RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY,
NKPOLU, PORT HARCOURT, NIGERIA.

A BIOCHEMICAL INVESTIGATION OF THE TAXONOMY OF THE CASSAVA GREEN MITE *Mononychellus* spp. (ACARI: TETRANYCHIDAE) IN KENYA.

BY

KENNETH KAMBONA OYUGI. BSc (Hons)
(UNIVERSITY OF NAIROBI)

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF A MASTER OF PHILOSOPHY (M.Phil) DEGREE IN APPLIED ENTOMOLOGY.

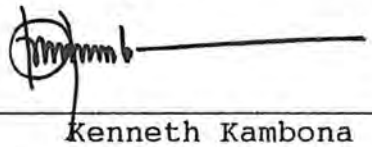
APRIL, 1989

"Africa has unfortunately concentrated on utilitarian sciences. These have been seen as passport to employment and success. We have neglected the old fashion idea of traininig the intellect, this is worrying"

Thomas.R.Odhiambo,

DECLARATIONS

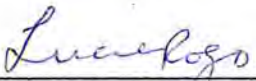
This thesis is my work and has not been presented for a degree in any other University.



Kenneth Kambona

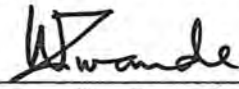
This thesis has been submitted for examination with our approvals as supervisors.

Dr. Lucie M. Rogo
The International Centre of Insect Physiology and Ecology, P.O Box 30772 Nairobi, Kenya.



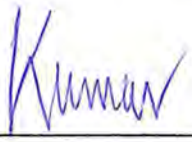
L.M.Rogo.

Dr. Wilber Lwande.
The International Center of Insect Physiology and Ecology, P.O.Box 30772 Nairobi, Kenya.



W. Lwande.

Prof. R. Kumar
University of Science and Technology, Nkpolu
P.M.B. 5080 Port Harcourt, Nigeria.



R. Kumar.

ACKNOWLEDGEMENTS

I wish to express my gratitude to the Director of the International Centre of Insect Physiology and Ecology (ICIPE), Professor Thomas R. Odhiambo for the permission to conduct this work at ICIPE. My sincere thanks to Dr. M.E. Smalley, the Academic cordinator of the African Regional Postgraduate Programme in Insect Science (ARPPIS), for the excellent way in which he handled my day to day problems during the study period.

I am most indebted to Dr (Mrs) L. M. Rogo, currently at the university of Umea, Sweden, for supervising this work and specifically for her many acts of goodwill and concern since its inception. I express my deep gratitude to Dr. W. Lwande, who as my second supervisor followed my progress throughout, giving valuable comments and suggestions. Special thanks to Prof. R. Kumar, my supervisor at The Rivers State University of Science and Technology, Nigeria for assisting me so much during the many times I had to call on his vast experience.

I wish to thank Dr A. Hassanali, unit head, Chemistry and Bioassay Research Unit, ICIPE, for extending me the unit facilities and for the useful advice throughout the entire period of my studies. It is my pleasure to thank Dr. S.K. Nokoe, unit head Biomathematics research unit (ICIPE) and Mr O. Okelo for the data analysis and interpretation.

I thank Dr. Ellie Osir of the Biochemistry Unit, ICIPE, Dr M. Hassane and Mr. F. Obunga Otieno of the University of Nairobi who offerred suggestions and critically read the manuscript. The technical assistance of Mr. J. Obara and Mr.L. Labongo of the Biological Control Sub-programme and the Biochemistry Research Unit respectively, is very much appreciated. Much thanks to all other technical staff of ICIPE who in their various capacities have contributed to the production of this work.

I would like to thank Mrs. A.A. Okumali and Mrs M.S. Myendo who made sure things went on right during my study period. Last, but not least, I owe a lot to my parents, for their understanding and constant encouragement during the course of my study.

ABSTRACT

Biochemical methods have been applied to elucidate the species systematics of cassava green mite (CGM). Traditionally CGM have been classified using the length of the dorso-central setae (D_1-D_3), which groups CGM into two species, the short setae form, ***Mononychellus tanajoa*** (Bondar) and the long setae form ***Mononychellus progresivus*** Doreste. However the validity of this method of classification is being questioned because the setae were found to vary from short to long in a continuous gradient. This characteristic, therefore becomes unreliable for species separation.

Isoenzyme analysis and cuticular hydrocarbon studies were carried out using isoelectric focusing and gas chromatography respectively. Thirteen enzyme/substrate systems were used in distinguishing closely related species. These were; Aconitate hydratase, alcohol dehydrogenase, glucose-6-phosphate dehydrogenase, isocitrate dehyrogenase, malate dehydrogenase, mannose phosphate isomerase malic enzyme, phosphoglucomutase, superoxide dimutase, nucleoside hydratase, hexokinase, glucose

phosphate isomerase and pyruvate kinase. Out of the thirteen, three enzymes proved most powerful as taxonomic indicators, namely; malic enzyme, glucose phosphate isomerase and malate dehydrogenase. CGM populations showed identically migrating bands for these enzymes. Multivariate analysis was applied to demonstrate the statistical significance of the electrophoretic data. No major significant differences were observed within the band parameters.

Hexane soluble cuticular hydrocarbons of the short and long setae forms, were separated by gas chromatography. The chromatographic profiles were identical for the two setal forms.

It is clear from these results that there are no biochemical variants in CGM populations of Kenya. Consequently, there is no evidence to warrant species differentiation. Only a single species of CGM, ***Mononychellus tanajoa*** can be considered to occur in Kenya.

DEDICATED

To

My grandfather, Ogot Oyugi,

TABLE OF CONTENTS

	pages
TITLE PAGE	i
DECLARATION	ii
ACKNOWLEDGEMENTS	ii-iv
ABSTRACTS	iv-vi
DEDICATION	vi
TABLE OF CONTENTS	vi-ix
LIST OF FIGURES	ix-xi
LIST OF TABLES	xi-xvi
LIST OF PLATES	xvi
1. GENERAL INTRODUCTION	1-9
Objective	9
2. LITERATURE REVIEW	9-24
2.1 Aspects of Tetranychid taxonomy	10-12
2.2 Taxonomic context of the genus *Mononychellus*	12-14
2.3 Application of biochemical systematics	14
2.3.1 Electrophoretic variation of enzymes as applied in taxonomic classification	14-19
2.3.2 Application of isoelectric focusing (IEF) technique in taxonomic classification	19-20
2.3.3 Cuticular hydrocarbons as taxonomic feature	20-21
2.3.4 Gas chromatography (GC) of cuticular hydrocarbons	21-24

3. MATERIALS AND METHODS..... 24-43

3.1 Collection of materials 24

3.2 Field sampling of Cassava green mites..... 24-27

3.3 Breeding Cassava green mites..... 27-29

3.4 Breeding red spider mites..... 29-30

3.5 Mounting..... 30-31

3.6 Measurements..... 31-33

3.7 Sample preparation and application..... 33

3.8 Running Isoelectric focusing..... 33-35

3.8 Staining method..... 35-40

3.10 Data recording and analysis..... 40-41

3.11 Gas chromatography..... 41

3.11.1 Sample preparation..... 41-42

3.11.2 Gas chromatographic separation..... 46-47

4 RESULTS..... 43-82

4.1 Measurements of setal lengths..... 43

4.2 Isoenzyme analysis..... 43-44

4.2.1 Malic enzyme 44-59

4.2.2 Glucose phosphate..... 62-70

4.2.3 Malate dehydrogenase..... 70-78

4.3 Cuticular hydrocarbon analysis..... 78-82

5. DISCUSSION..... 82-94

6. SUMMARY..... 94-98

7. CONCLUSION..... 98-99

8 LITERATURE CITED..... 99-119

9. APPENDICES..... 119-130

LIST OF FIGURES.

	Title	Pages
Figure 1	Initial discovery and spread of *Mononychellus* spp. across the African continent	2
Figure 2	Cassava growing areas and field collection sites	25
Figure 3	A schematic representation of the dorsal aspects of *Mononychellus* spp. showing nomenclature of body setae	32
Figure 4	A schematic representation of zymograms from IEF of malic enzyme (ME)	46
Figure 5	First and second principle component plot comparisons of the six populations of *Mononychellus* spp. using Malic enzyme	51
Figure 6	First and third principle component plot comparisons of the six populations of *Mononychellus* spp. using malic enzyme	52
Figure 7	First and fourth principle component plot comparisons of six populations of	

Mononychellus spp. using malic enzyme 53

Figure 8 Second and third principle component plot comparisons of six populations of *Mononychellus* spp. using malic enzyme 54

Figure 9 Second and fourth principle component plot comparisons of six populations of *Mononychellus* spp. using malic enzyme 55

Figure 10 Third and fourth principle component plot comparisons of six populations of *Mononychellus* spp. using malic enzyme 56

Figure 11 A schematic representation of bands from IEF of glucose phosphate isomerase 61

Figure 12 First and second principle component plot comparison of six populations of *Mononychellus* spp. using glucose phosphate isomerase 65

Figure 13 First and third principle component plot comparisons of six populations of *Mononychellus* spp. using glucose phosphate isomerase 66

Figure 14 Second and Third principle component plot comparisons of six populations of *Mononychellus* spp. using glucose phosphate isomerase 67

Figure 15 A schematic representation from IEF of malate dehydrogenase (MDH) 72

Figure 16 First and second principle component plot comparisons of six populations of *Mononychellus* spp. using malate dehydrogenase 75

Figure 17 Gas chromatogram of cuticular hydrocarbons isolated from Nairobi population of cassava green mite *Mononychellus* spp 79

Figure 18 Gas chromatogram of cuticular hydrocarbons isolated from Mombasa population of cassava green mites *Mononychellus* spp 80

Figure 19 Gas chromatogram of cuticular hydrocarbons isolated from red spider mite *Tetranychus urticae* 81

LIST OF TABLES.

	TITLE	Page.
Table 1	Sample collection sites representing various climatic zones	26
Table 2	Program for running IEF	36
Table 3	Staining conditions for isoenzymes	37-38
Table 4	Developer buffers	39
Table 5	Coefficient of correlation half matrix for variables of malic enzyme	48
Table 6	Normalized eigenvectors and variance explained by the best 4 principle components for the 10 variables of malic enzyme	50
Table 7	Analysis of variance for comparing locations using band widths of malic enzyme	57
Table 8	Analysis of variance for comparing locations using distances between bands of malic enzyme	58

Table 9 Coefficient of correlation half matrix for variables of glucose phosphate isomerase 62

Table 10 Normalized eigenvectors (weightings) and variations by the best 3 principle components for the 8 variables of glucose phosphate isomerase 63

Table 11 Analysis of variance for comparing locations using distances between bands of glucose phosphate isomerase 68

Table 12 Analysis of variance for comparing locations using band widths of glucose phosphate isomerase 68

Table 13 Coefficient of correlation half matrix for variables of malate dehydrogenase 73

Table 14 Normalized eigenvector and variance explained by the best two principle components for the six variables of malate dehydrogenase 74

Table 15 Analysis of variance for comparing locations using distances between bands of malate dehydrogenase 76

Table 16 Analysis of variance for comparing locations using band widths of malate dehydrogenase 77

LIST OF PLATES.

	TITLE	Page
Plate 1	Photograph of malic enzyme bands	45
Plate 2	Photograph of glucose phosphate isomerase bands	60
Plate 3	Photograph of malate dehydrogenase bands	71

1 GENERAL INTRODUCTION.

Cassava green mites (CGM), *Mononychellus* Wainstein belong to the subphylum Chelicerata. They are members of the subclass Acari, and are part of the agriculturally important family Tetranychidae Donnadieu. Cassava green mites are a serious pest of cassava, *Manihot esculenta* Crantz. The first records of these mites in Africa were near Kampala, Uganda in 1971, having been accidentally introduced with cassava cuttings from South America (Lyon, 1973). The mites spread rapidly in their new environment, with outbreaks reported in Kenya and Tanzania (Msabaha, 1984). Since, then this pest has been reported in 30 countries across the cassava belt of Africa as shown in fig 1 (Herren and Bennett, 1984).

