 and 4)

The performance of the aphids on young cocoa seedlings used as control is summarised in Fig. 4a. The aphids survived and multiplied on the young cocoa seedlings. The highest number of aphids were recorded on seedling infested five days after cotyledon opening, followed in that order by seedlings infested 3 days, 7 days and 10 days after cotyledon opening (Fig. 4a). As many as 68 aphids/seedling were recorded on seedlings infested 5 days after cotyledon opening compared to 34 aphids representing the lowest recorded on seedlings infested 10 days after cotyledon opening. In all cases the aphids were found at the undersurface of the young tender leaves.

The pattern of distribution appears to suggest differences in effect of the various ages of seedlings on aphid development. Analysis of variance to examine the effect of age (4 levels) of cocoa seedlings on the development of aphids is presented in Appendix 5 and Table 3.

Table 3
Mean number of aphids produced on cocoa seedlings infested at different periods after cotyledon opening

Infestation days after cotyledon opening	Mean No of aphids/seedling after two weeks
3	58 (1.71) ab
5	65 (1.81) a
7	48 (1.68) b
10	34 (1.53) c

Data represent mean of 4 replicates. Transformed means in brackets. Means in column followed by the same letter are not significantly different at (P=0.05).

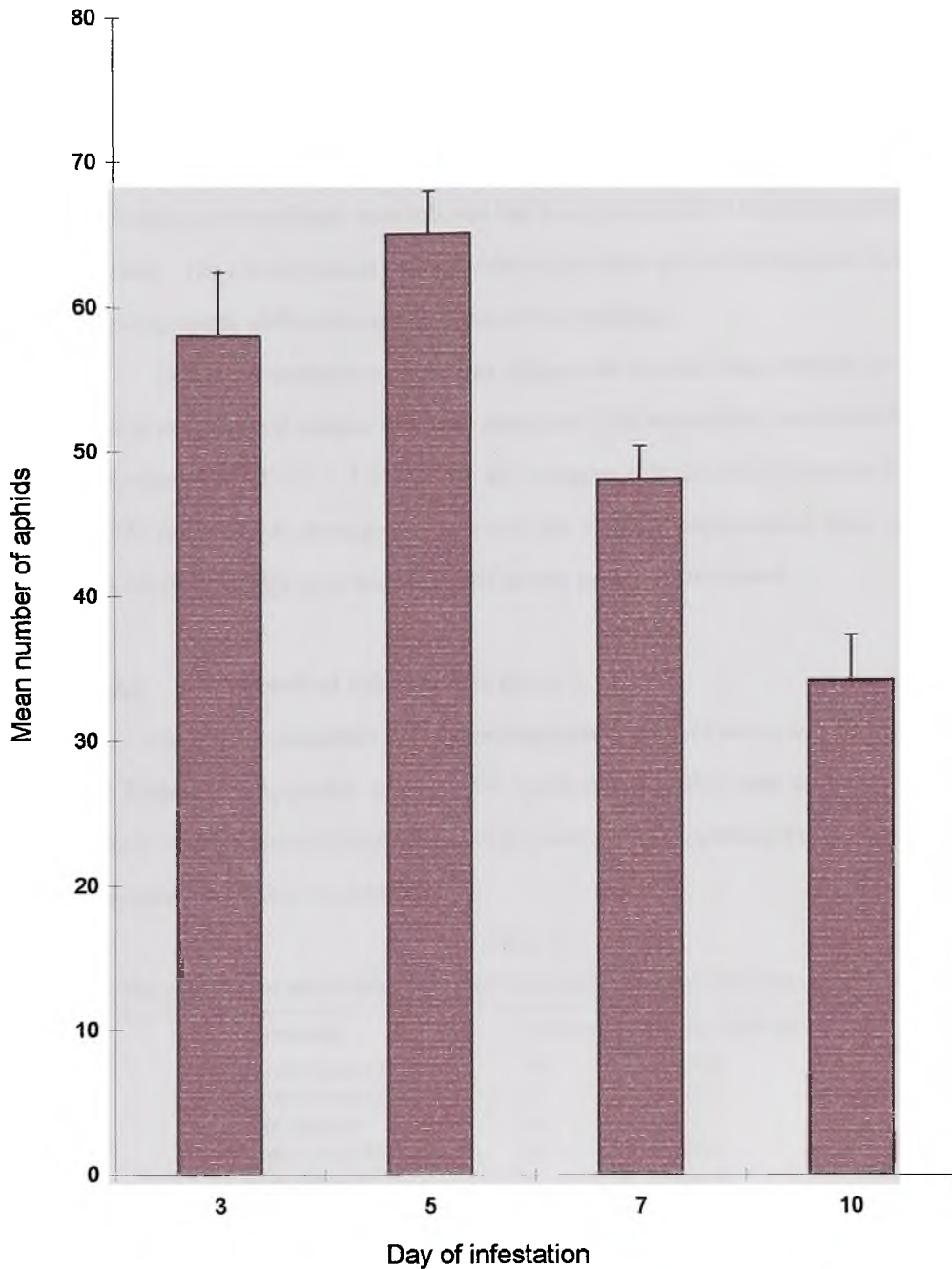


Fig. 4a: Mean number of aphids produced after two weeks on cocoa seedlings infested at four different ages (3, 5, 7 and 10 days) after cotyledon opening. (Data are means of 4 replicates)

There were no significant differences in the number of aphid produced on seedlings infested 3 and 5 days after cotyledon opening. However, aphid numbers produced on seedlings infested 3, 7 and 10 days after cotyledon opening were significantly different ($P < 0.05$) (Appendix 5). The overall results suggest that the age at which cocoa seedlings were infested had a significant effect on aphid population growth. The results present strong evidence that there are real differences in aphid developmental ability between the ages of the seedlings.

Detailed examination of the data suggests an inverse linear relation between age of seedling and number of aphids produced. This relationship, described by the equation $Y = -0.0307x + 1.8747$ (Fig. 4b) suggests that for a daily increase in age, 1.073 fewer aphids were produced within the limits of experimental data. As the leaves of seedlings aged the number of aphids produced decreased.

4.3.2 Survival of aphid on tree crops

The effect of six perennials on the developmental ability of cocoa aphids is shown in Table 4 and Appendix 6. Generally, aphid multiplication rates differed significantly on the different perennials ($P < 0.01$) with cocoa supporting the highest aphid population and cola the lowest.

Table 4

Mean number of aphids produced on different perennial after two weeks of infestation

Perennials	Mean number of aphids/seedling	
<i>Theobroma cacao</i>	107	(2.03) a
Mediterranean Sweet	50	(1.69) b
Late valencia	24	(1.38) c
<i>Coffea canephora</i>	18	(1.25) c
Washington navel	4	(0.69) d
<i>Cola nitida</i>	0	(0.00) e

Data represent mean of 4 replicates. Transformed means in brackets. Means in column followed with the same letter are not significantly different at ($P=0.05$) (LSD = 0.250).

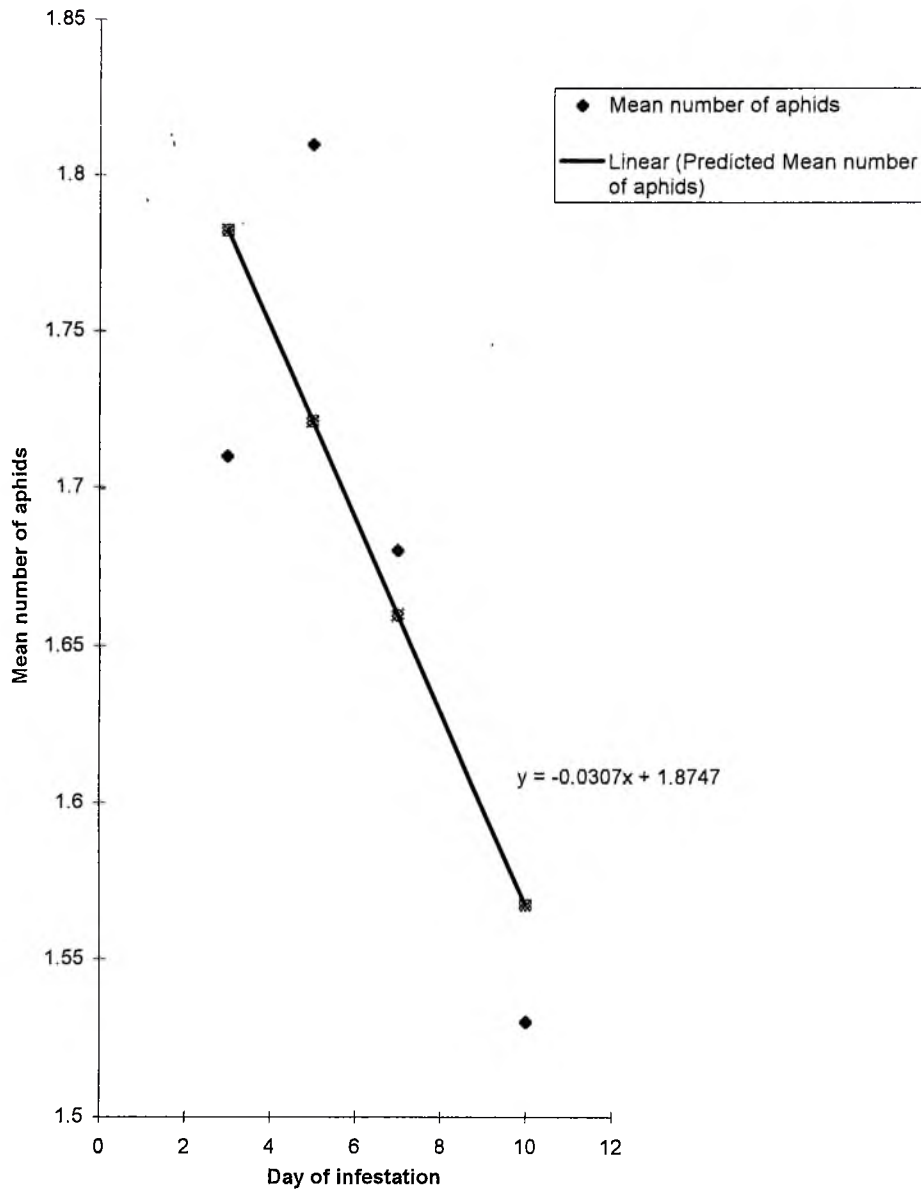


Fig. 4b: Relationship between age of cocoa seedling and the mean number of aphids produced

All aphids on the cola seedlings died four days after infestation. The Mediterranean sweet orange was the best host among the three citrus varieties.

On the basis of the number of live nymphs produced, cocoa was the best preferred, followed by Mediterranean sweet, Late valencia, coffee, Washington navel and cola in decreasing order (Fig.5).

4.3.3 Feeding Preference Test

4.3.3.1 *The Leaf disc method*

Result from the leaf disc preference test is summarized in Tables 5 and 6 below.