Jennings (1970) reported that *M. esculenta* was introduced into Africa in the later part of the sixteenth century. With an increasing world population and demand for drought resistant crops, there has been a recent surge of interest in the study of pests of cassava, particularly in Africa, where this crop presently constitutes an important source of carbohydrates for nearly 200 million people (Herren, 1987). It is also a basic

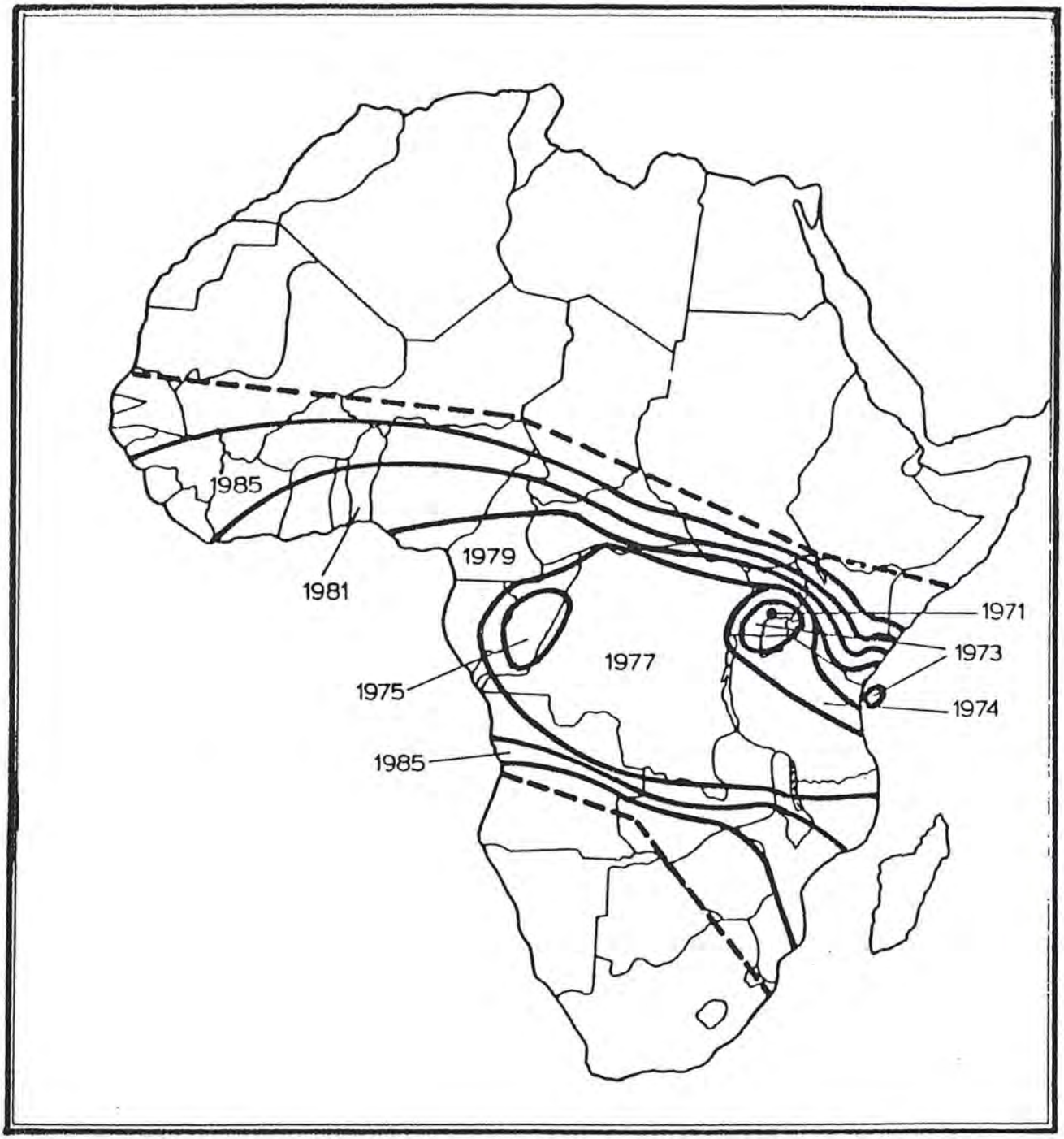


Fig 1. Initial discovery (dot) and spread (heavy solid lines) of *Mononychellus tanajoa* across the African continent. The dashed lines show the cassava belt. (Yaninek and Herren, 1988)

animal feed and a raw material for the manufacture of starch and the production of alcohol (Jennings, 1970; IITA, 1984). The increased importance of cassava has focused attention on the major constraints to its production.

A total of 10 million hectares of arable land in Africa is devoted to cassava production of which approximately 4.5 million hectares are already affected by CGM and the cassava mealybug, ***Phenococcus manihot*** Matile-Ferrero. Economic losses due to CGM have been variously estimated as follows; Doreste and Aponte (1978) estimated the loss to be 8-17% in Venezuela. In Colombia, yield reductions were 13% on susceptible varieties but only 5% on resistant varieties (Bryne *et al.*,1983), though Centro Internacional de Agricultura Tropical (CIAT, 1986) in the same region reported yield losses of 20%-53%.

Shukla (1976) while comparing infested and uninfested plants in Tanzania estimated yield losses at 80%. Nyiira (1972) reported a 3-46% loss for different varieties of cassava in Uganda. In Burundi, Ndayiragiye (1984) indicated losses of upto 32%. Sauti (1981) reported losses of upto 37% in Malawi. In all, an estimated US $ 2 billion is lost as a result of CGM infestation. This threatens the continual production of

cassava, calling for a long term effective control strategy for CGM (Yaninek *et al.*, 1987).

Chemical control of CGM is beyond the economic reach of the resource poor farmers of Africa and is ecologically unsafe. Cultural practices available to control CGM are limited and subsistence farmers in Africa grow cassava without much agronomic inputs. Resistant cultivars are not widely available in Africa either (Megevand *et al.*, 1987). Biological control has been recognized as the most promising solution to the CGM problems in Africa (Lyon, 1973). For successful classical biological control, accurate identification of the target pest is vital (Schelinger and Dout, 1964; Rosen and DeBach, 1973; Kumar, 1984; Danks, 1988).

Misidentification of the pests can often hamper the implementation of biological control. An example of this is the case of the Kenyan coffee mealybug, *Planococcus kenyae* (Le Pelley). The pest was first reported as the citrus mealybug *Pseudococcus citri* (Risso), and later to be *Pseudococcus lilacinus* Ukll. Time was wasted in exploration and introduction of the wrong natural enemies from four continents. Later, the pest was identified as an hitherto unknown species from Uganda, *Anagyrus* sp. nr. *Kivuensis*. Parasites

were subsequently introduced from that country which brought the mealybug under control (Greathead, 1970; Le Pelley, 1943). Similarly, a revisionary study of scale insects allowed the Califonian Red Scale *Aunidiella aurantii* (Maskell) to be identified and effective parasites were then found in China (Compere, 1961).

A similar situation is reported by Goto (1982) in the genus *Scirpophaga* whose larvae cause severe economic losses to rice and sugarcane. These moths have few reliable characters that can be used to separate them. They are mostly white with few or no markings and when the markings don't occur, identification becomes very difficult. This situation has led to frequent misidentification and a great deal of confusion. Inappropriate control measures have resulted from the incorrect identification. Later new characters, particularly the genitalia were found to be of taxonomic value. These have enabled correct identification to be made. It is now possible to separate *Scirpophaga nivella*, a very important pest of rice, from *S. excerptalis* that damages sugarcane. Other authors who have stressed on the importance of correct identification of species involved in biological control include, Kumar (1984) and Unruh and Goeden (1987)

Our knowledge of CGM taxonomy, particularly in Africa is far from satisfactory and has been a subject of controversy for sometime (Guttierrez, 1987). CGM infesting cassava in Africa have been identified using morphological characters. Two closely related species *Mononychellus tanajoa* (Bondar)and *Mononychellus progresivus* Doreste are said to occur. The two have been differentiated by the length of the dorsal-central body setae in relation to the longitudinal distances between the setae (Doreste, 1981). Rogo *et al.* (1987) have shown in their recent study that these characters are variable and cannot differentiate CGM into distinct taxa. The current state of confusion is best seen by examining the parallel courses of research in South America and Africa.

In South America, *Mononychellus* was first described by Bondar in 1938 as *Tetranychus tanajoa* (Bondar) using female mites. Flechtmann and Baker (1970) re-examined the female dorsum from the type region and assigned it to the genus *Mononychus* Wainstein. Doreste (1981) re-described the females using the relative lengths of the dorso-central setae in relation to the longitudinal distance between the setal bases and for the first

time, described males of *M. tanajoa* using specimen from Venezuela. Rogo *et al.* (1987) examined several females and males of both *M. tanajoa* and *M. progresivus* from their topotype and concluded that Doreste's (1981) description of the two species had several discrepancies. For example the length of dorsal-central body setae (D_2-D_3), used in the description vary from short to long in a continuous gradient. Examination of the males also showed that terminalia were all similar and resembled those described for *M. progresivus* males (Doreste, 1981).

Reporting on the situation in Africa, Lyon (1973) initially described CGM as *M. tanajoa*. Later Flechtmann *et al.*(1982) reported the presence of *M. progresivus* on cassava in Gabon and Nigeria. Macfarlen (1984) suggested that although both species were in Africa, *M. progresivus* was more abundant. Rogo *et al.* (1988) re-examined CGM from a number of African countries using Principle Component Analysis (PCA) and concluded that there was only one species, designated *M. tanajoa*.

Complimentary hybridization studies carried out on ecologically different CGM populations to provide quantitative assessment of the genetic relationships among the populations showed a high degree of genetic compatibility. The results

indicate that only one species exists (Murega, pers. comm.). The confusion that prevailed in the naming of cassava green mites has led certain authors to speak of a *Mononychellus* species complex infesting cassava in Africa.

Objective:

The primary objective of this work was to investigate characters other than morphological which can be used to clarify the taxonomic status of cassava green mites. For this purpose:

1. Measurements of the setal length were carried out to ascertain whether morphological criteria for characterization correlated with biochemical methods of classification.

2. Isoenzymes were analysed studied using isoelectric focusing. The banding patterns resulting from IEF separation were used to characterize CGM

3. Extracts of cuticular hydrocarbons were analysed using gas chromatography. In which the relationship between of cuticular hydrocarbon composition and taxonomic grouping of CGM were examined.

It is hoped that with the correct identity of CGM, Biological control workers would be able to design appropriate control strategies for the specific species of CGM devastating cassava in Africa.

2. LITERATURE REVIEW.

2.1 Aspects of Tetranychid taxonomy.

Members of the family Tetranychidae Donnadieu, as said earlier are probably the most serious plant feeding group of the Acari. They cause enormous loss of yield to various crops each year (Jeppson *et al.*, 1975). Inspite of their agricultural importance, the systematics of spider mites has not drawn the concerted effort of many acarologists (Baker, 1979).

The history of spider mite systematics dates back to 1758 when emphasis was placed primarily on the description of the species. This era began with Linnaeus, when he described twenty nine species. However the first semblance of a higher classification of spider mites was presented by Donnadieu in 1875 when he established the family name Tetranychidae. Murray (1877) in his "*Economic Entomology Aptera*" presented a comprehensive treatment of the spider mites. In the late 1800's meaningful descriptions and carefully constructed illustrations of spider

mites were made by Antonio Berlese who recognized the importance of the empodium of the tarsus as a taxonomic character.

Ewing (1913) described several spider mite species and for the first time used the profile of the male genitalia for species diagnosis. The setal arrangement, types of setae and striation patterns were still poorly understood. Major studies involving higher classification of Tetranychids have since been reported by Pritchad and Baker (1955), Wainstein (1960).