Table 5

Distribution of aphids on leaf discs of different vegetable plants

Crop	No of aphids settled				Total	Distribution (%)
	R1	R2	R3	R4		
Tomato	1	1	1	—	3	15
Sweet pepper	—	1	1	1	3	15
Garden eggs	—	1	1	—	2	10
Okro	2	—	1	1	4	20
Cocoa (control)	1	—	1	2	4	20
*Outside	1	2	—	1	4	20

Table 6

Distribution of aphids on leaf discs of different legumes

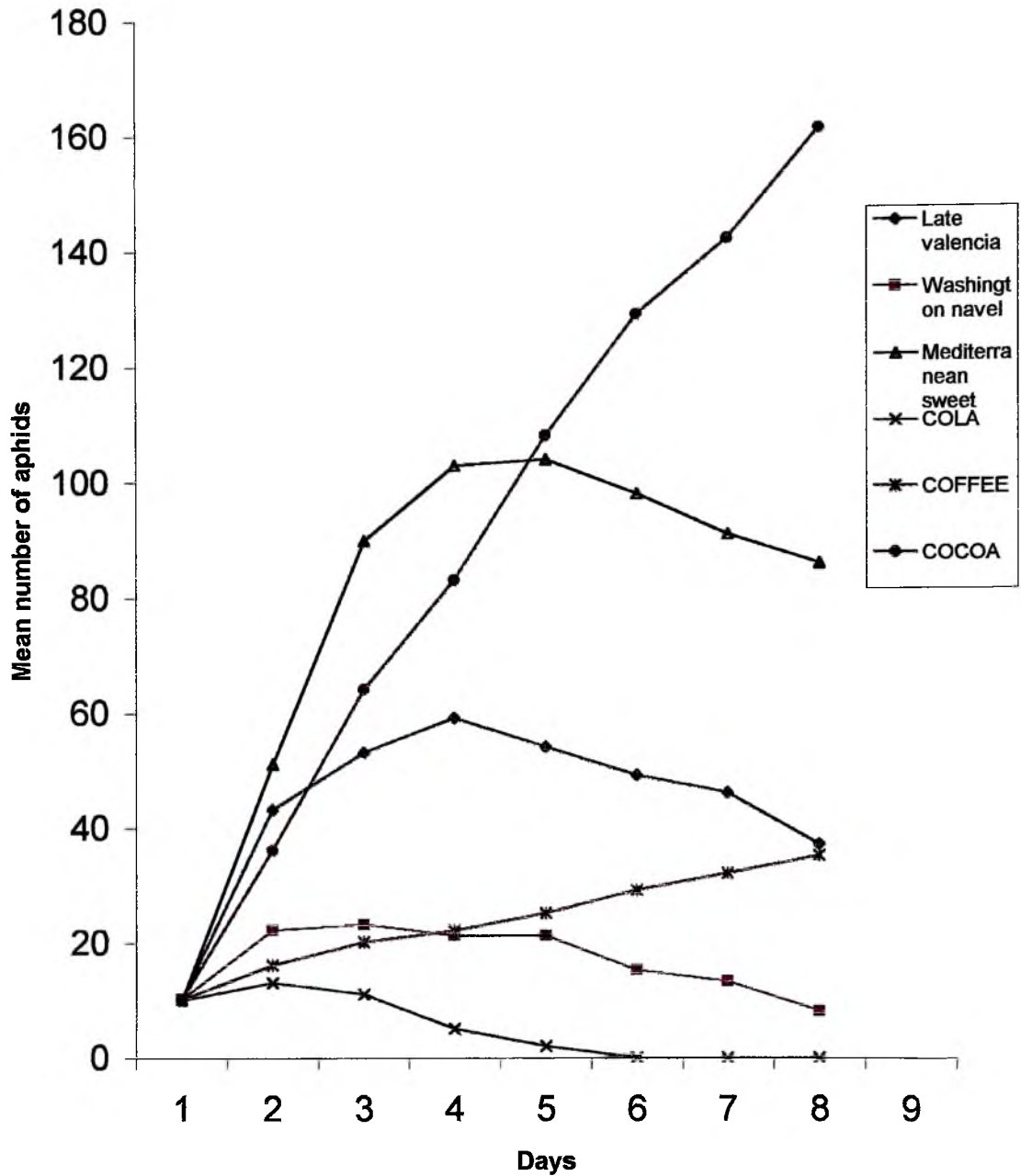


Fig. 5: Mean number of aphids produced on 6 different perennials infested with one adult aphid for seven days.

Crop	No. of aphids settled				Total	Distribution (%)
	Replicates	R1	R2	R3		
Groundnut	1	—	1	1	3	15
Cow pea	1	1	1	—	3	15
Broad bean	1	—	2	—	3	15
Green gram	—	1	—	1	2	10
Pigeon pea	—	1	—	1	2	10
Cocoa (control)	1	2	—	1	4	20
*Outside	1	—	1	1	3	15

*Outside represents aphids that settled outside leaf disc in petri dish.

The aphids did not move directly or specifically to any test plant but wandered from one leaf disc to another until they finally settled or died on a leaf disc or outside the disc. Twenty (20%) each of aphids finally settled and died on cocoa, okro or outside the disc; 15% settled on tomato and sweet pepper and 10% on garden eggs (Table 5). Thus the probability of locating and feeding on cocoa and okro was 0.2; 0.15 for tomato and sweet pepper and 0.1 for garden eggs.

With the legumes (Table 6) 20% each of the aphids settled and died on cocoa, 15% on groundnut; cowpea, broad bean and outside the disc whilst 10% settled on green gram and pigeon pea. Thus the probability of locating and feeding was highest on cocoa and least on green gram and pigeon pea.

4.3.3.2 Survival of aphids on leguminous and vegetable plants under semi field conditions

None of the test seedlings placed around the heavily infested cocoa trees got infested with the aphids. Aphids did not move from the cocoa chupons to the different test crops. On the other hand, the young cocoa seedlings were heavily infested throughout the period of observation.

With the infested vegetable and legume seedlings placed under the cocoa trees, all the aphids disappeared from the test seedlings within 5 days after their introduction. There was no sign that the aphids died since no dead bodies were found on any of the seedlings. The cocoa seedlings, on the other hand, continued to harbour the aphids throughout the observation period.

4.3.3.3 *Observation on the biology of T. aurantii*

Observations made on 50 individuals showed that *T. aurantii* is viviparous. The young stages differed from the adult only in size and the number of segments and length of the antennae. There were 4 nymphal instars and the mean duration for total nymphal development in the insectary was 6 days. The duration of the various instar stages are presented in Table 7.

Table 7

Nymphal developmental periods of *Toxoptera aurantii* on cocoa under insectary condition

(Mean of 50 individuals)

Stage	Duration (Days)
1st Instar	1
2nd Instar	2
3rd Instar	2
4th Instar	1
Total	6

Generally, reproduction by the mature adult started on or after the 6th day.

4.4 DISCUSSION

Results from the study have shown that all the seven leguminous and four vegetable plants tested were not suitable for raising cultures of the cocoa aphid, *Toxoptera aurantii*. This perhaps might be attributed to the lack of suitable and adequate nutritional composition of the plants. Other contributory factors might be anatomical or physiological characteristics of the plant. According to Painter (1951) the first stage in host-pest relationship at which resistance may find expression is in regard to insect oviposition or reproduction. Moreover, insects seldom oviposit indiscriminately over the plant surface. Instead, they characteristically deposit their eggs or give birth on selected plant parts (Panda, 1979). Thus, the legumes and vegetables probably failed to provide the appropriate reproduction inducing stimuli. The sites selected sometimes vary according to the stage of the plant development particularly with regard to leaf maturity and the presence or absence of reproductive parts (Blais 1952; Miller and Hibbs, 1963).

The fact that the leaves of the legumes and vegetables appeared more hairy than those of cocoa might also have been a deterrent factor as previously observed for the cotton jassid, *Empoasca* spp in Africa (Parnell *et. al.*, 1949) and among soybean and potato species (Genung and Green, 1962). They explained that the hairs initially arrest locomotion and with further sticky accumulation eventually traps aphids to the plant so that they are immobilized and starved to death. Plants with glandular hairs are thus promising candidates for pest resistance (Gibson, 1971).

In addition to providing a suitable ovipositional medium, the host plant must also serve as an adequate nutritional substrate requisite for the larval and adult insect survival through feeding. Resistance involving nutritional aspects may be expressed either by the lack of nutritional support or the presence of feeding deterrents in the host plant. Several chemical constituents of plants that serve as olfactory and

gustatory stimuli have been reported to differ qualitatively and quantitatively in different plants. They may be nutrient, for example, sugars, amino acids or no nutritive constituents e.g. glycosides, alkaloids and terpenoids. Such stimuli are specific and are regarded crucial in evoking preference or non preference response of insects to plants (Dethier, 1970; Schoonhoven, 1972). Plant biochemicals that have adverse effects on insect feeding behaviour may reduce the probability for survival. Mortality may then result from starvation or semi-starvation combined with other forces (Panda, 1979). In their work on aphids, Kindler and Staples (1969) showed that aphids starved to death on resistant or unsuitable plants because of their apparent inability to ingest sap from the plant. This might explain why the cocoa aphid could not survive beyond five (5) days on the legumes and vegetables.

It has always been assumed that both young and old leaves are particularly favourable for aphids because such leaves are likely to be rich in translocated nutrients, particularly nitrogen (van Emden and Bashford, 1971). However, in the present work the mature leaves were found to be unsuitable for rearing of *T. aurantii*. The multiplication rate of the aphid was highest on younger leaves less than 7 days old. The inverse relationship between age of seedling and number of aphids produced, clearly suggests that there could be a substance in the cocoa plant, the increase or decrease of which adversely affects aphids developmental/reproductive ability, but this needs to be verified by future researchers. White (1970) remarked that aphids which continue to develop and reproduce over a long period would certainly require a high quality nitrogen source. Thus, it seems likely that the newly developed leaves preferred by *T. aurantii* were richer in the nitrogen content but this needs to be confirmed in future studies. The present findings are consistent with observation by Firempong (1975) that the cocoa aphid is found on young flush leaves of apical

shoots and chupons and by Stroyan (1961) that the foliage of *Citrus* is only suitable for the nutrition of aphids during its pale green juvenile phase. The present results are also in conformity with the observation by Entwistle (1972) that *T. aurantii* seems to be found on perennials rather than on herbaceous plants.

Reviewing host-plant specificity, Blackman and Eastop (1984) reported that aphids tend to be associated with particular plant families, such that each species within an aphid genus tends to restrict its feeding to a certain genus or species of host plant. Adams and van Emden (1972) suggested that the past history of aphid populations may be the factor responsible for the observed differences.

Owusu *et. al.* (1996) also conducted experiment on effect of host plant change on survival of host-adapted colonies of cotton aphid *A gossypii* and noted that in all cases, adult survival and fecundity decreased significantly when insects were transferred to a host other than the original. Reasons for the low reproductive rate and shorter survival period by the aphids on non-original hosts could be attributed to difference in host nutritional composition.

The results of the preference test in which the vegetables and legumes were heavily infested with aphids and placed under mature cocoa trees but all the aphids disappeared after five (5) days confirm the unsuitability of these crops in rearing the aphid. This is in agreement with the findings of McMurtry and Standford (1960) that although aphids settle and start to feed as quickly on resistant as on susceptible plants, they become restless on resistant plants after some time, and eventually die or leave the plant.

Although broad bean *vicia faba*, has been used by many workers to raise populations of some aphid species for resistance screening (Maxwell and Jennings, 1980), from the present study it was found unsuitable for the cocoa aphid.

The present findings point to the fact that cocoa seedling is the most suitable

host for rearing the black citrus aphid *Toxoptera aurantii*. A number of methods have previously been employed for the rearing of the cocoa aphid. For example, cut citrus twigs immersed in glass vials (Rivnay, 1938) and use of young cocoa seedlings, 30–76 cm tall in black polythene bags (Firempong, 1975). In these experiments the cut citrus shoots and cocoa seedlings had to be replaced every two or three days. The terminal shoot of the cut shoots wilt after some days even though the shoots were immersed in water.

It is concluded from the present study that young cocoa seedling of Trinitario, Y44, not more than a week after cotyledon opening, and placed in ventilated cage is the best of all the materials tested for rearing *T. aurantii*.

The seedlings do not wilt and the cages exclude natural enemies and other unwanted insects. This present method also provided a more constant environment and ideal conditions for both plant and insect growth and permitted direct observation of the insect in the cage without disturbance. Optimum temperature and relative humidity for the rearing of the aphid were between $24 \pm 3^\circ\text{C}$ and 72–85% respectively.

From the mass rearing of *T. aurantii* in the present study the young seedlings had to be replaced after one week, (not 2 or 3 days as reported by Firempong, 1975) When aphids were subcultured on new young seedlings on regular basis, it was possible by this method to produce aphids in sufficient numbers for the resistant screening studies.

CHAPTER FIVE

5.0 DEVELOPMENT OF INFESTATION/EVALUATION METHOD AND ASSESSMENT OF APHID RESISTANCE IN COCOA

5.1 INTRODUCTION

In agricultural terms plant resistance to insects is a property that enables a plant to avoid, tolerate or recover from the injurious effects of insect feeding and oviposition. In order to screen crop varieties for resistance, efficient methods must be developed to evaluate plants both at the seedling and more mature stages (Russel, 1978).

According to Maxwell and Jennings (1980) evaluation of resistance to aphids are on death or adverse effects on the insect and death or alteration of the plant. In some cases resistance is the result of the insect not preferring the plant for oviposition; in such cases no damage occurs from larval or nymphal feeding because no reproduction takes place. Panda (1979) also observed that the degree of insect damage to crop plant is due to the pest density, the characteristic feeding or oviposition of the pest species and the biological plant characteristics. Each of these factors is affected by environmental and other biotic factors, and a correlation between population levels and plant damage is sometimes possible.