The division of the Tetranychidae is currently based on a combination of characters which have proved valuable in erecting various taxa. Most of these characters are morphological for example, the aedeagus, the peritremes, empodium, leg setae, placement and relative sizes of body setae, egg shape, and the appendages. Recently the nature and distribution of the cuticular lobes and colour of the haemolymph have been adopted as taxonomic features (Boudreaux, 1963). Bob-Manuel (1987) in her study of cassava green mite taxonomy, used the ontogeny of body setae in the classification.

2.2 Taxonomic context of the genus *Mononychellus*.

Cassava green mite was originally classified in the genus *Tetranychus* (Dufour). Bondar (1938) described CGM as *Tetranychus tanajoa* (Bondar) using females from Bahia, Brazil. His description was inadequate since it was done using only females, but he did state that *T. tanajoa* had short strong dorsal central body setae. Flechtmann and Baker (1970) later studied specimens collected from *Manihot* sp. in the type region and transferred *tanajoa* to a new genus *Mononychus*. Wainstein (1971) however found that the genus *Mononychus* was preoccupied and changed the generic name to *Mononychellus*. Subsequent authors recognized the species as *Mononychellus tanajoa* (Flechtmann, 1977).

The name *Mononychellus* was given to all cassava green mites collected from Africa for many years since it's discovery in the continent. However in 1979 Doreste reported another species *Mononychellus estradai* (Pritchard and Baker) in South America and then the same author in 1981 described 2 new species of *Mononychellus* collected from the same cassava host, also in South America. These were, *Mononychellus progresivus* and

Mononychellus manihoti (Doreste, 1981). He also reported the occurrence of *M. tanajoa*.

Doreste, (1981) distinguished females of *M. progresivus* from *M. tanajoa* on the basis of the lengths of the dorso-central body setae and the distances between them. *M. tanajoa* was described as having short dorsal-central body setae (D_1-D_3) and a strong straight aedeagus, ending in a bulge with two projections sharply directed forward at the basal part. Doreste further described the presence of nine tactile setae, one slender solenidion on tibia I and seven tactile setae on tibia II. On tarsi I are five proximal tactile setae and one solenidion, while on tarsi II are three proximal tactile setae and one solenidion. *M. progresivus* was described as having long dorso-central body setae (D_1-D_3), and the aedeagus which lacked a strong ventral hook. Tarsus I had four proximal tactile setae and one solenidion . Tibia I had eight tactile setae and one solenidion.

However Rogo *et al.*(1987) examined materials from south America and concluded that the length of the dorsal-central body setae (D_1-D_3), the tibial count and the male terminalia could not differentiate the two species since no clear distinction of these characters was apparent in cassava green mite specimens examined. The

validity of the differentiation of CGM, based on setal lengths is questionable particularly for specimens collected from Africa. There is need for a revisionary study of the genus *Mononychellus* using characters other than morphology.

2.3 Application of Biochemical Systematics.

Goto (1982) reported that for many years it had been known that there are demonstrable differences in the biochemical composition of different organisms. In cases where distinction between closely related species becomes difficult on morphological grounds, the value of biochemical data in systematic studies is widely acknowledged. Two common characters used in biochemical systematic are:

(a) Enzymes

(b) Cuticular hydrocarbons.

2.3.1 Eletrophoretic variation of enzymes as applied in taxonomic classification.

Electrophoretic techniques have become principal tools for characterizing enzymes in organisms. The method is based on the fact that proteins have a charge and are therefore able to

move when placed in an electric field. Electrophoretic techniques were first used to distinguish between multiple fractions of serum proteins. These techniques were sufficiently reformed to permit examination of large numbers of different proteins in organisms (Ayala and Powel, 1972).

In recent years, electrophoretic separations of multiple molecular forms of enzymes (isoenzymes) have been applied in clarifying the often confusing taxonomic status of biotypes, races, or sibling species of pests and vector species complexes. Differentiation of species by isoenzyme analysis has been reported by Petersen (1982) who characterized five *Lutzomyia* Franca spp. collected in Panama using cellulose acetate electrophoresis. Miles and Ward (1978) performed preliminary isoenzyme studies employing starch gel electrophoresis to characterize two colonies of *L.flaviscutella* (Mangabeira). They showed that malate dehydrogenase (MDH) and glucose phosphate isomerase (GPI) are good diagnostic features.

Ward *et al.* (1982) investigated enzymatic variation in *Phlebotomus permicoccosus* and other sandfly species collected in Southern France and Tunisia, and concluded that phosphoglucomutase (PGM), hexokinase (HK), alpha glucose-phosphate

dehydrogenase (G6PD) and glutamine oxaloacetate (GOT) could be used to separate *Phlebotomus* (Rondani) species.

Krutzer (1979) used cellulose acetate to investigate isoenzymes in two Melanoconian mosquitoes; *Culex ocossa* Dyar and *Culex panacossa* Dyar and Panacossa. He successfully classified various forms of these species. Saul *et al.*(1977) employed polyacrylamide gel electrophoresis to separate *Culex papiens* Weidemann, *Culex territans* Walker and *Culex restuans* Theobald. Saul and Gimstad (1977) differentiated *Culex isalinerius* (Coq.) and *Culex papiens quinquefasciatus* (Say) using a similar method.

Characterization of *Aedes scutellaris* Theobald complex by enzyme variants was reported by Townson *et al.*(1977) in which *Aedes polynesiensis* Marks and *Aedes pseudoscutellaris* could be differentiated by esterases. Alpha-glycerophosphate dehydrogenase was also used to distinguish *Aedes malayensis* Colles from *Aedes scutellaris* Theobald. Alcohol dehydrogenase (ADH) allows the separation of the closely related *Aedes polynesiensis* and the 'Tufahi' forms.

In the identification of *Anopheles* Meigen complex, Bianchi and Chesa.(1970), distinguished *An.atroparvus* (Van Thiel) from *An.*

labranchidae Falleroni by gel electrophoresis. They also used hydroxybutyrate dehydrogenase and superoxide dimutase (SOD) to distinguish *An. beklemishevlvi* (Stegnii and Kabanova) from *An. messeae*. Steiner *et al.* (1982) detected enzyme differences among, *An. argyritarsis* Theobald, *An.albitarsis* Lynch, *An. braziliensis* and *An. darlingi* (Root) using cellulose acetate electrophoresis. Miles (1979) surveyed 22 enzyme systems in *An. gambiae* Giles complex using starch gel electrophoresis and distinguished between the various strains.

May *et al.* (1977) found interspecific differences in allelic frequencies in four enzymes of the *Simulium jenningsi* group using starch gel electrophoresis. Synder (1982) investigated enzyme characters of *Simulium venustum verecundum* complex by starch gel electrophoresis. Meredith (1982) established that a variant of Xanthine dehydrogenase (XDH) can distinguish the 'Sanje' and 'Turiani' forms of *Simulium* Latrielle spp. Meredith and Townson (1981) screened 44 enzyme systems of which 2 enzyme systems, phosphoglucomutase and Trehalase were found to separate *S. yahense* Vajime and Dunbar from *S. squamosum* (Enderlein). In addition these two could be separated from *S. sirbanum* Vajime and

Dunbar, *S. damnosum* Theobald, *S. sactipauli* and *S. soubrense* Vajime and Dunbar.

Pashley (1987) conducted electrophoretic surveys of *Spodoptera frugiperda* Smith in which 35 enzymes were examined, five of these were able to differentiate between the various strains of *S. frugiperda.* Menken (1988) carried out electrophoretic separation on geographical populations, host races and sibling species of Lepidoptera and Diptera and was able to distinguish between species within these orders.

Daly and Fisk (1988) used cellulose acetate electrophoresis to examine enzymatic variation in two Australian species of *Heliothes* Hubner; *H. armigera* (Hubner) and *H. punctigera* Wallengren. ICD-1 ICD-2 and PGD clearly distinguished these two forms. Singh and Cunningham (1981) reported on intra and inter generic variation in aphid enzymes.

In mites electrophoretic separation include the work of Ward *et al.*(1982) who examined enzyme polymorphism and differentiation in three species of spider mites using starch gel electrophoresis. Sula and Weyda (1983) investigated esterase polymorphism of two spotted spider mites, *Tetranychus urticae* Koch.

Cardwell-Grafton et al. (1988) distinguished

among females of three spider mite species *Tetranychus turkestani* Ugarov and Nikolski, *T. urticae* koch and *T. pacificus* McGregor using cellulose acetate electrophoresis. Blank (1979) used disc electrophoresis to separate *Sancassania berlesii* Michael from *Tetranychus urticae* and *T.turkestani*. Cicolani *et al.* (1981) used starch gel electrophoresis to clarify the relationship of mesostigmatid mites.

2.3.2 Application of Isoelectric focusing (IEF) technique in taxonomic classification.

Isoelectric focusing is an electrophoretic separation method in which a pH gradient is established between two electrodes and is stabilized by carrier ampholytes. Proteins focus at their isoelectric points (pI). The pI defines a physical parameter of the protein and therefore has great taxonomic significance.

Bark *et al.* (1976) used IEF in analysing polymorphic phosphoglucomutase (PGM) and reported the method to be more sensitive in showing three PGM phenotypes (PGM 1, PGM 2, PGM -2-1). Allsops and Gibson (1983) compared the effectiveness of starch gel electrophoresis and IEF in agarose for the separation of lactate dehydrogenase (LDH) of

Theilaria parva. Similarly glucose phosphate isomerase (GPI) of various cestode orders were analyzed using IEF. This technique proved more discriminatory than starch gel electrophoresis.

Allen *et al*.(1974) separated proteins differing in their pI by only 0.0025 pH units using IEF. This established IEF's effectiveness over the other methods of protein separation used in biochemical taxonomy. Heinz and Buschinger (1988) studied variability of esterases in species complexes of the *Leptothoracini* using IEF, and concluded that IEF offered a higher degree of reproducibility and resolution required for the critical analysis of the esterases.

2.3.3 Cuticular hydrocarbons as taxonomic features

The surface of arthropods is covered with a complex mixture of aliphatic compounds. These surface lipids play a key role in the survival of the organisms by providing protection from desiccation, as well as serving as a barrier to abrasion, micro-organisms and chemicals. They have been shown to be important in chemical communication, as sex attractants and

2.3.4 Gas Chromatography (GC) of Cuticular hydrocarbons

Carlson and Walsh (1981) differentiated two West African female black flies, *Simulium sirbanum* V.and D. and *Simulium squamosum* (Enderlain) which are vectors of *Onchocerca volvulus* (Leuckart) by GC analysis of their cuticular hydrocarbon . Differentiation between *Anopheles gambiae* Giles complex by GC analysis is reported by Carlson (1980).

Carlson (1986) also analyzed the cuticular hydrocarbons in species of *Glossina* Weidemann which showed marked differences in chromatographic profiles of the males. Similarly subspecies of *Glossina palpalis* R-D.,*G. palpalis palpalis* R-D. and *G. palpalis gambiensis* were distinguished by GC separations of their hydrocarbon components.

Nelson *et al.* (1988) examined the methyl-alkenes of four species of the *Palpalis* R-D group *Glossina fuscipes fuscipes, G. palpalis palpalis, G. palpalis gambiense, G. tachninoides* Westwood, and one species of the *Fusca* Walk. group *G. brevipalpalis*, to determine the presence of unique or specific methyl-alkanes which could be used as taxonomic indicators.