There are different methods used in infesting test plants with insects. Brushes and aspirators of various sizes and designs are useful for handling and transferring individuals or small groups of sedentary insects such as aphids (van Emden, 1972, 1977). The reaction of plants exposed to insect attack must be measured at the proper stage of growth. This can be done by visual observations or by actual measurements of the effects of insects on plants or the effects of plants on insect (Tingey, 1986).

Utilization of rating scales in evaluation of resistance at earlier stages of plants has been very valuable. Most rating scales include five or more classes that describe the insect damage or the plant response (Maxwell and Jennings, 1980).

Tingey (1986) also suggested the use of growth parameters in the case of insects that do not produce immediate or obvious feeding damage symptoms until late in plant development. Plant growth criteria useful for measurement of response to insect attack include:

- (i) plant weight, volume, area and growth habit;
- (ii) growth, elongation and maturity of plant organs;
- (iii) number, size, weight and location of vegetative and fruiting organs.
- (iv) ability for recovery or replacement.

In this chapter an infestation/evaluation method is developed for (i) very young cocoa seedlings using T85/799 and Y44 open pollinated pods; (ii) older seedlings using pairwise crosses and clonal materials. Resistance in terms of antibiosis is investigated.

5.2 MATERIALS AND METHODS

5.2.1 Young cocoa seedlings

5.2.1.1 Insectary screening of seedlings (aphids)

Cocoa beans from two types of open pollinated pods, Amazon (T85/799) and Trinitario (Y44), origins were sown in alternate rows in aluminium trays (72 cm and 35 cm) at a distance of 16 cm between and 10 cm within rows (Plate 3). Six seedlings of each type were in a row in a tray.



Plate 3. Arrangement of young seedlings of two cocoa types (T85/799 and Y44) in trays.

The seedlings were infested one week (P_1), two weeks (P_2) and three weeks (P_3) after cotyledon opening by introducing ten adult apterous cocoa aphids on each seedling using thin soft camel hair brush. The aphids used in this study were raised by the method developed in this work. A control was set up in which the seedlings were not infested (P_4).

The four treatments were replicated four times using Completely Randomised Design. All the trays were kept in the insectary and the seedlings watered as necessary. The seedlings and aphids were observed and monitored for a period of eight weeks ($W_1 - W_8$). The following records were taken weekly:

- i. Number of aphids per plant
- ii. Height of each seedling
- iii. Girth of each seedling
- iv. Number of crinkled leaves (after 8 weeks).

Aphid counts were transformed by $\log(x + 1)$ and subjected to analysis of variance. The differences between means were separated by Least Significant Difference test (LSD).

The number of crinkled leaves produced was used to establish criteria for resistance

Rating	Damage Level	Susceptibility Classification
1	No leaf symptom	Highly Resistant
2	1 crinkled leaf	Resistant
3	2 crinkled leaves	Moderately Resistant
4.	3 crinkled leaves	Susceptible
5	4 or more crinkled leaves	Highly susceptible

5.2.1.2 Determination of the level of attractiveness of the two cocoa types to mirids using the "microtest" method

To determine the level of attractiveness of the two cocoa types, Amazon T85/799 and Trinitario Y44 to mirids, a laboratory screening was conducted using the microtest method described by Nguyen-Ban (1993).

Fourth and fifth instar larvae of mirids (*Sahlbergella singularis*), starved overnight to encourage their feeding on the plant materials and fresh twigs of the seedlings of the two cocoa types were used as follows: The twigs were cut into 50mm pieces. Two pieces of twigs of each type were stapled together to form a square layout (Plate 4). The assembled twigs were placed in 120-mm diameter petri dishes, the bottom lined with filter paper. One fourth or fifth instar larva of *Sahlbergella Singularis* was placed in the middle. The experiment was replicated 30 times. The set up was kept in the laboratory for 24 hours. The total feeding punctures (lesions) on the two twigs per each cocoa type were counted and recorded. The counts were square-root transformed to break the relationship between means and variances to meet analysis of variance (ANOVA) assumption of normality.

5.2.2 Older cocoa seedlings (pairwise crosses and clonal materials).

5.2.2.1 Pairwise crosses

Seedlings of 25 different pairwise crosses were raised in black polythene bags measuring 18 cm wide and 25 cm high. The following 25 pairwise crosses were used:

1. T79/501 x T79/467
2. T85/799 x T79/501
3. T63/967 x T65/328
4. T79/467 x T63/967
5. T79/501 x SCA 6
6. Pound 7 x Pound 10
7. PA 150 x PA 7

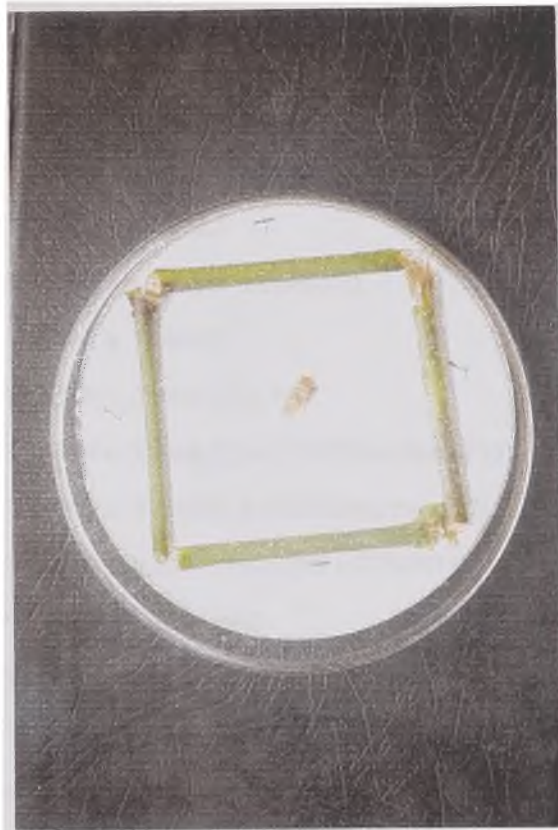


Plate 4. Arrangement of twigs of two cocoa types (T85/799 and Y44) in mirid attractive test (microtest).

8. Pound 15 x NA 32
9. T60/887 x T63/971
10. T16/613 x P30
11. PA 7 x PA 150
12. Pound 10 x Pound 15
13. IMC 85 x IMC 47
14. T65.328 x T65/326
15. NA 32 x IMC 60
16. T65/326 x T60/887
17. IMC 47 x (T60/887 x Pound 7)
18. T63/971 x T16/613
19. (T85/799 x Pound 15) x PA7
20. (T85/799 x Pound 25) x (T85/799 x Pound 15)
21. (T85/799 x T79/501) x (T85/799 x Pound 7)
22. (T85/799 x Amel) x (T85/799 x T79/501)
23. P30 x (T85/799 x Amel)
24. (T60/887 x Pound 7) x (T60/887 x Pound 15)
25. (T85/799 x Pound 7) x (T60/887 x Pound 25)

These crosses were selected for this work on the basis that they are high yielding and resistant to CSSV and blackpod disease (Adu-Ampomah *et. al.*, 1999).

After eight weeks of germination, one fresh flush leaf of each seedling was infested with one adult apterous 'virgin' aphid (i.e. adult aphids which have not yet reproduced) using soft camel hair brush and the aphids allowed to multiply. Four seedlings of each cross was used in a replicate. There were four replicates in a completely randomised design. The seedlings were kept in cages in the insectary.

Seedlings were examined everyday for seven days after infestation and the number of aphids recorded. Average multiplication rate for each cross was determined. The crosses were rated on a five-point scale, as follows:

Rating	Infestation level	Susceptibility Classification
1	No aphid survival	Highly Resistant
2	1–12 live aphids	Resistant
3.	13-24 live aphids	Moderately Resistant
4	25–48 live aphids	Susceptible
5	Over 48 live aphids	Highly susceptible

5.2.2.2 Clonal materials

Twenty five (25) different clonal materials were supplied by the Plant Breeding Division of CRIG for this work. The clonal materials aged eight weeks included:

1. T85/799
2. T85/799 x T79/501
3. T85/799 x Pound 25
4. Pound 7
5. Pound 15
6. Pa 7/808
7. T63/967
8. Pa 150
9. T63/971
10. T79/467
11. T65/238

12. T85/799 x Pound 7
13. T85/799 x Amel
14. T60/887 x Pound 15
T60/887
16. T17/524
17. IMC 67
18. T65/326
19. T79/501
20. T60/887 x Pound 7
21. IMC 76
22. T85/799 x Pound 15
23. Pound 10
24. Na 32
25. P 30

These clones were used for this test because they are high yielding and have shown resistance to CSSV and blackpod disease (Adu-Ampomah *et al*, 1999).

Four plants of each clone were used in a replicate. There were four replicates in a completely randomised design. Thus, 16 plants per treatment were used. One fresh flush leaf of each clone was infested with one adult virgin apterous aphid using soft camel hair brush and the aphid allowed to multiply. The seedlings were kept in cages in the insectary and examined everyday for seven days. Aphid populations were recorded and average multiplication rate determined. The same five-point scale described above was used to rate the different clones.

5.3 RESULTS

5.3.1 Very Young Cocoa Seedlings of T85/799 and Y44 origins.

5.3.1.1 Number of aphids/aphid infestation levels.

Tables 8 and 9 show aphid infestation levels eight weeks after infestation for the Amazon (T85/799) and Trinitario (Y44) cocoa types.

The aphid population increased rapidly during the first week (W_1) of infestation, followed by a steep decline in the subsequent weeks (W_2 – W_8).

TABLE 8

Mean number of aphids per seedling after eight weeks for Amazon T85/799

Period	Weeks							
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8
P_1	91	35	16	6	1	0	0	0
P_2	22	11	5	2	0	0	0	0
P_3	9	4	3	1	0	0	0	0
P_4	—	—	—	—	—	—	—	—

TABLE 9

Mean number of aphids per seedling after eight weeks for Trinitario Y44

Period	Weeks							
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8
P_1	179	96	41	13	10	4	1	0
P_2	68	45	13	6	2	0	0	0
P_3	38	14	6	1	0	0	0	0
P_4	—	—	—	—	—	—	—	—

Seedlings infested one week (P_1) after cotyledon openings showed the highest rate of multiplication of aphids. These seedlings were also able to maintain or support aphid populations for a longer period of up to five and seven weeks for the Amazon and Trinitario seedlings, respectively (Figs.6 & 7).

Statistical analysis of the aphid population levels produced on the two cocoa types one week after infestation and mean separation are presented in Appendix 7 and Table 10, respectively.

Table 10

Mean number of aphids produced after one week on two cocoa types infested at 3 different periods (P_1 , P_2 and P_3) after cotyledon opening.

Period	T85/799	Y44
P_1	91 (1.964) a	179 (2.255) a
P_2	22 (1.362) b	68 (1.949) b
P_3	9 (1.000) c	38 (1.591) c

Numbers followed with different letters in the same column are statistically different at ($P < 0.05$) (LSD = 0.30), P_1 = one week; P_2 = two weeks; P_3 = three weeks.

The two types of cocoa differed significantly ($P < 0.05$) in aphid multiplication rates with the Trinitario (Y44) supporting the higher population level. There were also significant differences among the periods of infestation (P_1 , P_2 & P_3). Seedlings infested one week after cotyledon opening (P_1) supported the highest aphid numbers whereas seedling infested three weeks after cotyledon opening (P_3) supported the lowest aphid population.