Cuticular hydrocarbons of nine Acridids; *Schistocerca vaga* Scudder , *Schistocerca americana*, *Schistocerca gregaria* (Forsk) (Jackson, 1982), *Melanoplus sanguinipes* (Fabricious), *Melanoplus parckardii*, *Melanoplus bivittatus* (Say), *Melanoplus fermurrumbrum* (DeGeer), *Melanoplus differentiales* (Thomas) and *Melanoplus dawsoni* have been studied for taxonomic purposes (Howard, 1982)

Lockey (1980) examined the extent to which cuticular hydrocarbons could be used in classifying groups within the family Tenebrionidae and found that hydrocarbons of *Onymacris plana*, *O.rugatipennis*, *Physadesmia globosa* (Haag) and *Stenocara gracilipes* (Haag) had several features in common. At the same time certain hydrocarbon components could be used to distinguish the two species of *Onymacris* Allard from *P. globosa* and *S. gracilipes*, and to distinguish *S. gracilipes* from *P. globosa*.

Haverty *et al.*(1988) evaluated the quantitative and qualitative variation in cuticular hydrocarbons of the Dampwood termites *Zootermopsis* as a step towards the revision of the genus. Similarly, Howard *et al.* (1988) characterized *Nasutitermes corniger* (Motshulsky)

and ***N. ephratae*** (Holmgren) by capillary gas chromatography. The same author (Horward, 1988) also differentiated two sympatric North American ***Rhinotermitids*** Light species; ***Reticulitermes flavipes*** (Kollar) and ***Reticulitermes virginicus,*** (Banks) using gas chromatography.

Jackson (1971) reported on the qualitative and quantitative analysis of cuticular hydrocarbons of two cockroach species, ***Periplaneta japonica*** and ***Periplaneta americana*** (L.) as a method of separating the species. Similarly Carlson and Brenner (1988) used the quantitative differences in cuticular hydrocarbon profiles to identify the various stages of three cockroach species , ***Blattella asahinai*** Mizukubo, ***Blatella germanica*** (L.) and ***Blattella vaga*** Hebard.

To the best of my knowledge this is the first time that GC has been used to study cassava green mites. The degree of success demonstrated by the above, and similar investigations using biochemical techniques show that electrophoresis and chromatography could serve in resolving the taxonomic problem of CGM.

3. MATERIALS AND METHODS

3.1 Collection of materials

Cassava green mites were collected from six localities which were representative of the major cassava growing areas of Kenya as shown in fig 2. The collection was done during the dry season (November-January), when mite populations were high (Akinlosotu, 1981; Yaninek *et al.*, 1987). The sampling method was based on earlier distribution and taxonomic studies of *Mononychellus* spp. in Kenya by Rogo *et al.* (1987, unpublished). Sampling design took into account possible variations in the mites based on different eco-climatic conditions within Kenya (Table 1).

3.2 Field Sampling of Cassava Green Mite

Sampling techniques used in this study are like those reported by Nachman (1984). Mite collections were carried out in fields at every 15

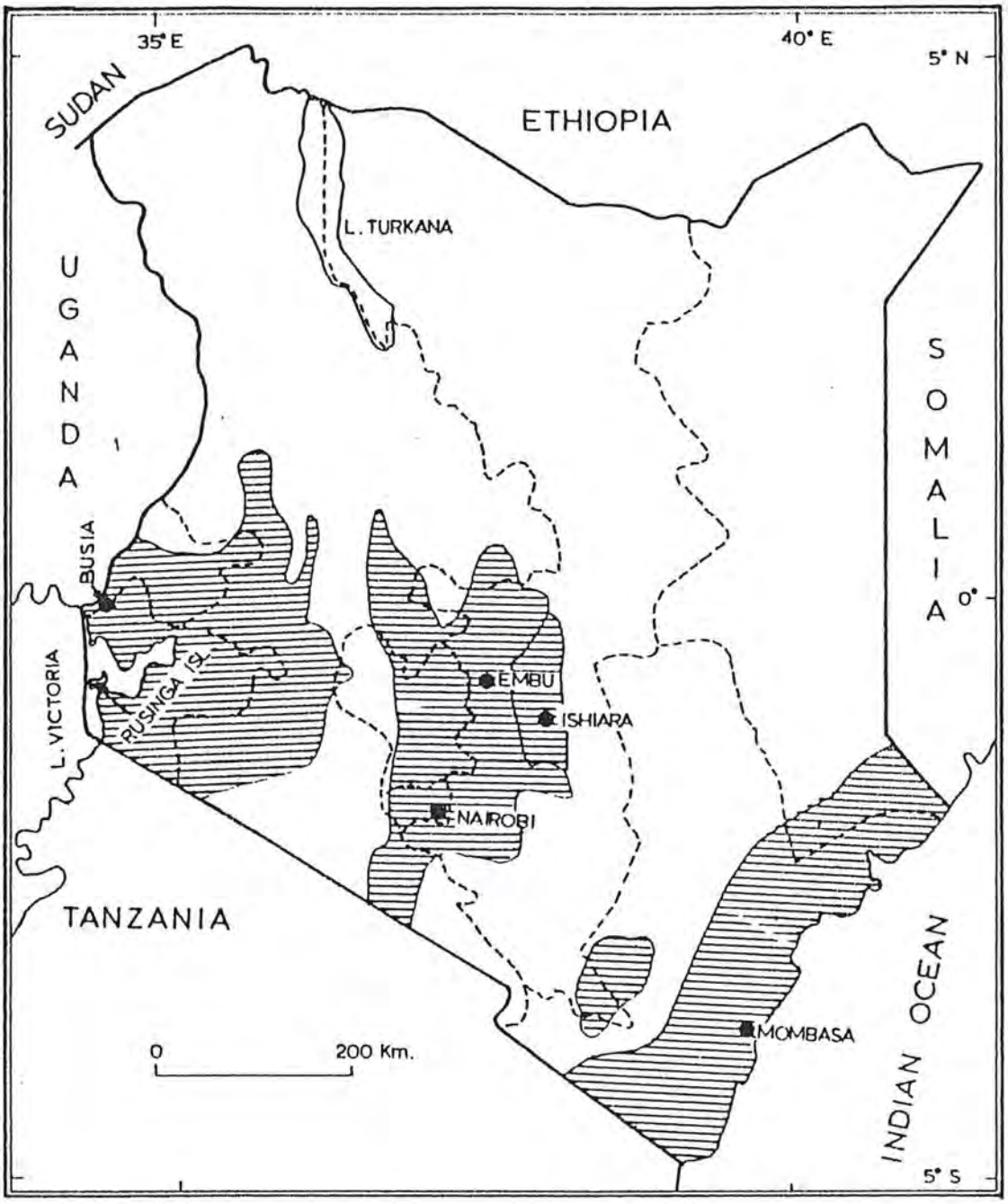


Fig 2. Cassava growing areas of Kenya and the collection site of cassava green mites

Tables 1: Sample collection sites representing various climatic zones.

Collection Site	Altitude (meters)	Climatic Zone
Busia	1500	Equatorial
Rusinga (Lake Basin)	1000	Dry sub-humid
Embu (Central Highlands)	1524	Sub-humid
Mombasa (Coastal Plains)	0	Dry sub-humid
Nairobi	2134	Semi-arid
Ishiara	914	Arid

kilometres interval along a road transect, within a cassava growing area. Plant samples were chosen at random. At every stopping point, a 40 cm long wooden peg, with one sharpened end was thrown from the edge of the cassava field. The nearest cassava plant to the sharpened end was sampled.

From each sample plant, the first 7 fully expanded young leaves from the shoot tip were taken and placed singly in a cellophane bag (7 cm x 12 cm). The bag was in turn placed in a plastic sandwich box measuring 16 cm x 10 cm x 6 cm and its lid replaced immediately to avoid desiccation. Leaves handled this way remained in perfect condition for 7 days at ambient temperature and humidity conditions of about 22 ^{o}C -29 ^{o}C and 50% - 70% RH respectively.

3.3 Breeding cassava green mites.

Pure-line progenies were required for biochemical studies (Weyda, 1983). Breeding experiments were therefore performed among populations of cassava green mites. Female specimens from the field were made to lay eggs on fresh leaf discs measuring 15 mm in diameter. On hatching the larvae developed through the succeeding stages into adults. A quiescent female

deutonymph and an active adult male of the same progeny were transferred to a separate leaf disc and allowed to mate. Males were removed a day after and adult females left undisturbed to oviposit eggs for 10 days. The resulting progeny were reared to adult stage.

The leaf disc method of rearing, described by Helle and Overmeer (1985) was employed. This method enables easy observation of the mites under a stereo-microscope. Young, fully expanded cassava leaves of a variety locally called 'kibandameno' were made into leaf discs using a cork borer. The leaf discs were washed, dried and then put with their upper surface on a wad of water saturated cotton wool placed in a petri-dish. The petri-dishes were in turn placed in trays measuring 380 mm x 230 mm x 30 mm. Water was added to the trays.

The trays were put in a growth chamber covered with fine netting material to keep out flying insects. A 60 W incandescent bulb was used to illuminate the chamber and provide the necessary warmth. Crawling insects were avoided by placing the growth chamber in a galvanized trough. Conditions within the chamber were maintained at 26 ^{o}C - 28 ^{o}C and 65% - 75% relative humidity. Four day old adult females of every

generation upto the fifth generation were picked into a Beckmann^{TM} polyethylene tube and stored in a deep freezer at -20 ^{o}C until required for electrophoresis. The fifth generation mites were used in the electrophoresis to avoid the problem of in-breeding which might result in laboratory specimens differing from the wild type.

3.4 Breeding red spider mites (RSM)

The red spider mite, *Tetranychus urticae* Koch, was used as a reference material. These were reared on potted kidney bean plants, *Phaseolus vulgaris* (L.) which were placed in a green house as described by Helle and Overmeer, (1985). Young bean plants were initially infested with ten males and the same number of adult females of RSM. These were subsequently left to multiply on the foliage. When the plants became densely populated, they were replaced with fresh ones, thus assuring availability of a large colony of the red mites. Adults were collected by tapping the leaves over a white piece of paper and then picking those that fell on the paper using a fine camel hair brush.

3.5 Mounting

Mounts of cassava green mites were made for microscopic examination. CGM were killed in 70% ethyl alcohol and mounted in Hoyer's medium according to the method of Pritchard and Baker (1955). Hoyer's medium consists of; distilled water (50 ml), gum Arabic (30 g), chloral hydrate (200 g) and glycerol (16 ml). The ingredients were mixed at room temperature in the sequence listed above. Periodic stirring for about 14 days was necessary.

A drop of Hoyer's solution was placed at the centre of a clean 20 mm x 60 mm microscope slide, mites were carefully placed onto the centre of the drop. Females were oriented with the ventral side down, and all appendages extended. Males were mounted in profile in order to enable examination of the aedeagus. A cover slip was then placed over the specimen. To expand and clear the specimen, the slide was warmed over a spirit lamp and left to dry at 55 ^{o}C in a Fischer slide warmer for 5 - 7 days. Dry slides were sealed with neutral nail polish.

3.6 Measurements.

Measurements of the dorsal central body setae, D_1-D_2 (Fig. 3) were made using a Lietz phase contrast microscope. A stage micrometer calibrated in millimicrons (µm) was mapped onto an eye piece graticule. Dimensions on the stage micrometer were then calibrated with the divisions on the eye piece graticule under a x40 objective.

For example 0.05 mm of the stage micrometer was equivalent to 18 divisions on the eye piece graticule. To convert to millimicrons the values of setal lengths is multiplied by value equivalent to a single division in the eye piece;

E.g. $0.05 \times 1/18$

$= 2.78$ µm

The setal lengths were categorized according to Rogo *et al.* (1986) as follows;

D1	D2	D3	
$\leq$ 7 µm	$\leq$ 9 µm	$\leq$11 µm	short setae
$\geq$ 10 µm	$\geq$ 10 µm	$\geq$15 µm	long setal

Values of intermediate setae forms fall in between those of the long and short forms. Measurements were done for five mites from each study region. These measurements were important in ascertaining whether the setal length variations correlated with possible biochemical variation.

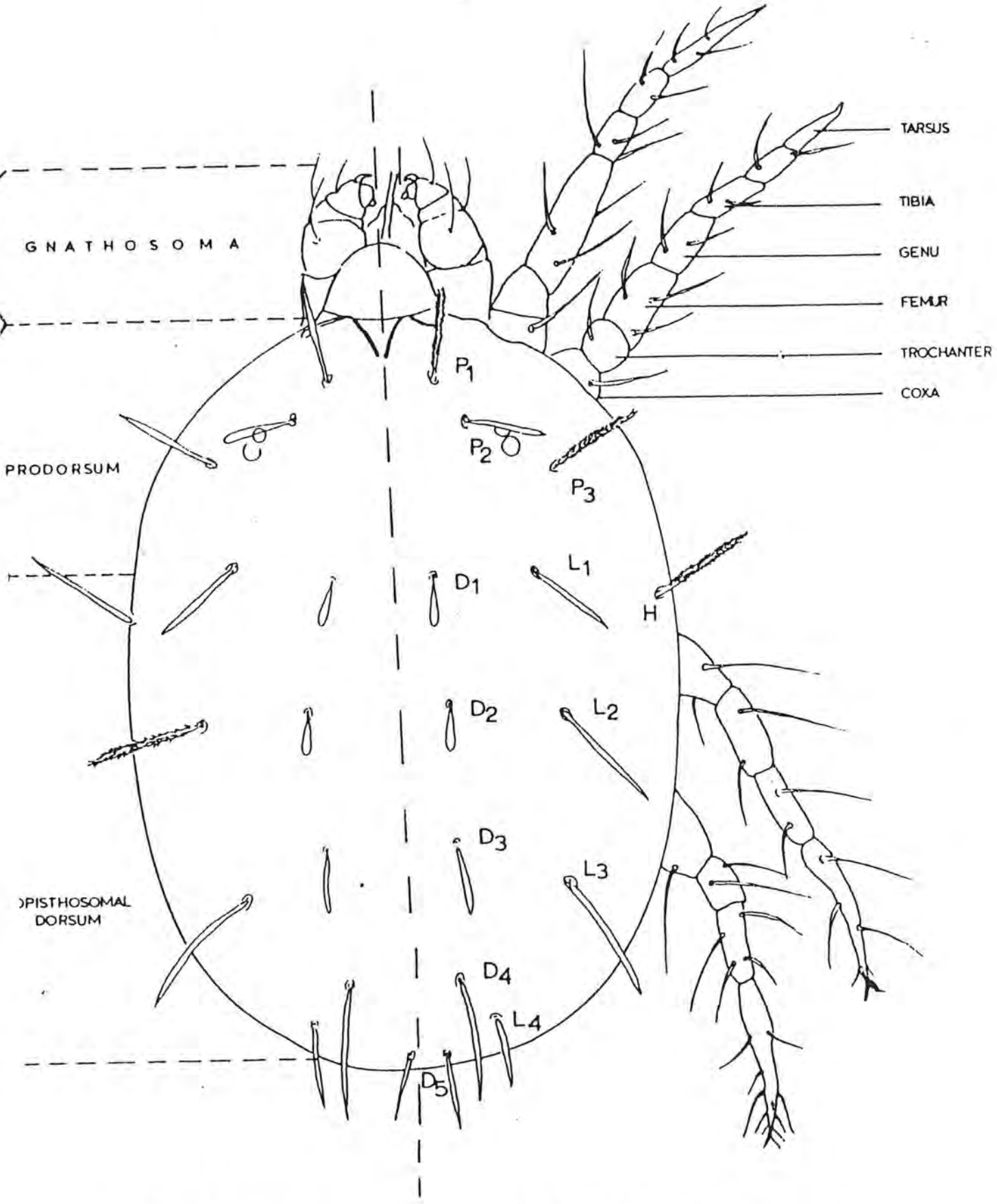


Fig 3 Schematic representation of the dorsal aspect of *Mononychellus* spp. showing the nomenclature of body setae (Helle and Sabelis, 1985)

3.7 Sample preparation and application.

Mite samples were homogenized in freshly prepared solutions by the method of Sula and Weyda (1982, 1983). Thirty single line adult females of the CGM and RSM were manually macerated in polyethylene disposable microtubes using a close fitting stainless steel pestle. 1 µl of 0.1% phenylmethylsulphonylfluoride (PMSF), 5 µl of 0.1% phosphate buffer solution (PBS) and 1 µl of 0.1% triton X-100 detergent were used as homogenization buffer. Triton X-100 extracts membrane-bound enzymes, making them available for analysis. The crude homogenate was allowed to settle for one hour before centrifugation.

Samples were spinned in a Beckmann™ microfuge at 10,000 g for 10 minutes at 4 ^{o}C. The homogenate supernatant was carefully transferred to clean polyethylene microtubes and used for electrophoresis.

3.8 Running Isoelectrofocusing

Isoelectrofocusing was done according to the technique of Cox and Willis (1985) with several modifications for optimal

separation. The gels were then stained for thirteen enzymes listed below ; Aconitate hydratase (Acon. E.C. 4.2.1.3), alcohol dehydrogenase (ADH. E.C. 1.1.1.2), glucose-6-phosphate dehydrogenase (G6PD E.C. 1.1.1.49), isocitrate dehydrogenase (ICD. E.C. 1.1.1.42), malate dehydrogenase (MDH. E.C. 1.1.1.37), mannose phosphate isomerase (MPI. E.C. 5.3.1.8), malic enzyme (ME. E.C. 1.1.1.40), phosphoglucomutase (PGM. E.C. 2.7.5.1), superoxide dimutase (SOD. E.C. 1.15.1.1), nucleoside hydratase (NH. E.C. 3.2.2.1), hexokinase (HK. E.C. 2.7.1.1), phosphoglucose isomerase (GPI. E.C. 5.3.1.9), pyruvate kinase (PK. E.C. 2.7.1.40).

A commercially available, already prepared, high performance agarose PhastGel^{TM} media was used. The gel dimensions were; 43 mm X 50 mm, length 37 mm, gel matrix 3%, Pharmalyte concentration of 0.22 (um), and a 3-9 pH gradient. The homogenate was transferred, using a micropipette, into wells formed out of a sample-well stamp using a parafilm. A PhastGel^{TM} applicator was then loaded by dipping into the wells containing the homogenate. The applicator took up samples on its grooves by capillary action. The PhastGel was placed in the separation chamber and the system programmed to perform a

prefocusing for 75 volt hours (vh). The PhastSystem was also programmed to lower and raise the sample applicator thereby applying the samples for 15 vh onto the gel automatically. The duration of separation (focusing) was 410 vh. All populations and the reference material were uniformly applied on the gel. The running conditions are shown in Table 2.

3.9 Staining method

Isoelectrofocused gels were stained using specific histochemical techniques for isoenzymes and general proteins. The reaction mixtures were modifications of those developed by Shaw and Prasad (1970), Blanq *et al.* (1986). The isoelectrofocused gel was immersed in the staining solution specific for each isoenzyme (Tables 3 and 4). The gel was incubated for 10-15 minutes in the dark at 37 ^{o}C until the bands were visible. Thereafter, it was removed from the reaction mixture, rinsed and fixed in 7% acetic acid at room temperature for 15 minutes. A hair drier was used in drying the gel.

Table 2: Program for running IEF (After Olsson et. al. 1988)

Step	Stage	Voltage V	Current in mA	Power in Watts	Temperature in °C	vh
1	Prefocusing	2000	2	3.5	15	75
2	Sample application (a)	200	2	3.5	15	15
3	Focussing (b)	2000	5	3.5	15	410

(a) Sample applicator down at Step 2 after 0 vh (accumulated 75 vh)

(b) Sample applicator up at Step 3 after 0 vh (accumulated 90 vh)

TABLE 3: STAINING CONDITIONS FOR ISOENZYMES (letters refer to developer buffers on table 4)

ENZYME	DEVELOPER BUFFER	H_2O	ADDITIONAL IONS	COENYMES	LINKING ENZYMES	SUBSTRATES	VISUALIZTION METHOD
1 Aconitate hydratase	A 6.0ml	0.2ml	1M mgcl2 0.1ml	NADP (mg/ml) 0.3ml	ICD (E.C.1.1.1.42) 2 units	n-propanol 0.7ml	MTT 5mg/ml 1ml PMS 2mg/ml 0.5ml
2 Alcohol dehydrogenase	A 6.5ml	0.8ml		NADP 0.5ml		n-propanol 0.7ml	MTT 1ml PMS 1.ml
3 Glucose-6-phosphate dehydrogenase	E 7.0ml	0.8ml		NADP 0.5ml		D-glucose-6-phosphate 10mg/ml	MTT 1ml PMS 1ml
4 Glucose phosphate isomerase	A 6.5ml	0.5ml	1.0M mncl 0.2ml	NADP 0.5ml	G6PD 5ml	D-fructose-6-phosphate (10mg/ml) 0.8ml	MTT 1ml PMS 0.5ml
5 Isocitrate dehydrogenase	E 6.5ml		1.0M $Mncl_2$ 2ml	NADP 0.3ml		DL-isocitric acid 0.5ml	MTT 1ml PMS 1ml
6 Malate dehydrogenase	A 6.5ml	3.8ml		NAD 0.5ml		L-malic acid 1m (neutralised with NaOH) 0.2ml	MTT 1ml PMS 0.5ml
7 Malic enzyme (ME)	E 6.5ml	1ml	1.0M $Mgcl_2$ 0.1ml	NADP 0.2ml		1.0M L-malic acid (neautralized with NaOH) 0.2ml	MTT 1ml PMS 0.5ml
8 Mannose phosphate isomerase	E 6.5ml		1.0M $Mgcl_2$	NADP 0.2ml	GPI 30 units (E.C.5.3.1.9.)	Mannose-6-phosphate (Na_2) 2.5mg	MTT 1.0ml PMS 0.5ml
9 Nucleoside hydrolase (NH)	D 6.5ml	2ml			Xanthine oxidase (E.C.1.2.3.2.)	Inosine 10mg	MTT 1ml PMS 0.5ml
10 Phosph0glucomutase (PGM)	A 6.5ml	1ml	1.0M $Mgcl_2$ 0.2ml	NADP 0.2ml	G6PD (E.C.1.1.1.49) 0.5ml	Glucose-1-phosphate containing 1% glucose (1,6-biphosphate 1ml	MTT 1ml PMS 0.5ml

Table 3 cont.

11 Phosphogluconate dehydrogenase (6PGD)	E 6.5ml	1.ml	1.0M $MgCl_2$ 0.2ml	NADP 0.2 ml		6-phosphogluconate (10mg/ml 0.5ml	MTT 1ml PMS 0.5ml
12 Proline peptidase (PEP-D	E 6.5ml	1.ml 0.2ml	1.0M MnCl 0.2ml		Peroxidase E.C.1.11.1.7.)1mg L-amino acid oxidase (E.c.1.4.3.2.) 2mg	L-Leucyl-L-Proline 10mg	3-amino-9-ethyl carbazole,25mg 0.7 ethanol Agar 10ml
13 Pyruvate kinase (PK)	A 6.5ml		1.0M $MnCl_2$ 0.4ml 1.0M KCl 2.4ml		G6Pd (E.C.1.1.1.49) 10 units	Phosphate pyruvate 2mg	MTT 1ml PMS 0.5ml

Tables 4: Developer buffers. (After Miles *et al.*, 1980)

A. 0.3M Tris/HCl pH 8.0

B. 0.019M NaH_2PO_4/0.081M $NaHPO_4$ pH 7.4

C. 0.05M Tris/HCl pH 8.5

D. 0.03M Tris/HCl pH 7.0

E. 0.03M Tris/HCl pH 7.4

F. 0.004M NaH_2PO_4/0.096M Na_2HPO_4 pH 8.0

3.10 Data recording and Analysis

Bands were photographed immediately to obtain a permanent record for each sample studied. Isoenzyme mobility was defined as the distance, in millimeters at which the bands form, relative to the starting slots. Mobility was compared amongst the populations and then relative to the reference material (RSM).