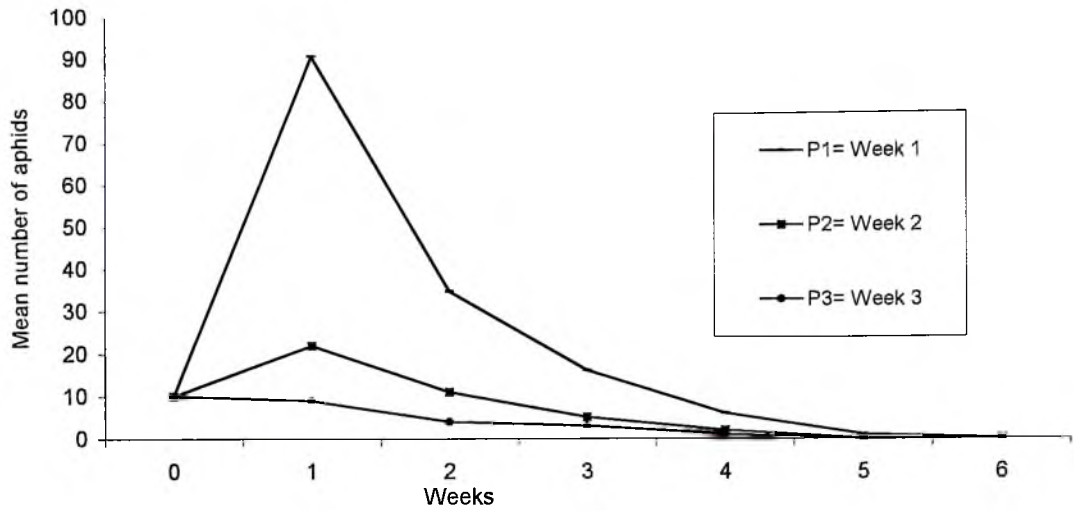


Fig 6: Mean number of aphids produced on cocoa type T85/799 infested at different periods (P1, P2 and P3) after cotyledon opening.

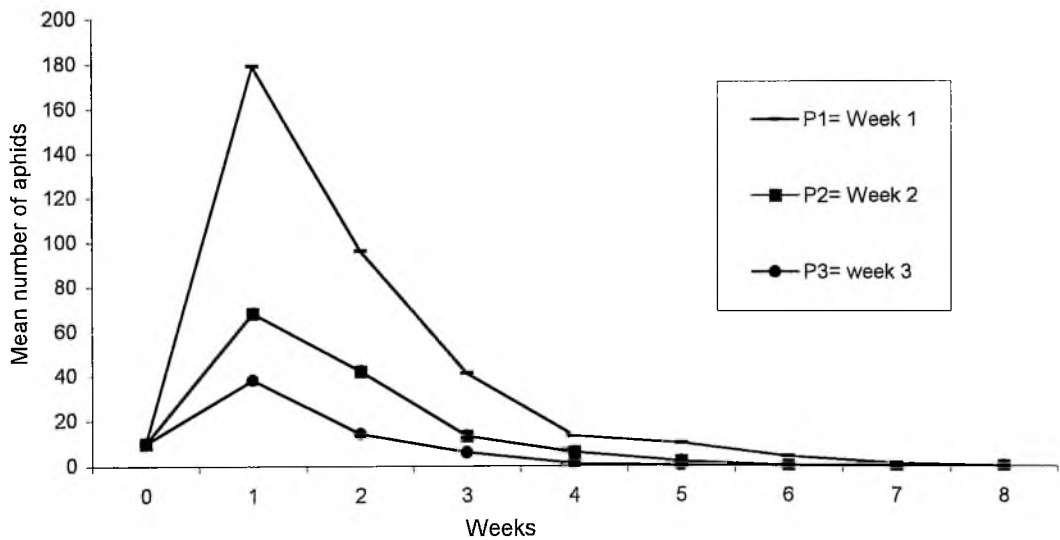


Fig. 7: Mean number of aphids produced on cocoa type Y44 infested at different periods (P1, P2 and P3) after cotyledon opening.

5.3.1.2 Aphid infestation on growth of young cocoa seedlings

Growth measurement data recorded included height, girth and number of crinkled leaves of seedlings

Tables 11 and 12 present data on mean height and girth increments eight weeks after infestation.

The results indicated that *T. aurantii* infestation had significant effect on the growth of very young seedlings. Among the infested seedlings, those infested 3 weeks (P_3) after cotyledon opening produced seedlings having growth rate closest to that exhibited by the control (P_4) seedlings. This was more obvious from the girth measurements.

TABLE 11

Effect of aphid infestation on height (cm) of two cocoa types (T85/799 and Y44) infested at different periods (P_1 , P_2 and P_3 . P_4 =control)

Period	Pre-infestation		After eight weeks		Height increment	
	T85/799	Y44	T85/799	Y44	T85/799	Y44
P_1	20.65	22.40	27.65	29.37	7.00a	6.97 a
P_2	23.32	25.22	31.40	34.15	8.08a	8.93 b
P_3	24.45	27.10	32.55	37.00	8.10a	9.90 bc
P_4	20.57	22.48	30.13	33.60	9.56 b	10.12 c

Numbers followed with the same letters in the same column are statistically insignificant at ($P=0.05$)

(LSD=1.21). P_1 = one week; P_2 = two weeks; P_3 = three weeks;

P_4 = control.

Table 12

Effect of aphid infestation on girth (mm) of two cocoa types (T85/799 and Y44) infested at different periods (P_1 , P_2 and P_3) after cotyledon opening

Period	Pre-infestation		Post-infestation		Girth increment	
	T85/799	Y44	T85/799	Y44	T85/799	Y44
P_1	7.38	7.13	10.62	10.21	3.24	3.08
P_2	7.58	8.01	11.15	11.31	3.30	3.30
P_3	8.17	8.23	11.69	11.78	3.52	3.55
P_4	7.23	7.13	10.78	11.15	3.55	4.02

P_1 = one week; P_2 = two weeks; P_3 = three weeks; P_4 = control.

There was no significant difference ($P=0.05$) in height increment between the two cocoa types. However, there were significant differences ($P < 0.05$) in height increment among the Trinitario treatments infested at different periods ($P_1 - P_3$). Moreover height increment in all the plants infested one and two weeks after cotyledon opening (P_1 & P_2) were significantly lower than increment recorded for the untreated plants (P_4).

Although there were no significant differences among the Amazon (T85/799) treatments (P_1 , P_2 and P_3), the control (P_4) produced a significantly higher height increment. The effect of aphid infestation on both types of cocoa was most pronounced on seedlings infested a week after cotyledon opening (P_1).

Statistical analysis (Appendix 9) showed that there were no significant differences ($P = 0.05$) in girth increments between the cocoa types and among the different periods of infestation. The control plants also showed no significant girth

increments from the treated plants most likely because a period of eight weeks is too short for girth increments to manifest.

5.3.1.3 Number of crinkled leaves due to aphid infestation

The mean number of crinkled leaves produced per each cocoa type eight weeks after infestation. (Fig. 8) showed that there was increasing resistance to aphids as the plant aged. Observations made on the leaves at the end of the experiment revealed that, with the exception of the control, *T. aurantii* damage (leaf crinkling) was recorded on all treatments.

Seedlings infested one week (P_1) after cotyledon opening produced more crinkled leaves with the effect being more pronounced on the Y44 than on the T85/799 plants (Table 13). For both cocoa types, the number of crinkled leaves was lowest in seedlings infested three weeks after cotyledon opening (P_3).

Tables 13
Relationship between aphid density and number of crinkled leaves for two types of cocoa infested at different periods

Period	Mean number of aphids		Mean No of crinkled leaves	
	T85/799	Y44	T85/799	Y44
P1	91	179	2	4
P2	22	88	1	3
P3	9	38	0	1
P4	—	—	—	—

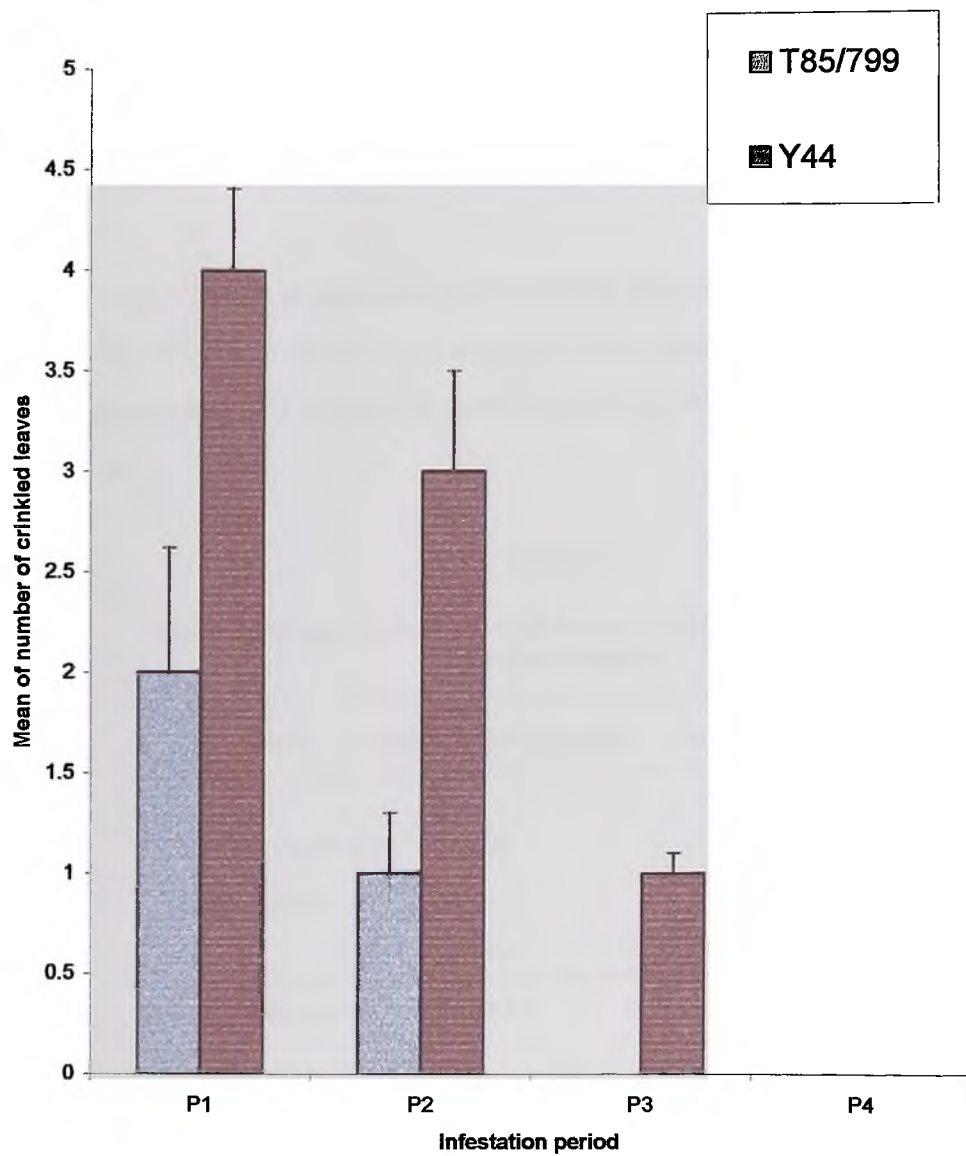


Fig. 8: Mean number of crinkled leaves produced after eight weeks on two cocoa types infested at different periods(1, 2 and 3 weeks, P4=control) after cotyledon opening.

Statistical analysis showed that the mean number of crinkled leaves was positively correlated with the mean aphid infestation level ($r = 0.94$ for Y44 and $r = 0.93$ for T85/799). As the number of aphids increased on the seedlings the effect of their feeding was more pronounced and, thus, resulted in the formation of more crinkled leaves.

5.3.2 Level of attractiveness of two cocoa types to mirids

Out of a total of 154 feeding punctures, 116 were counted on the Trinitario (Y44) representing 75% as against 38 on the Amazon (T85/799) representing 25% (Table 14).

Table 14

Number of feeding punctures produced on two cocoa types (T85/799 and Y44) by *Sahlbergella singularis*

Cocoa type	Number of feeding punctures	Percentage(%)
Amazon (T85/799)	38	25
Trinitario (Y44)	116	75
Total	154	100

Data represents totals of 30 replicates

Analysis of variance (Appendix 10) of the data indicated that the two cocoa types were significantly different ($P < 0.01$) in their level of attractiveness to the mirids. The Trinitario Y44 was more preferred to the Amazon T85/799.

5.3.3 Aphid multiplication rate on older seedlings

5.3.3.1 Pairwise Crosses

The results of this study (Table 15) and analysis of variance (Appendix 11) showed varietal differences in susceptibility among crosses.