3.11 Gas chromatography (GC)

3.11.1 Sample Preparation

Cassava green mites used in the GC study were obtained in the same way as described for IEF analysis. Extracts of mites were prepared as previously described by, Baker *et al.* (1979). Ten females each of the long and short setae forms of CGM were immobilized by freezing, then put into a perspex glass tube 15 mm long and 1.5 mm internal diameter with one end sealed. 10 ul of Purified hexane was added to the mite samples. To another glass tube of similar dimensions was added the same amount of purified n-hexane as a control.

Both sample and control glass tubes were placed inside a sample vial containing 20 ul of hexane. The hexane in the sample vial helped to minimize evaporation of hexane from the sample and control tubes. The sample was left to soak for 24 hours at room temperature.

3.11.2 Gas chromatographic separation.

Analysis of cuticular hydrocarbons was conducted according to the method of Peschke and Metzter (1986). A Hewlett-Parkard 5890A series gas chromatograph, fitted with a flame ionization detector was used in the analysis. The gas chromatograph was equipped with a 50 meter long Hewlett-Packard fused silica capillary column, cross linked with methyl silicon and having an internal diameter (ID) of 0.31 mm.

Samples were analysed with the initial oven temperature programming from 100 ^{o}C to 270 ^{o}C at an increase rate of 4 ^{o}C per minute. The injector temperature was 220 ^{o}C and the detector temperature was 280 ^{o}C. Nitrogen was used as the carrier gas. Samples of the short and long setae forms were run separately. A Hewlett-Packard 3393A computing integrator was used to determine the area and peak heights of the samples.

4. RESULTS

4.1 Setal length measurement

Measurements of the dorsal central body setae (D_1-D_3) for the first five generations of cassava green mite populations are given in appendices 1-6. Results indicate that adult CGM of different generations of a single line, in all the populations, do not show significant variation in the setal lengths. This means that mites from any of the generations could be used in the biochemical analysis. Rearing the mites upto the fifth generation did not affect the eventual length of setae. Mombasa and Rusinga population had long setae while Nairobi, Embu, Ishiara and Busia had short setae. In the locations where long and short setae forms occured, intermediate forms were also recorded.

4.2 Isoenzyme analysis

Three out of the thirteen enzyme/substrate systems assayed gave consistent and high

resolution bands which could easily be interpreted for taxonomic purposes. These included malic enzyme, glucose phosphate isomerase and malate dehydrogenase. The other isoenzymes assayed either produced very faint bands, and were unreproducible in which case they could not be used for meaningful taxonomic interpretation or did not show any measurable activity at all. Since these bands were unreproducible they were not represented diagramatically.

4.2.1 Malic enzyme

Malic enzyme (Plate 1 and figure 4) were detected in all the populations of Cassava Green Mite studied. Identical banding pattern characterizes all populations Three prominent bands occur in each population. Two bands, much broader with a slow mobility and rather faint compared to the electrophoretically faster bands also occur in all the populations of CGM. These bands are not seen in the in RSM.

Table 5 gives the results of coefficient of correlation half matrix of variables, "LB" and "WB" which represent distances between bands and the band widths respectively, considered under malic enzyme. The normalized eigenvectors of the

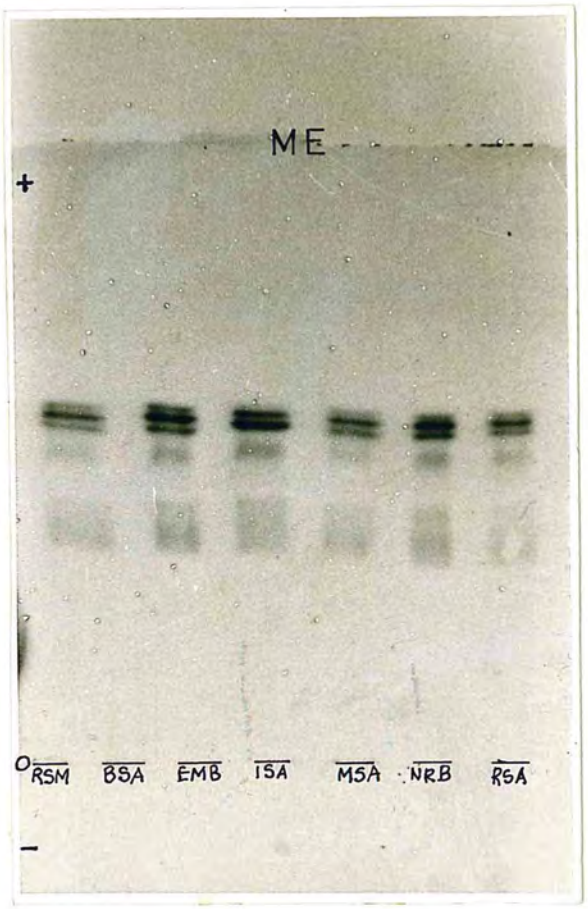


PLATE 1: Photograph of malic enzyme bands.

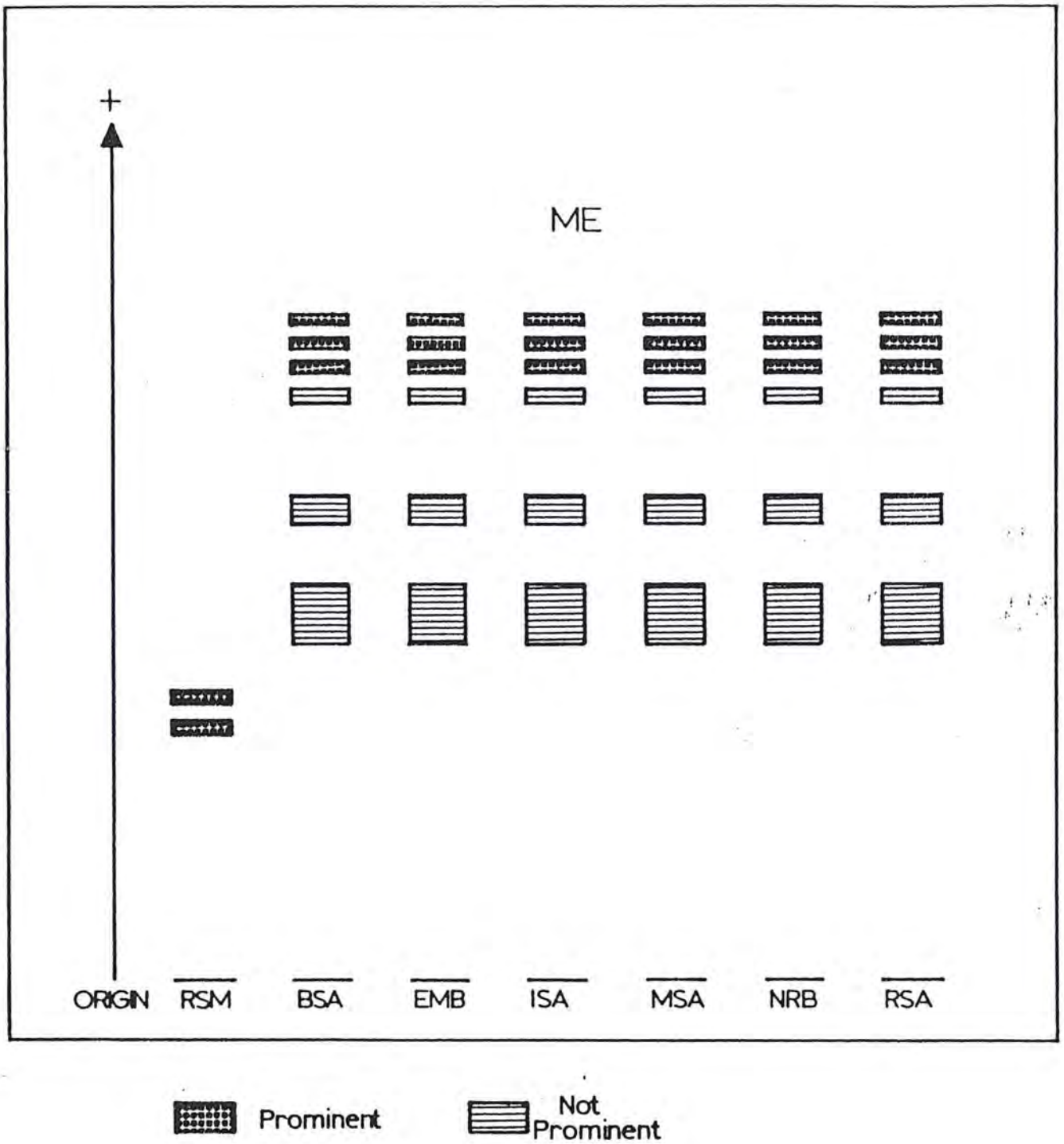


Fig. 4 A schematic representation of zymograms from the IEF of malic enzyme (ME)

RSM = Red Spider Mite, BSA = Busia, EMB = Embu,
ISA = Ishiara, MSA = Mombasa, RSA = Rusinga
NRB = Nairobi

Table: 5 Coefficient of Correlation half matrix for the variables of malic enzyme (ME)

	LB12	LB23	LB34	LB45	LB56	WB2	WB3	WB4	WB5	WB6
LB12	1.00	-0.47	-0.68	-0.54	-0.22	-0.49	0.26	0.16	0.09	0.32
LB23		1.00	0.61	0.49	0.07	0.49	0.13	-0.14	0.08	0.079
LB34			1.00	0.39	0.10	0.63	0.12	0.06	0.21	-0.34
LB45				1.00	0.24	0.31	0.20	-0.37	0.05	0.33
LB56					1.00	0.07	0.39	0.21	0.02	0.02
WB2						1.00	0.26	0.10	-1.20	0.08
WB3							1.00	0.71	0.21	0.02
WB4								1.00	0.51	0.10
WB5									1.00	-0.65
WB6										1.00

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands).

"WB" denotes the band width (e.g.WB1 is the width of the first band from the application point).

correlation matrix are presented in table 6. These values represent the best four components of the variables that effectively describe the relationship of band widths as well as distances between bands. Variables with high positive or negative (> 0.7) weightings are said to reflect significant variation within a population. None of the principal components account for more than half the variance. The first component accounts for 32.58% of the total variance and is clearly the most important. The other three components, 2,3 and 4 each account for much less.

Figure 5 shows a plot of the computed values for the first two principle components, which between them accounts for 55.49% of the variation in the data. The plot shows a high degree of correlation within each population and subsequently between the populations. A similar trend is seen in all the principal component plots shown on Figs 6 - 10.

Further analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) are shown on Table 7 and 8. The results show that means do not differ significantly at a given level for both the band width and the distances in between bands. Analysis of band widths also shows high

Table 6. Normalised eigenvectors (weightings) and variance explained by the best 4 principal components for the 10 variables of malic enzyme.

	PRINCIPAL COMPONENTS			
	1	2	3	4
LB12	-0.828	0.182	0.096	-0.095
LB23	0.733	-0.132	0.287	-0.156
LB34	0.863	0.110	0.044	-0.252
LB45	0.708	-0.309	-0.155	-0.390
LB56	0.240	0.320	0.346	-0.819
WB2	0.695	0.066	0.419	0.337
WB3	0.056	0.811	0.462	0.010
WB4	-0.066	0.922	0.114	-0.112
WB5	0.091	0.649	-0.659	-0.010
WB6	-0.453	0.309	0.720	-0.022
Cummulative % of Variance	**32.58**	**55.49**	**71.43**	**81.89**

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands)

"WB" denotes the band width (e.g. WB1 is the width of the first band from the application point)

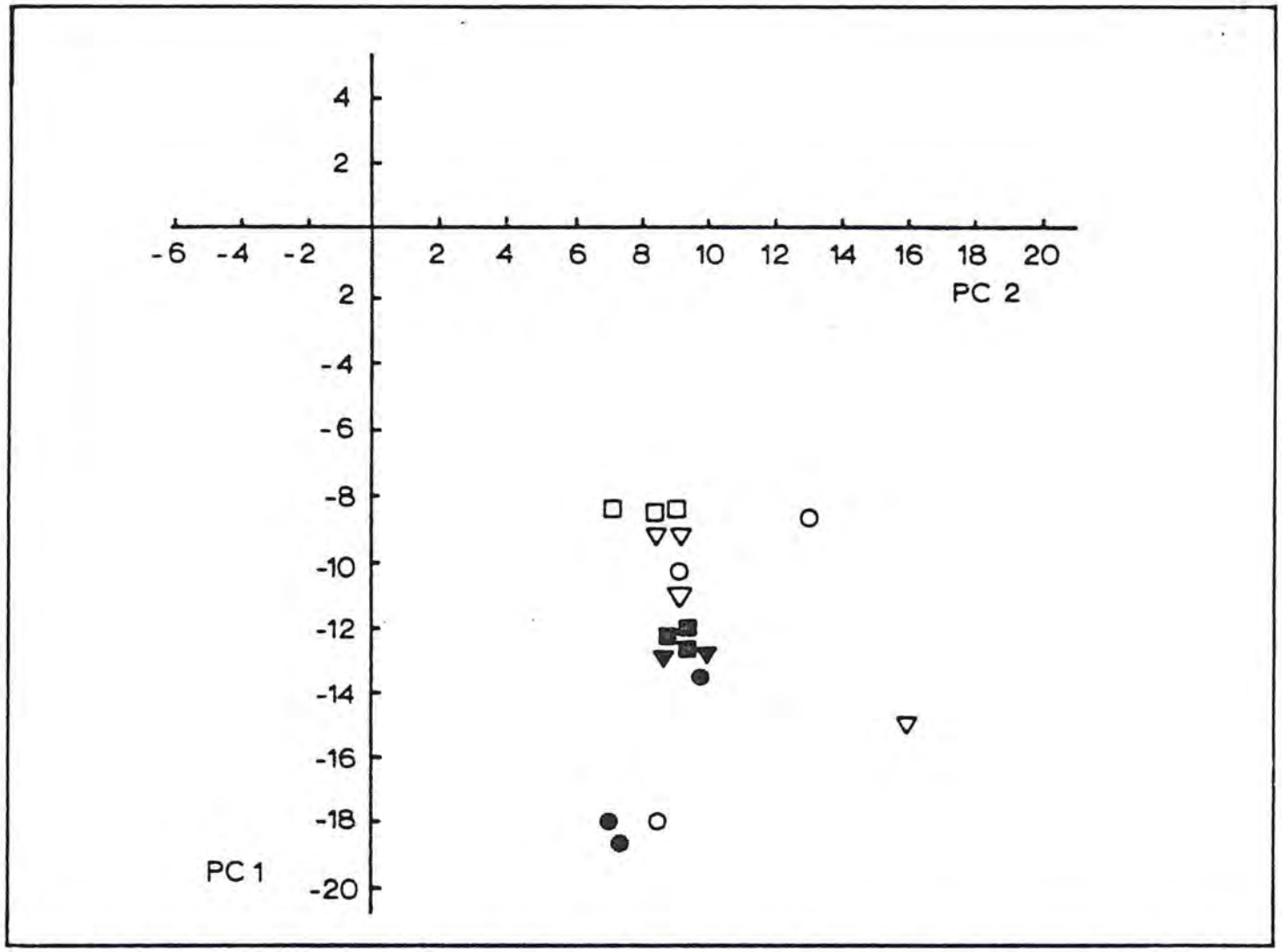


Fig. 5. First and second principle component plot comparisons of the six population of Mononychellus spp. using Malic Enzyme (ME)

● Busia ▼ Ishiara □ Nairobi
■ Embu ○ Mombasa ▽ Rusinga

PC = Principle Component

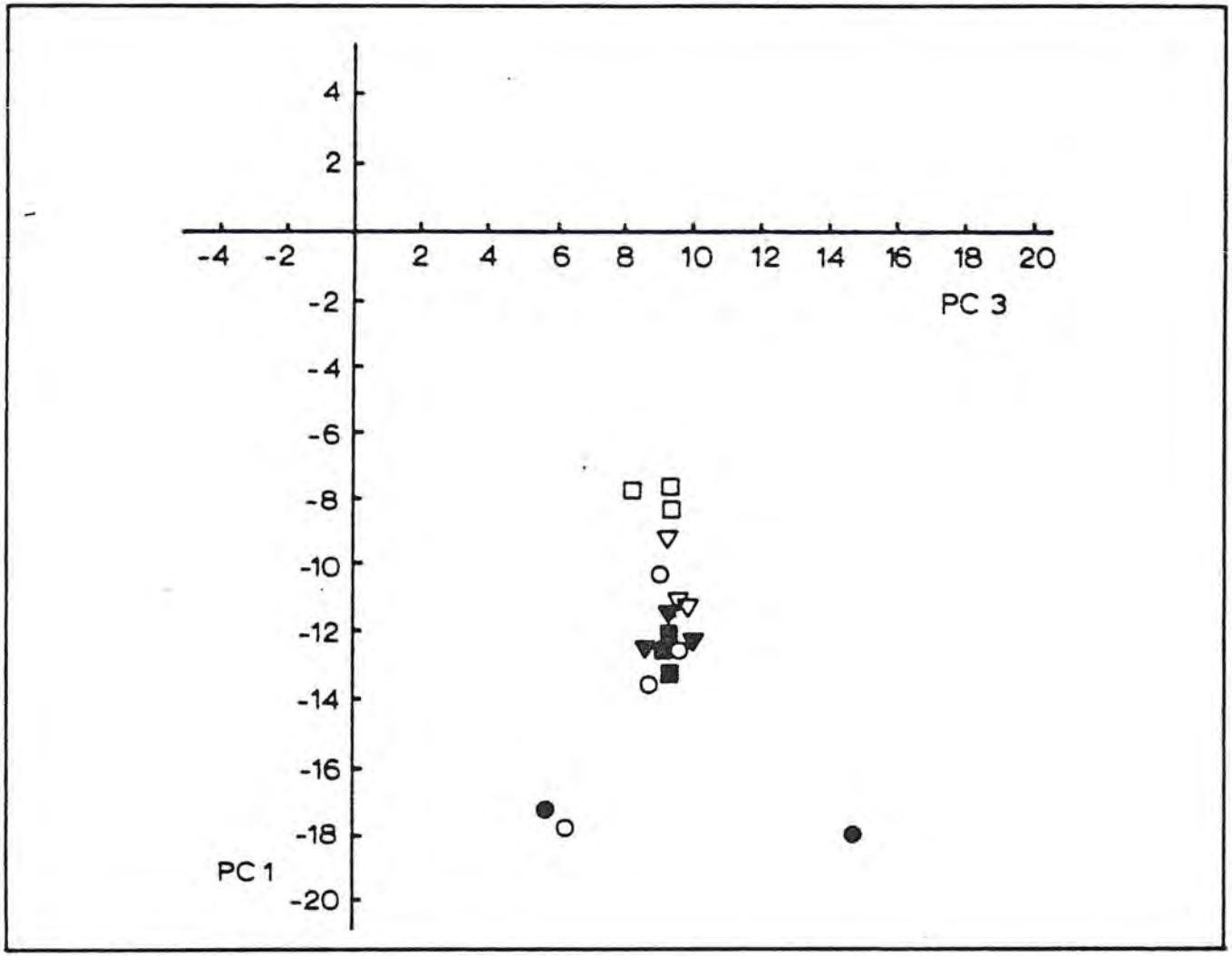


Fig. 6 First and third principle component plot comparisons of the six populations of Mononychellus spp. using Malic Enzyme (ME)

● Busia ▼ Ishiara □ Nairobi

■ Embu ○ Mombasa ▽ Rusinga

PC = Principle Component

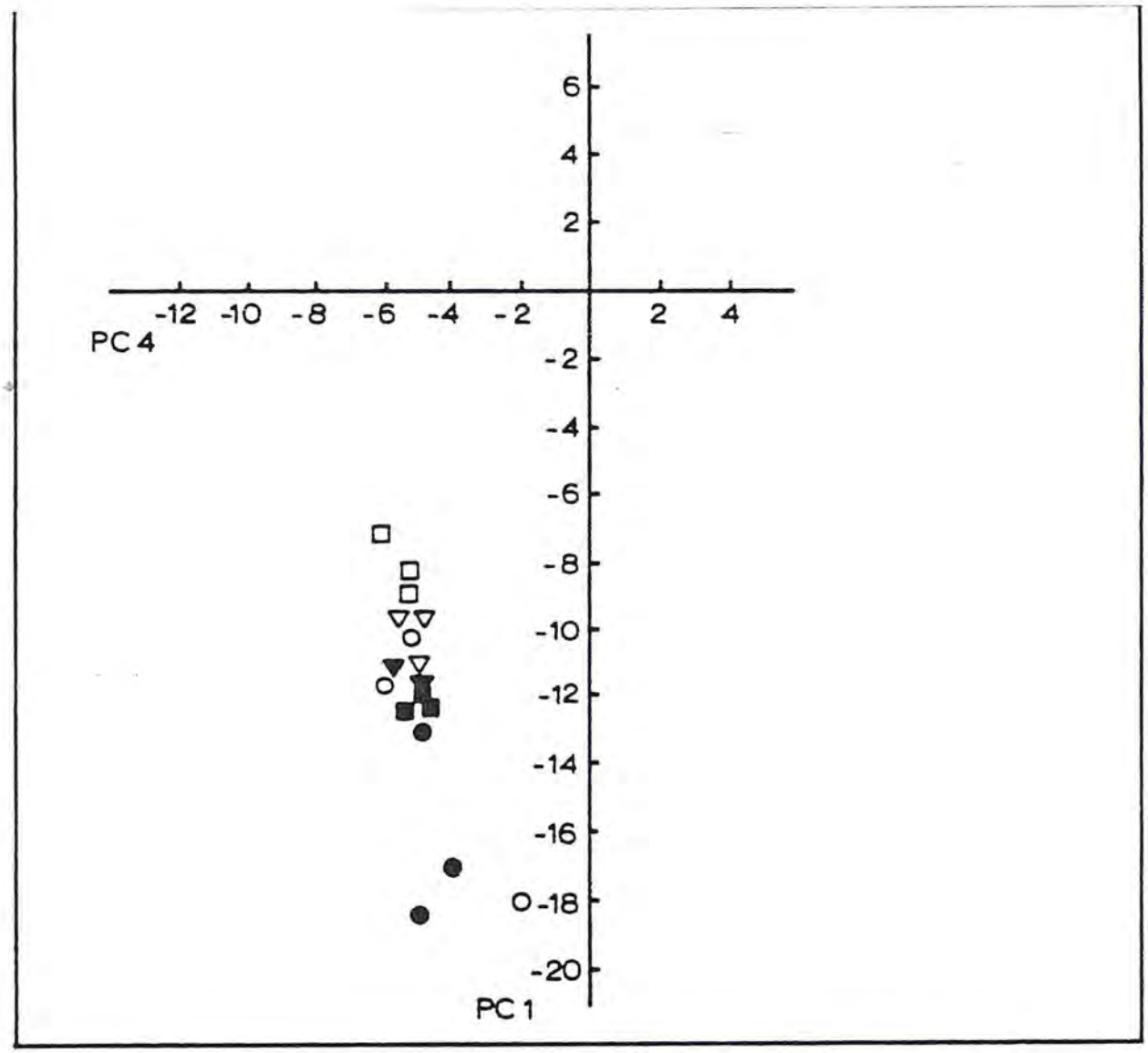


Fig. 7 First and fourth principle component plot comparisons of the six populations of Mononychellus spp. using Malic Enzyme (ME)