Table 15
Rating of 25 pairwise crosses by multiplication rate

Code	Pairwise Cross	No of aphids by 6th day	No of nymphs on 7th day	Total Aphids produced	Rating
A	T79/501 x T79/467	14	0	14	3
B	T85/799 x T79/501	10	0	10	2
C	T63/967 x T65/328	15	4	19	3
D	T79/467 x T63/967	29	14	43	4
E	T79/501 x SCA6	20	9	29	4
F	Pound 7 x Pound 10	15	1	16	3
G	PA 150 x PA 7	30	7	37	4
H	Pound 15 x Na 32	31	20	51	5
I	T60/887 x T63/971	8	2	10	2
J	T16/613 x P30	20	8	28	3
K	PA 7 x PA 150	20	6	26	4
L	Pound 10 x Pound 15	4	0	4	2
M	IMC 85 x IMC 47	28	29	57	5
N	T65/328 x T65/326	12	0	12	2
O	Na 32 x IMC 60	18	3	21	3
P	T65/326 x T60/887	30	15	45	4
Q	IMC 47 x (T60/887 x Pound 7)	15	5	20	3
R	T63/971 x T16/613	20	8	28	4
S	*T85/799 x Pound 15) x PA 7	21	10	31	4
T	(T60/887 x Pound 25) x (T85/799 x Pound 15)	28	20	48	4
U	(T85/799 x T79/501) x (T85/799 x Pound 7)	14	0	14	3
V	(T85/799 x Amel) x (T85/799 x T79/501)	26	18	44	4
W	P30 x (T85/799 x Amel)	21	13	34	4
X	(T60/887 x Pound 7) x (T60 x Pound 15)	30	8	38	4
Y	(T85/799 x Pound 7) x (T60/887 x Pound 25)	34	28	62	5

1= highly resistant (no aphid survival); 2= resistant (1-12 live aphids)
3= moderately resistant (13-24 live aphids); 4= susceptible (25-48 live aphids)
5= highly resistant (over 48 live aphids)



Significant differences ($P < 0.01$) in multiplication rate were observed among the crosses (Appendix 11). On the basis of live nymphs produced per adult, crosses Y M and H were the most susceptible (Class 5) while crosses L, B and I were the least susceptible (Class 2). The remaining crosses were intermediate in susceptibility between the two extremes.

The outstanding cocoa crosses from this trial in terms of resistance to aphid multiplication were L, B & I in decreasing order. The three most susceptible ones were Y, M and H in a decreasing order.

5.3.3.2 *Clonal materials*

Results from the multiplication rate of the cocoa aphid on the 25 different clones infested (Table 16) and analysis of variance (Appendix 12) indicate that the clones differed significantly ($P < 0.01$) in their level of susceptibility.

Table 16
Rating of 25 cocoa clones by multiplication rate

Code	Clone	Number of aphids by 6th day	Number of aphids on 7th day	Total Aphids produced	Rating
A	T85/799	30	5	35	4
B	Pound 7	28	6	34	4
C	Pound 15	26	6	32	4
D	PA 7/808	23	7	30	4
E	T63/967	34	14	48	4
F	Pa 150	18	12	30	4
G	T63/971	7	0	7	2
H	T79/467	35	11	46	4
I	T65/238	22	9	31	4
J	T60/887	35	10	45	4
K	T17/524	20	6	26	4
L	IMC 67	12	3	15	3
M	IMC 76	27	7	34	4
N	T79/501	23	13	36	4

Table 16 cont.

Code	Clone	Number of aphids by 6th day	Number of aphids on 7th day	Total Aphids produced	Rating
O	T65/326	9	0	9	2
P	Pound 10	20	7	27	4
Q	Na 32	17	4	21	3
R	P30	5	0	5	2
S	T85/799 x T79/501	34	12	46	4
T	T85/799 x Pound 25	42	16	58	5
U	T85/799 x Pound 7	33	13	46	4
V	T85/799 x Pound 15	20	10	30	4
W	T85/799 x Amel	16	4	20	3
X	T60/887 x Pound 7	36	16	52	5
Y	T60/887 x Pound 15	34	10	44	4

1= highly resistant (no aphid survival); 2= resistant (1-12 live aphids)

3= moderately resistant (13-24 live aphids); 4= susceptible (25-48 live aphids)

5= highly susceptible (over 48 live aphids)

Considering the number of live nymphs produced per adult aphid, clones T and X emerged as the most susceptible (Class 5) while the three most resistant clones (Class 2) were R, G and O in decreasing order. The remaining clones were intermediate between the two extremes.

5.4 DISCUSSION

5.4.1 Very Young Seedlings

Results from this study have shown that very young seedlings were extremely susceptible to and maintained high populations of *T. aurantii*. On older seedlings the rate of reproduction was lower and plants maintained very low aphid population which were progressively reduced until they all died. The observation that the populations of *Toxoptera aurantii* survived and multiplied fast on fresh flush leaves



is in conformity with the findings of other workers (Stroyan, 1961; Firempong, 1975). Such behaviour is not limited to *T. aurantii*. Kennedy (1958) and Muller (1958) indicated that the effect of leaf age and physiological state of a plant on the fecundity of aphids can be explained on the basis of nutritive changes. The relative hardness of the leaves at the different periods of infestation was identified as the major factor which determined the rate of aphid multiplication. Emery (1946) found that the water content of alfalfa plants was a primary factor in temporary resistance to aphids. Even though this factor may not significantly alter the atmospheric humidity, it may influence aphid feeding by causing variations in the turgor pressure. Grison (1952, 1958) found that the sugar and lecithin contents of potato foliage fed to the Colorado potato beetle exerted a marked influence on insects fecundity. Old leaves were found to contain relatively high sugar but low lecithin concentrations as compared to young leaves. The beetles fed on old leaves laid fewer eggs per day than when fed on young leaves. El-Ibrashy and Riad (1972) reported that reproduction of the aphid *Rhopalsiphum maidis* on barley had an early maximum performance which peaked at five days followed by a progressive fall.

The effect of *T. aurantii* infestation on growth of cocoa seedlings was tremendous on very young seedlings infested a week after cotyledon opening. The early infestation and faster multiplication rate of the aphid on the young seedlings resulted in significant reduction of height and leaf deformations. Dixon (1973) made similar observation that aphid infestation in both lime and sycamore resulted in reduced growth and that the higher the number of aphids infesting a tree, the greater the reduction in growth of the plant. He indicated that the whole plant may be affected by an aphid infestation even though parts of the plant may be distant from the site of infestation.

The finding that the number of crinkled leaves was positively correlated with the aphid infestation level is in conformity with the observation by Panda (1979). He reported that the degree of insect damage of crop plants is due to the pest density and the biological plant characteristics.

Resistance related to age has also been observed in field plants and in plant tissue. Bong *et al.* (1994) found that in general, field resistance improved with maturity of the plant. Stroyan (1961) in his study on aphids on *Citrus* indicated that the foliage of *Citrus* is, in general, only suitable for nutrition of aphids during its pale green juvenile phase. This showed that young leaves are extremely susceptible to damage and that matured plants could tolerate aphid attack. Saunders (1979) also observed that the pest status of the cocoa aphid *T. aurantii* is most apparent in the nursery, where it may severely retard the growth of young seedlings if not controlled. Ingram (1964) also reported that in Uganda, the aphid causes severe deformation of young cocoa seedlings.

The difference in susceptibility levels of the two cocoa types tested to both aphids and capsids have been demonstrated. The rate of multiplication of the aphid was significantly higher on Trinitario (Y44) than on Amazon (T85/799). The Trinitario also supported the aphid population for a longer period indicating that it was better preferred. With the laboratory microtest, it was observed that the capsid, *Sahlbergella singularis* also showed significant preference for the Trinitario (Y44), which harboured 75% of the feeding lesions. These two results seem to indicate that cocoa varieties that are resistant to *T. aurantii* may tend to be resistant to capsids and are in agreement with the suggestions by Campbell (1990) and Eskes *et al.* (1998) that resistance to aphids could be an indication of general insect resistance in cocoa. However, further experiments must be conducted to ascertain the validity of this observation in relation to other cocoa genotypes.

5.4.2 Older Seedlings: Clones and Pairwise crosses

Resistance to pest attack is characterized by the resistant plants having a lower pest population density, or few damage symptoms, than the other plants which are termed susceptible. The mechanism of resistance exhibited in the older cocoa seedlings to *T. aurantii* according to the results of this study is antibiosis. Morphological and physical characteristics of host plants are known in some instances to protect the host from insect attack (Howe, 1950), however, with this study since the aphid reproduced at least on all the clones and crosses tested, resistance could not be antixenosis (non-preference). None of the clones or crosses was found to be completely immune to *T. aurantii* attack. Antibiosis in this respect may be due to the deficiency of certain nutritional elements in the host plant. The role of nutritional factors in resistance has been more pronounced in a number of aphid resistant plant species.

Panda (1979) indicated that the nutritional value of the plant and the absence of toxic compounds determine the adequacy of food to sustain various physiological processes related to the growth, longevity and fecundity of adult aphids. Russel (1978) also reports that the rate of population increase of a pest on a host plant is reduced by antibiosis because it causes death of the pest, or decreases its rate of development or reproductive potential.

The rate of multiplication of the cocoa aphid *T. aurantii* on different varieties of cocoa can, therefore, be used to determine their preferences and, their susceptibility or resistance levels. The sap sucking aphid *T. aurantii* showed significant preference for some of the clones and crosses evaluated. This is in contrast to earlier findings by Firemong (1984) and Bassie (1997) who observed no significant preference by *T. aurantii* for any of the progenies they evaluated. The disparity could be due to the fact that they worked on mature progenies in the field without considering young plants or seedlings. Amongst the 25 clones evaluated in the present study, *T. aurantii*

appeared to prefer T85/799 x Pound 25 and T60/887 x Pound 7, which were rated highly susceptible, indicating that the clones probably offer more nutritious foliage which contributed to the high multiplication rate as explained by Thorsteinson (1960). The relatively low preference shown for P30, T63/971 and T65/326 might have resulted from the presence of feeding deterrent or low amino acid content which is needed in high concentration for reproduction (Dethier, 1970). Additionally, the relative hardness of the different clones, coupled with their juice/water content are factors which could determine the suitability of the plant for feeding and the number of nymphs produced per adult.

With the pairwise crosses, Pound 10 x Pound 15, T85/799 x T79/501, T65/328 x T65/326 and T60/887 x T63/971 showed the most promising resistance to the aphid. The highly susceptible ones included (T85/799 x Pound 7) x (T60/887 x Pound 25), IMC 85 x IMC 47 and Pound 15 x Na 32.

These differences observed in the clones and crosses were as expected since the clones and crosses were selected on the basis of different mechanisms and levels of resistance and from widely differing genetic sources.

Comparing the crosses and the clones, it was observed that the best crosses have at least one resistant parent, but the other parent is either moderately resistant or susceptible. The worst crosses had parents which were both susceptible. For example, in the case of cross Y (T85/799 x Pound 7) x (T60/887 x Pound 25), the susceptible parents are T85/799 x Pound 7, T60/887 x Pound 7 and T85/799 x Pound 25.

An observed anomaly was with the cultivar T85/799 x T79/501. Both parents came out as susceptible in the clonal screening, but as a pairwise cross it was resistant. This anomaly could be due to the susceptibility genes in the clones being recessive.



sive and the resistant genes being more pronounced in the cross. This however, requires further investigation. With the exception of this anomaly, the results followed the general trend that in a group of cultivars the higher the average resistance observed in the parents, the higher the resistance of their progeny as pointed out by Phillips-Mora (1996).

CHAPTER SIX

6.0 SCREENING COCOA PROGENIES FOR THEIR RESISTANCE TO THE COCOA APHID *TOXOPTERA AURANTII*

6.1 INTRODUCTION

Some control of cocoa insect pest has been achieved by chemical means, cultural practices or a combination of these (Owusu-Manu, 1997). The use of genetic resistance as a control strategy has not been fully exploited. While chemical control has been successful in the short term more long term strategies which would include the use of resistant cultivars is highly desirable.

Disease and pest resistant should be a breeding objective of highest priority but resistance of a new cocoa cultivar is only meaningful to the cocoa growers in combination with other required agronomic characteristics, such as vigour and uniform plant type, early and total yield capacity, bean size and cocoa quality (Van der Vossen, 1996). Rapid advances in cocoa variety improvement programmes are best achieved by the (reciprocal) recurrent selection schemes with distinct sub-populations presently adopted in Ivory Coast (Paulin and Eskes, 1995) Malaysia (Lockwood and Pang, 1994) and Brazil (Pires *et. al.*, 1996).

Field data on disease and pest resistance are very useful to start a programme for the detection of resistant genotypes, but efficient and reliable pre-selection tests (screening methods) are essential in obtaining satisfactory progress in breeding programmes.

Screening of varietal material for insect preferences has been in progress at the Cocoa Research Institute of Ghana (CRIG) for some time now (Anon, 1946, Bigger, 1972, Bigger 1975a, Ackonor *et. al.*, 1993, Bassie, 1997).

Collingwood and Marchart (1971) identified some varietal differences in susceptibility of different cocoa types to capsid attack. A survey of insects occurring on two cocoa progenies at Tafo, indicated that one of the progenies was more susceptible to attack by a range of sucking insects including mealy bugs, capsids, some ants and cocoa aphids (Bigger, 1972). In his work, Campbell (1990) examined 68 progenies of cocoa in the field and concluded that progenies differed in their levels of infestation to mealy bugs and other honey dew producing Homoptera. In Cote d'Ivoire Nguyen Ban (1993) conducted resistance studies to evaluate several clonal and hybrid cocoa for their susceptibility to capsid attack.

Resistance of cocoa to insects appears to be quite general. Criollo and Trinitario germplasm are generally more susceptible than Forastero to any type of damaging insects. In this chapter four progenies derived from different clones with expectedly different level of insect resistance were screened for aphid resistance using the method developed in the previous chapter.

6.2 MATERIALS AND METHODS

The four progenies screened were derived from the following crosses

Progeny A — NA32 x T60/887

Progeny B — P 30 x P 30 (selfed)

Progeny C — ICS39 x Y44

Progeny D — T79/501x Pa 150

Both young germinating and older seedlings were used in this test.

6.2.1 Young germinating seedlings

Cocoa beans from the four progenies were sown in alternate rows in aluminium trays (72 cm by 35 cm) at a distance of 16 cm between and 10 cm within rows. Six seed-

lings of each type was in a row in a tray. The seedlings were infested one week after cotyledon opening by introducing ten adult apterous cocoa aphids on each seedling using a thin soft camel hair brush. A control treatment was set up in which the seedlings were not infested. Each treatment was replicated four times in a completely randomised design.

All the trays were kept in the insectary and the seedlings were watered as necessary. Temperature and relative humidity at the insectary during the period of the studies fluctuated between $24 \pm 3^{\circ}\text{C}$ and 72 – 86%, respectively. The seedlings and aphids were observed and monitored for a period of eight weeks. The following records were taken weekly:

- i. Number of aphids per plant
- ii. Height of each seedling
- iii. Girth of each seedling

The number of crinkled leaves were recorded after the eighth week.

Aphids count was undertaken with the aid of a magnifying glass. The data was transformed by $\log(x+1)$ and subjected to ANOVA. Differences between means were separated by the Least Significant Difference test (LSD). Data on height and girth differences were also subjected to ANOVA and differences between means separated using LSD.

Using the aphid resistance criteria developed in chapter 5 the progenies were ranked accordingly.

6.2.2 Using flush leaves of older seedlings

Seedlings of the four progenies were raised in black polythene bags measuring 18 cm

wide and 25 cm high. After eight weeks of growth, one fresh flush leaf of each seedling was infested with one adult "virgin" apterous aphid using soft camel hair brush, and the aphids allowed to multiply. Four seedlings of each progeny was used in a replicate. There were four replicates in a completely randomised design. Sixteen (16) seedlings were therefore used in a treatment. The seedlings were kept in cages in the insectary and watered as necessary. The seedlings were examined everyday for seven days after infestation and the number of aphids recorded. Average multiplication rate for each progeny was determined. The data was transformed using $\log(x + 1)$ and subjected to ANOVA. Differences between means were separated by the Least Significant Difference test (LSD). Using the five point resistant scale developed in chapter five, the progenies were rated.

6.3 RESULTS

6.3.1 Young germinating seedlings

6.3.1.1. *Number of aphids per plant*

Fig. 9 presents average number of aphids per plant produced on the four cocoa progenies for seven days. Aphid numbers increased daily on all the four progenies during the first week of infestation. The rate of increase was highest on progeny C (ICS 39 x Y44) and lowest on progeny B (P 30 selfed). Progenies A (Na 32 x T60/887) and D (T79/501 x Pa 150) were intermediate

Statistical analysis of aphid number after one week (Appendix 13 and Table 17a) indicated that the four progenies differed significantly ($P < 0.05$) in aphid multiplication rates with progeny C supporting the highest aphids population level and progeny B the least. Progenies A and D were not significantly different.

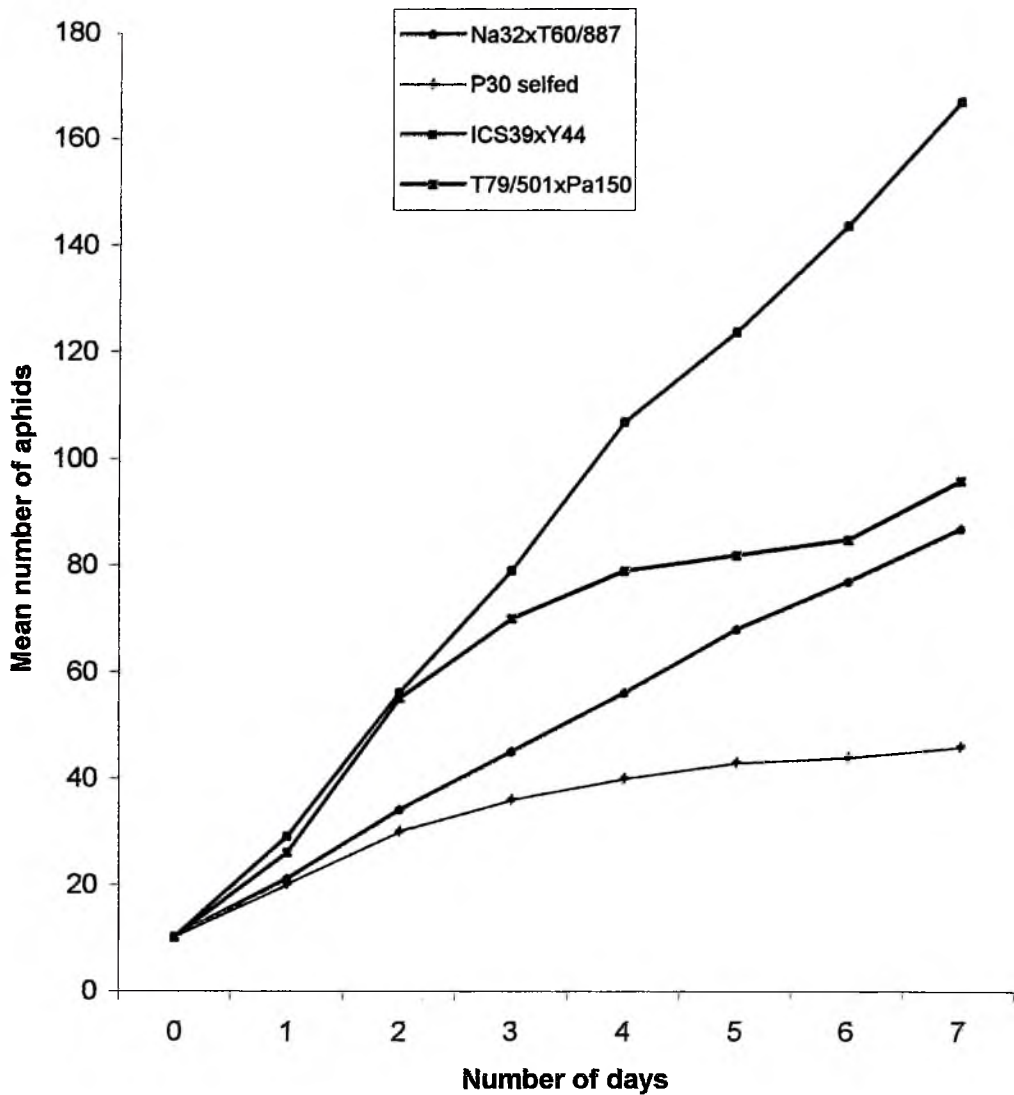


Fig. 9: Mean number of aphids produced on young seedlings of four cocoa progenies for seven days

Table 17a

Mean number of aphids produced by *T. aurantii* reared on the young seedlings of four cocoa progenies after a week

Code	Progeny	Number of aphids	Percentage of total
A	Na 32 x T60/887	87	21.97 b
B	P 30 selfed	46	11.62 c
C	ICS 39 x Y44	167	42.17 a
D	T79/501 x Pa 150	96	24.24 b
	Total	396	100

Numbers represent mean of 4 replicates. Numbers in column followed by the same letter are not significantly different at ($P=0.05$)

Considering the entire experimental period of 8 weeks, it was observed that the aphid population increased rapidly during the first week W_1 , followed by a steep decline in the subsequent weeks $W_2 - W_8$ (Table 17b)

Table 17b

Mean number of aphids per seedling produced from the first to the eighth week (W_1-W_8) after infestation on four cocoa progenies

Progeny	Weeks							
	W_1	W_2	W_3	W_4	W_5	W_6	W_7	W_8
Na 32 x T60/887	87	67	39	21	9	5	0	0
P 30 selfed	46	12	3	2	1	0	0	0
ICS 39 x Y44	167	111	57	45	21	15	9	5
T79/501 x Pa 150	96	49	22	9	3	1	0	0

Whereas progeny C maintained and supported aphid population throughout the eight weeks, progenies A and D supported them for 6 weeks and progeny B for 5 weeks. Thus the preference for the cocoa progenies by *Toxoptera aurantii* was in the following order; C was the most preferred followed by D, A and B.

6.3.1.2 *Aphid infestation on growth of young seedlings*

Growth measurements data recorded included height, girth and the number of crinkled leaves. Height difference among the four progenies and their control (untreated plants) are shown in Table 18.

Table 18

Effect of aphid feeding on height(cm) of four progenies after eight weeks of infestation

Treatment	Pre-infestation height	Height after 8 weeks	Height increment
A	12.75	23.57	10.82 ab
B	12.65	24.85	12.20 bc
C	14.45	23.75	9.30 a
D	14.27	24.90	10.63 ab
Control			
A ₁	13.05	24.97	11.92 bc
B ₁	13.00	25.00	12.00 bc
C ₁	13.70	26.92	13.22 c
D ₁	13.78	25.70	11.92 bc



Analysis of variance (Appendix 14) showed that there were significant differences ($P < 0.05$) in height increments among the treated plants of the progenies. Progeny C recorded the lowest height increment whereas progeny B produced the highest increment. The two differed significantly. However, there were no significant differences in height increment among progenies A B and D.

Comparing the four treated plants with their controls, only progeny C showed significant difference in height increment. Height differences observed between the remaining progenies and their controls were not statistically significant.

The effect of aphid infestation and feeding was most pronounced on progeny C which makes it the most preferred and, therefore, the most susceptible. Progeny B was not significantly affected by the aphid infestation and feeding.

Girth increments of the treated plants of the four progenies and their controls and analysis of variance are presented in Table 19 and Appendix 15 respectively.

Table 19
Effect of aphid feeding on girth (mm) of four progenies after eight weeks

Code	Progeny	Pre-treatment	Girth after eight weeks	Girth increment
A	Na 32 x T60/887	6.20	11.39	5.19 a
B	P 30 selfed	6.60	11.23	4.63 b
C	ICS 39 x Y44	7.38	12.65	5.27 a
D	T79/501 x Pa 150	6.75	11.39	4.64 b
Controls				
A1	Na 32 x T60/887	6.05	11.31	5.26 a
B1	P 30 selfed	6.52	11.15	4.63 b
C1	ICS 39 x Y44	7.31	12.88	5.57 a
D1	T79/501 x Pa 150	6.68	11.39	4.71 b

Numbers followed by the same letter are not significant at ($P=0.05$)

There were significant differences ($P < 0.05$) in girth increments among the progenies. Progeny C showed the highest girth increment whereas progeny B showed the least. Progenies A and C were statistically different from progenies B and D.

The control plants (untreated) of all the progenies showed no significant girth increment from their treated counterparts. The infestation and feeding of *T. aurantii* could not cause any significant effect on the girth of the plants.

6.3.1.3 Number of crinkled leaves due to aphid infestation

The mean number of crinkled leaves produced on the four progenies after eight weeks of infestation is presented by Fig 10.

Progeny C produced the highest number of crinkled leaves suggesting it was the most susceptible. It was followed by progenies D, A and B in decreasing order.

Table 20
Relationship between aphid density and the number of crinkled leaves produced after eight weeks for four cocoa progenies

Code	Progeny	Mean number of aphid	Mean number of crinkled leaves
A	Na32 x T60/887	87	2.0
B	P 30 selfed	46	1.0
C	ICS 39 x Y44	167	4.0
D	T79/501 x Pa 150	96	2.0

The relationship between aphid infestation level and the number of crinkled leaves is shown in Table 20. Statistical analysis indicated that mean number of crinkled leaves was positively correlated with aphid infestation level ($r = 0.99$).

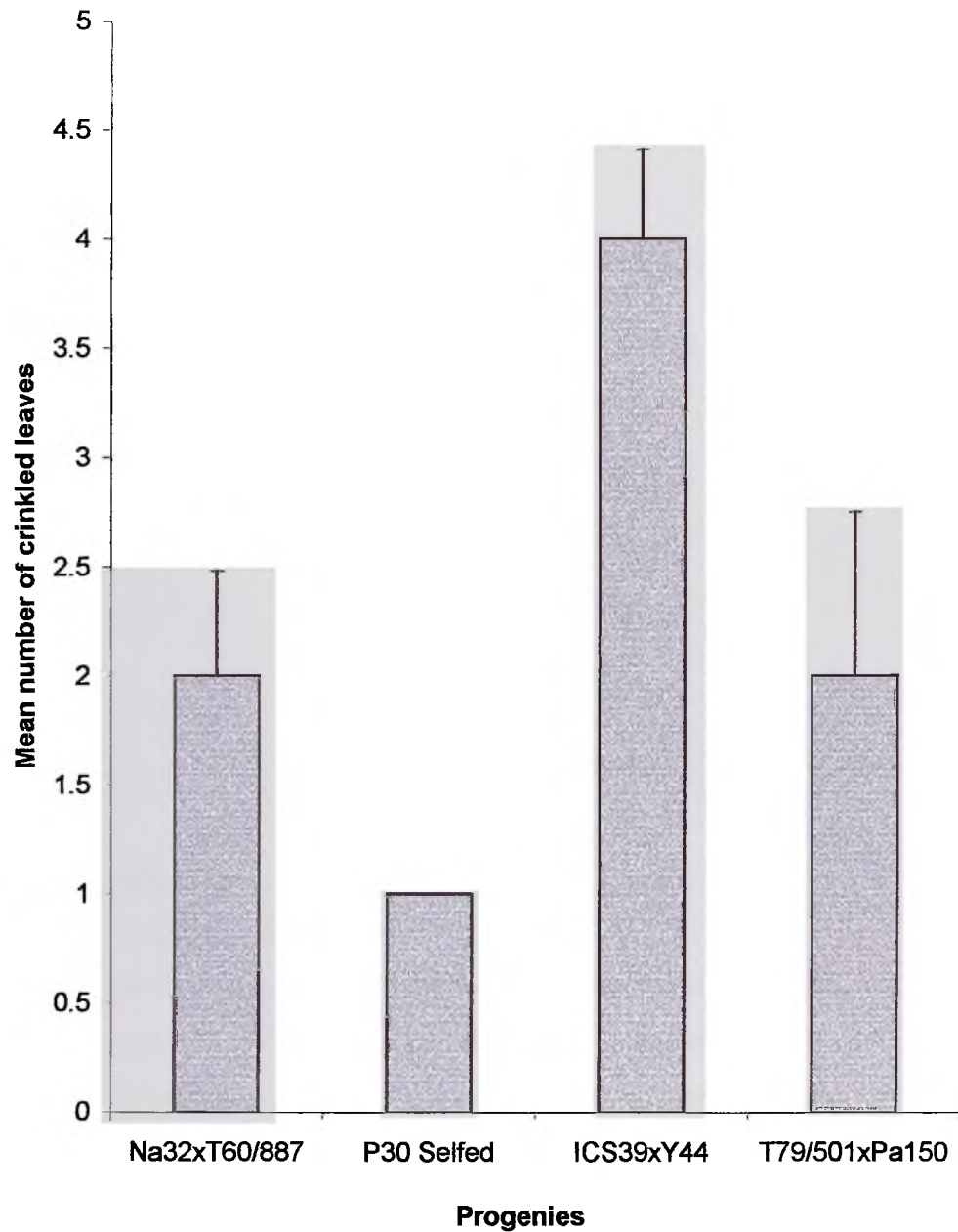


Fig10: Mean number of crinkled leaves produced after eight weeks on four cocoa progenies.

6.3.2 Aphid multiplication rate on older seedlings

The average number of live nymphs produced per adult aphid varied significantly among the progenies (Appendix 16 and Table 21)

Table 21
Mean number of aphids produced by one adult aphid on older seedlings of four cocoa progenies after 7 days

Progeny	Replicates				Mean	
	1	2	3	4		
A	29	37	33	31	32.5	b
B	19	12	16	10	14.3	c
C	47	56	50	41	48.5	a
D	60	51	54	43	52.0	a

Aphid multiplication rate was highest on progeny D, followed by C, A and B in decreasing order (Fig. 11).

The results of this study showed varietal differences in susceptibility among progenies. Using the susceptibility scale developed in chapter 5, with respect to *T. aurantii* infestation, progenies D and C are rated highly susceptible, progeny A susceptible and B resistant.

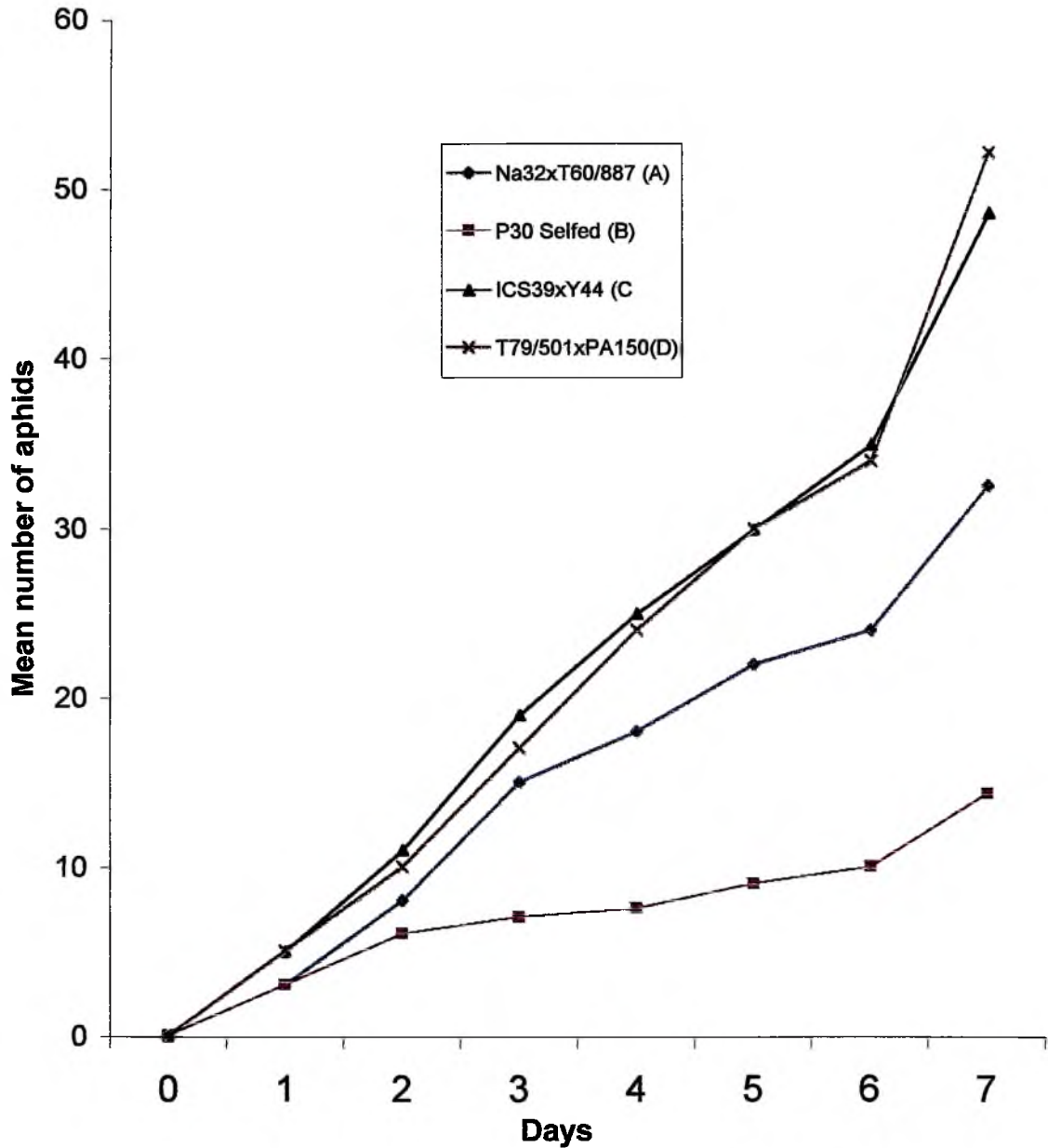


Fig11:Mean number of aphids produced on older seedlings of four cocoa progenies infested with one adult aphid for one week.

6.4 DISCUSSION

Significant differences were observed in the multiplication rates of the cocoa aphid *Toxoptera aurantii* and the number of crinkled leaves produced among the four different progenies. There were also significant differences in height and girth increments among the progenies and between the treated progenies and their controls. Distinct responses for these characters may result from the existence of different genes controlling resistance. The different aphid multiplication rates observed among the different progenies is a clear indication of the differences in susceptibility or resistance levels of the different progenies. Van Emden *et. al.* (1969) noted that the final selection of plants by aphids is favoured by the same characteristics that favour subsequent aphid multiplication. This agrees with the findings of Lowe (1974) that resistant varieties of crop plants generally support smaller aphid populations in both field and glasshouse experiments, irrespective of the aphid colonies involved. On the basis of the two evaluation methods, Y44 x ICS 39, a Trinitario progeny came out as the most preferred, followed by the Amazon progenies T79/501 x Pa 150, Na 32 x T60/887 and an Amelonado progeny, P30 selfed in decreasing order. The two methods were to a high extent consistent; in both methods the Amelonado progeny, P30 selfed, was the least preferred and, therefore, the most resistant to aphid infestation. This is consistent with the finding of Ackonor *et. al.* (1993). Working on field evaluation of insect preferences for cocoa progenies, these authors observed that some of the progenies that attracted the least aphid numbers had P 30 as one of the parents. Bassie (1997) also made a similar observation that the least preferred progenies to *T. aurantii* had P30 as one of the parents. These were Macorango x P30 and MA 12 x P30. The methods were able to differentiate between the Trinitario progeny as being more susceptible than the Amazon progenies to *T. aurantii* infestation. This confirms the well known fact that "Criollos" and "Trinitarios" are generally more

susceptible than "Forasteros". According to Pires *et al.* (1996) there is a clear association between the geographic origin of the various cocoa types and their levels of resistance/susceptibility. In his work on Witches Broom Resistance, Pires *et al.* (1996) observed that series originating in Upper Amazon and Peru or their descendants showed lower total disease infection values while those from Mexico, Central America, the Caribbean and Bahia showed high disease values. He concluded, among other things, that series that are constituted primarily by "Criollos" and "Trinitarios" presented the highest values of diseases.

In a long term study of two cocoa progenies T85/799 x T17/359 and series II B (a local Trinitario x T17/359), Bigger (1975a) found that the latter was consistently more infested by all Homoptera including *T. aurantii*. In an earlier work, he had showed that *Distantiella theobroma* was more prevalent on series II B hybrids (Bigger, 1972).

The methods used in the present work, have confirmed these observations and have established that differences exist in different cocoa progenies in their resistance or susceptibility levels. The progenies were selected from different genetic sources and, therefore, the differences observed were expected. Nicol (1945) observed severe attack by *D. theobroma* over three years on a 16 acre progeny trial at Tafo which killed many young plants and caused a general retardation in growth. He remarked that there were very marked differences in the level of damage between the seedlings and that the progeny of certain selections seemed to have escaped damage and made excellent growth. Other workers had also made similar observations that cocoa progenies vary in their suitability for and colonization by mealy bugs and other insects. (Bigger 1975a, 1975b, Firempong 1982, 1984).

The interaction of factors which lead to one progeny being less susceptible to damage by the aphid, *T. aurantii*, and for that matter other cocoa insects, than an-

other is obviously quite complex. Mc Callan (1943) working on cocoa thrips attributed resistance to difficulty in puncturing the leaf. Nguyen-Ban (1972) working on *Earias biplaga* attributed the susceptibility of UF 667 to the hairiness of its young leaves; according to him, the leaves appear to stimulate oviposition. Resistance to some cocoa clones, notably ICS 1, ICS 95, SCA 12 and SCA 6, to the cocoa beetle *Xyleborus ferrugineous* F in Ecuador was studied by Soria and Saunders (1966). They postulate that a natural toxin in the bark of SCA 12, is produced in quantities that are not sufficient to kill insects but rather makes them more active and consequently increases the number of attacks.

Again, Nguyen-Ban (1993) attributed the differences in mirid choice of cocoa varieties to differences in physical nature of chupons as well as the relative water status. Cros *et. al.*, (1995) also reported that the attractiveness of specific cocoa trees to mirids is related to the amount of flavonol present in the tree. Thus the four progenies tested in this study might have differences in the physical nature of their leaves, differences in natural toxin levels and different nutrient/water content levels which accounted for the differences in susceptibility/resistance levels.

The differences observed suggest that ample possibilities exist for genetic improvement following the selection of the most resistant individuals. It also offers the possibility of incorporating host plant resistance and a selection criterion for plant breeders attempting to expand cocoa insect control strategies.

CHAPTER SEVEN

CONCLUSIONS

A mass rearing technique which could provide abundant and reliable supply of the cocoa aphid *T. aurantii* for screening different cocoa types in the laboratory as an index for capsid resistance was developed.

Young cocoa seedlings, not more than a week after cotyledon opening and placed in ventilated cages, emerged as the best of all the plant materials tested (Leguminous, vegetable and tree crops) for rearing the aphid.

Young cocoa seedlings provided consistently adequate numbers of aphids for laboratory screening studies. The obvious advantages of the technique were that it prevented the wilting associated with cut stems used in earlier studies, permitted direct observation of insects in a cage without having to disturb them, excluded predators, and provided a more constant environment and optimum conditions for both plant and insect growth.

The adverse effect of aphid infestation and feeding was most pronounced when cocoa seedlings were infested before seven days after cotyledon opening. Clear differences in susceptibility levels were manifested in aphid multiplication rates and the number of crinkled leaves produced. The number of aphids produced after one week of infestation and the number of crinkled leaves after two months were, therefore, used to establish criteria for susceptibility for the young seedlings. With the two types of cocoa evaluated, Trinitario Y44 was significantly better preferred than the Amazon T85/799 by the aphids. In a laboratory microtest conducted to determine the level of attractiveness of the two cocoa types to mirids, *Sahlbergella singularis* showed significant preference for the Trinitario Y44 which recorded 75% of the total

feeding lesions.

Older cocoa seedlings showed significant differences in aphid multiplication rates among different clones and crosses when their flush leaves were infested and the number of aphids produced evaluated after seven days. The aphid multiplication rate level on plants infested one week after cotyledon opening was used to establish a criterion for susceptibility rating. Differences in susceptibility levels were established among the four cocoa progenies screened. The Trinitario progeny Y44 x ICS 39, was the most preferred by *T. aurantii*, followed by the Amazons T79/501 x Pa 150, Na 32 x T60/887 and the Amelonado P 30 selfed, was the least preferred.

Results from the evaluation of the two cocoa types Trinitario Y44 and Amazon T85/799 and the screening of the four progenies seem to confirm the views of earlier workers that resistance of different cocoa types to *T. aurantii* could be an indication of general insect resistance in cocoa.

The differences observed suggest that ample possibilities exist for genetic improvement following the selection of resistant individuals. It also offers a selection criterion for cocoa plant breeders and the possibility of incorporating host plant resistance in cocoa insect control strategies.

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APPENDICES

APPENDIX 1

Monthly mean maximum and minimum temperatures for ten years (Jan. 1990 –Dec. 1999) and the year 2000 for Tafo

Month	Maximum		Minimum	
	10 Yrs Ave.	Year 2000	10 Yrs Ave.	Year 2000
January	32.9	32.3	19.5	20.9
February	34.7	34.2	20.9	18.9
March	33.8	34.7	21.7	22.1
April	33.1	33.0	21.9	22.2
May	31.9	32.4	22.0	21.9
June	30.4	30.2	22.0	22.1
July	28.5	29.1	21.6	21.5
August	28.6	28.6	21.4	22.7
September	29.6	29.6	21.8	22.2
October	30.8	31.2	21.6	21.8
November	32.1	32.3	21.2	21.9
December	31.6	32.4	20.6	21.0

APPENDIX 2

Survival of aphids under starvation with time (hours)

Time (Hrs)	No of aphids alive	Percent mortality (%)
0	100	—
1	100	—
2	100	—
3	100	—
4	100	—
5	100	—
6	100	—
7	100	—
8	100	—
9	100	—
10	100	—
11	98	2
12	97	3
13	95	5
14	92	8
15	90	10
16	85	15
17	82	18
18	80	20
19	76	24
20	75	25
21	75	25
22	70	30
23	66	34
24	63	37
25	62	38
26	60	40

Appendix 2 cont.

Time (Hrs)	No of aphids alive	Percent mortality (%)
27	54	46
28	50	50
29	46	54
30	40	60
31	40	60
32	36	64
33	32	68
34	30	70
35	26	74
36	22	78
37	22	78
38	20	80
39	20	80
40	17	83
41	15	85
42	10	90
43	8	92
44	4	96
45	4	96
46	3	97
47	2	98
48	2	98
49	0	0



APPENDIX 3

Daily survival of aphids starved for eight hours on seedlings of leguminous plants, infested 3, 5, 7 and 10 days after cotyledon opening

Legumes	Day of infestation after cotyledon opening	Number of aphids on day:						
		0	1	2	3	4	5	6
Cowpea	3	5	4	2	1	0	0	0
	5	5	3	2	0	0	0	0
	7	5	2	1	0	0	0	0
	10	5	2	0	0	0	0	0
Broad bean	3	5	4	1	0	0	0	0
	5	5	3	2	0	0	0	0
	7	5	2	1	0	0	0	0
	10	5	2	0	0	0	0	0
Groundnut	3	5	4	2	1	1	0	0
	5	5	3	2	0	0	0	0
	7	5	3	2	0	0	0	0
	10	5	1	0	0	0	0	0
Green gram	3	5	3	2	0	0	0	0
	5	5	2	1	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	1	0	0	0	0	0
Pigeon pea	3	5	4	2	0	0	0	0
	5	5	3	2	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	1	0	0	0	0	0
Glyricidia spp	3	5	3	1	0	0	0	0
	5	5	2	0	0	0	0	0
	7	5	2	0	0	0	0	0
	10	5	1	0	0	0	0	0
Winged bean	3	5	3	2	0	0	0	0
	5	5	2	0	0	0	0	0
	7	5	1	0	0	0	0	0
	10	5	1	0	0	0	0	0

APPENDIX 4

Daily survival of aphids starved for eight hours on seedlings of vegetable plants infested 3, 5, 7 and 10 days after germination

Vegetable	Day of infestation after germination	Number of aphids on day:				
		0	1	2	3	4
Tomato	3	5	4	3	1	0
	5	5	4	2	0	0
	7	5	2	0	0	0
	10	5	2	0	0	0
Sweet pepper	3	5	3	1	0	0
	5	5	2	1	0	0
	7	5	1	0	0	0
	10	5	1	0	0	0
Okro	3	5	3	1	0	0
	5	3	1	0	0	0
	7	5	2	0	0	0
	10	5	1	0	0	0
Garden egg	3	5	4	2	1	0
	5	5	2	1	0	0
	7	5	2	0	0	0
	10	5	1	0	0	0

APPENDIX 5

ANOVA on aphid numbers produced on cocoa seedlings infested at four different periods (3, 5, 7, and 10 days) after cotyledon opening to examine the effect of age on aphid development

Source of variation	DF	SS	MS	F	P
Periods	3	0.17215	0.05738	19.00**	0.00
Error	12	0.03605	0.00300		
Total	15	0.20820			

**Significant at $P < 0.01$

APPENDIX 6

ANOVA to determine the effect of six different perennials on aphid reproduction after two weeks of infestation

Source of variation	DF	SS	MS	F	P
Perennials	5	10.8937	2.1787	93.21**	0.00
Error	18	0.4207	0.0234		
Total	23	11.3144			

**Signification at $P < 0.01$

APPENDIX 7

ANOVA on aphid numbers produced after one week on two cocoa types (T85/799 & Y44) infested at 3 different periods (P_1 , P_2 and P_3) to examine the effect of age on aphid development

Source of variation	DF	SS	MS	F	P
Type	1	0.3620	0.3620	23.3548**	0.00
Period	2	0.6650	0.3325	21.4516**	
Error	2	0.0310	0.0155		
Total	5	1.0580			

** Highly significant at $P < 0.01$

APPENDIX 8

ANOVA to determine the effect of aphid infestation on height increments of two cocoa types (T85/799 & Y44) infested at 3 different periods (P_1 , P_2 and P_3) after 8 weeks

Source of variation	DF	SS	MS	F	P
Type	1	1.2645	1.2645	4.3409 N.S.	10.13
Period	3	8.6277	2.8759	9.8726*	
Error	3	0.8740	0.2913		
Total	7	10.7662			

N.S. Not significant at $P = 0.05$

*Significant at $P < 0.05$

APPENDIX 9

ANOVA to determine the effect of aphid infestation on girth increments of two cocoa types (T85/799 & Y44) infested at 3 different periods (P_1 , P_2 and P_3) after 8 weeks

Source of variation	DF	SS	MS	F	P
Type	1	0.0144	0.0144	0.3956 N.S.	10.13
Period	3	0.4519	0.1506	4.1374 N.S.	9.28
Error	3	0.1093	0.0364		
Total	7	0.5756			

N.S. Not significant at $P = 0.05$

APPENDIX 10

ANOVA on number of feeding punctures made by *S. singularis* on two cocoa types (T85/799 & Y44) to determine their levels of attractiveness to capsids

Source of variation	DF	SS	MS	F	P
Type	1	7.2870	7.2870	18.07**	0.00
Error	58	23.3900	0.4030		
Total	59	30.6770			

**Significant at $P < 0.01$

APPENDIX 11

ANOVA on aphid multiplication rates for 25 pairwise crosses to examine their levels of susceptibility

Source of variation	DF	SS	MS	F	P
Crosses	24	8.23583	0.34316	67.69**	0.000
Error	75	0.38023	6.00507		
Total	99	8.61605			

**Significant at $P < 0.01$

APPENDIX 12

ANOVA on aphid multiplication rates for 25 clonal materials to examine their levels of susceptibility

Source of variation	DF	SS	MS	F	P
Clones	24	7.24049	0.30169	55.30**	0.00
Error	75	0.40913	0.00546		
Total	99	7.64962			

**Significant at $P < 0.01$

APPENDIX 13

ANOVA on the effect of young seedlings of four cocoa progenies on aphid development after one week of infestation

Source of variance	DF	SS	MS	F	P
Progenies	3	0.6581	0.2194	9.08*	0.02
Error	12	0.2898	0.0241		
Total	15	0.9479			

Significant at $P < 0.05$

APPENDIX 14

ANOVA to determine the effect of aphid infestation on height increments of four cocoa progenies and their controls after eight weeks

Source of variation	DF	SS	MS	F	P
Progenies	7	40.04	5.72	4.81*	0.002
Error	24	28.56	1.92		
Total	31	68.60			

*Significant at $P < 0.05$

APPENDIX 15

ANOVA to determine the effect of aphid infestation on girth increments of four cocoa progenies and their controls after 8 weeks

Source of variation	DF	SS	MS	F	P
Progenies	7	3.933	0.562	4.98*	0.001
Error	24	2.707	0.113		
Total	31	6.640			

*Significant at $P < 0.05$

APPENDIX 16

ANOVA to determine differences in aphid multiplication rates on older seedlings of 4 cocoa progenies after one week of infestation

Source of variation	DF	SS	MS	F	P
Progenies	3	0.8305	0.2768	45.38**	0.00
Error	12	0.0732	0.0061		
Total	15	0.9037			

**Significant at $P < 0.01$