● Busia ▼ Ishiara □ Nairobi
■ Embu ▽ Rusinga o Mombasa

PC = Principle Component

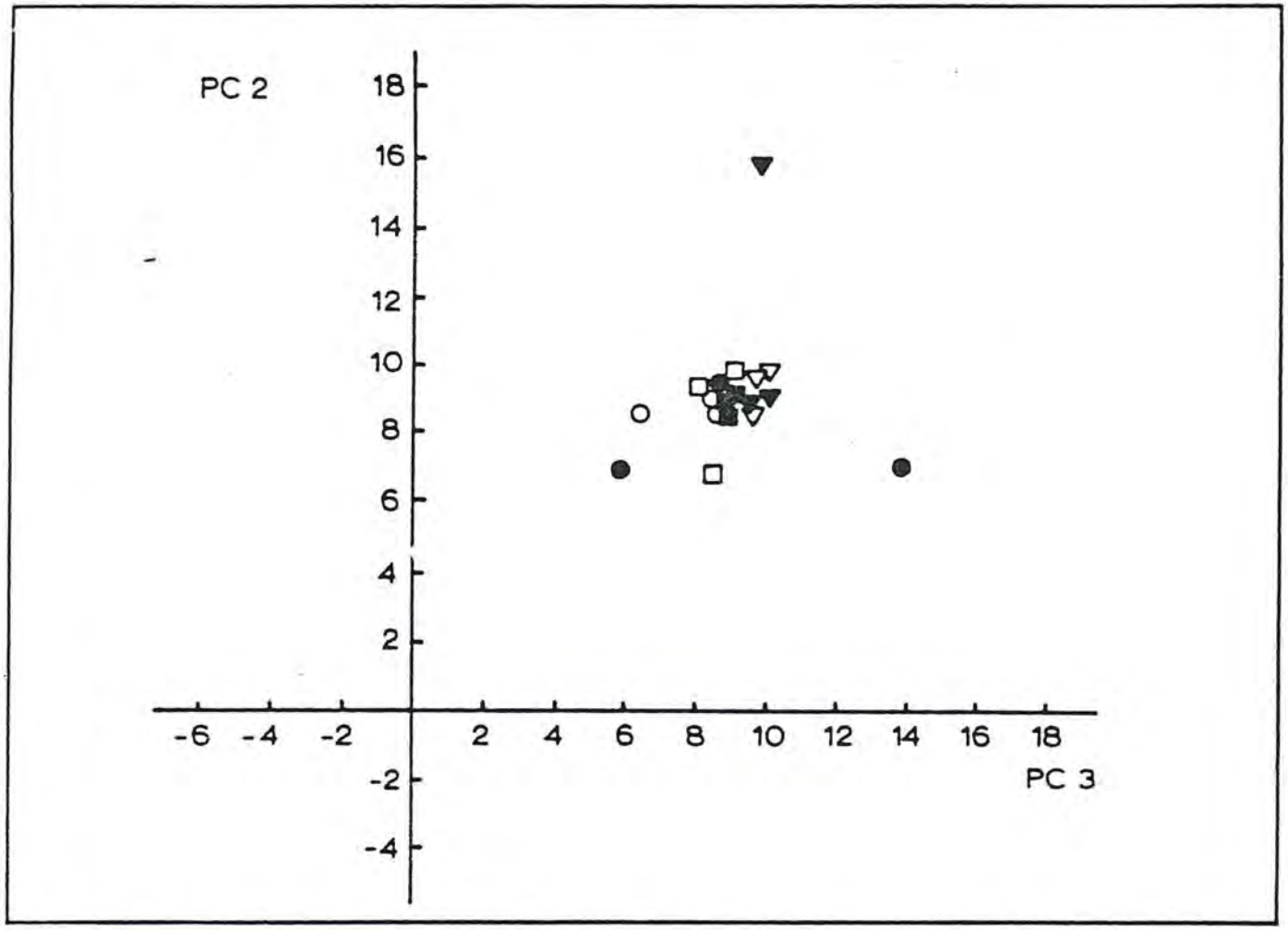


Fig. 8 Second and third principle component plot comparisons of six populations of Mononychellus spp. using Malic Enzyme (ME)

● Busia ▼ Ishiara □ Nairobi

■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

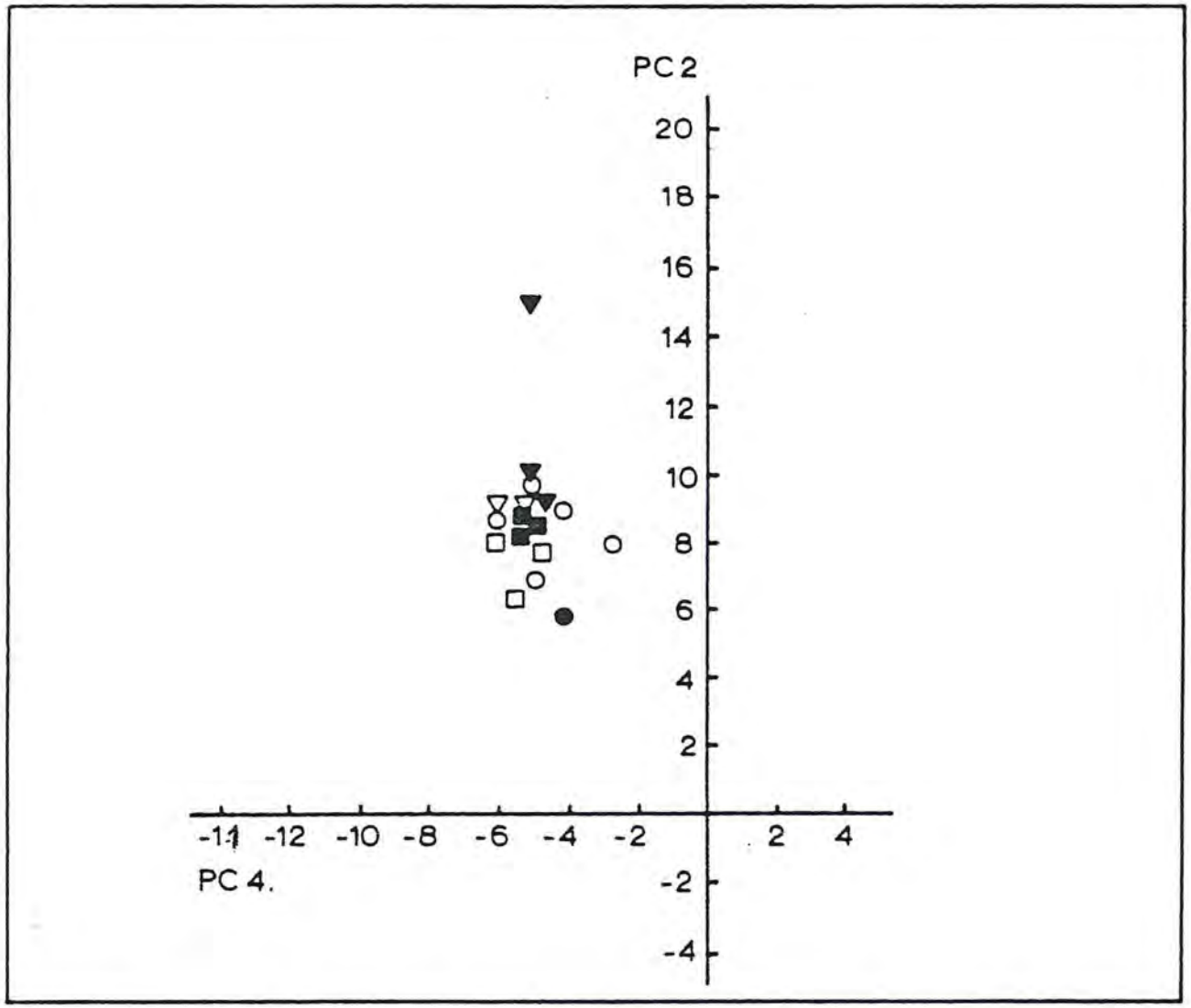


Fig. 9 Second and fourth principle component plot comparisons of six populations of Mononychellus spp. using Malic Enzyme (ME)

● Busia ▼ Ishiara □ Nairobi

■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

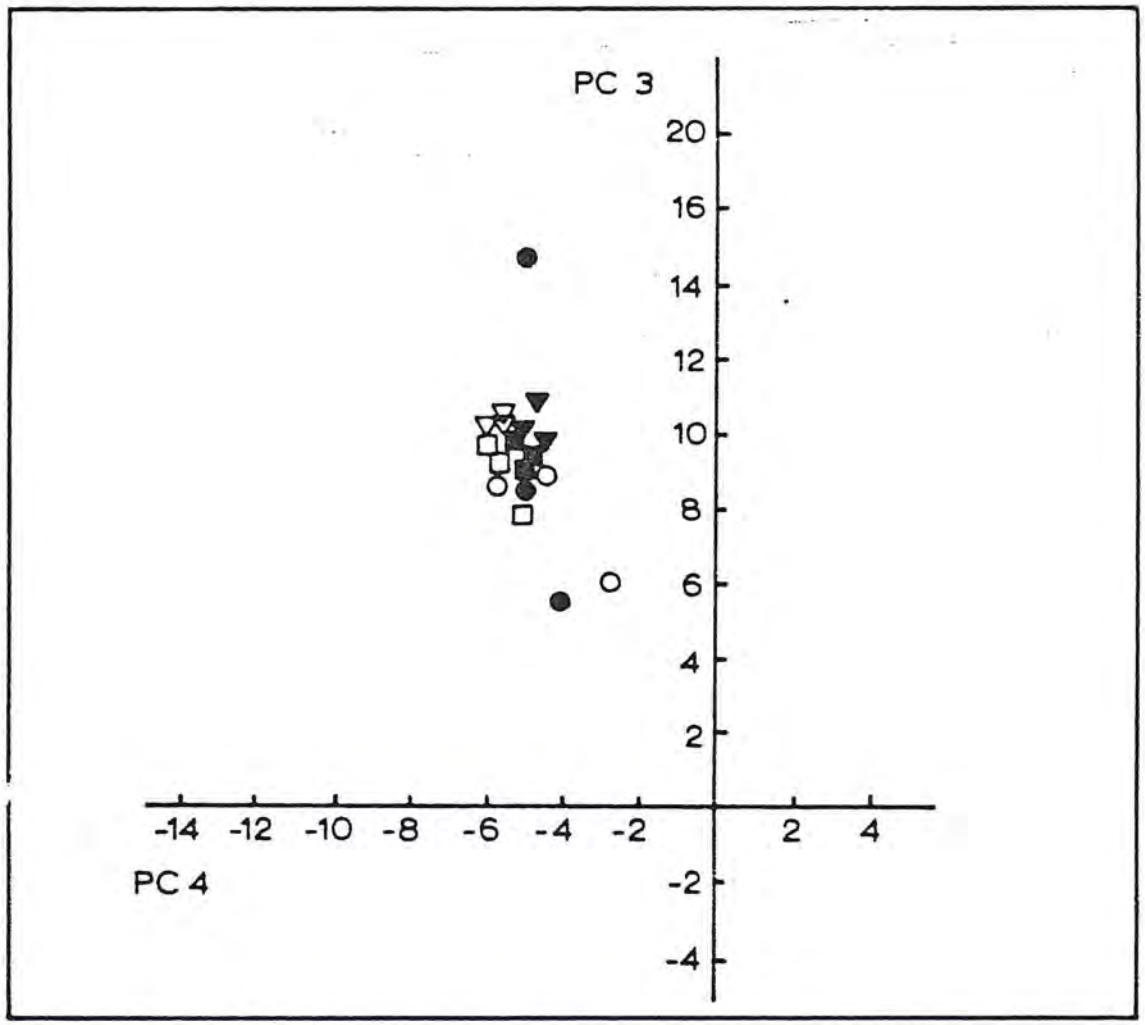


Fig. 10 Third and fourth principle plot comparisons of six populations of Mononychellus spp. using Malic Enyme (ME)

● Busia ▼ Ishiara □ Nairobi

■ Embu ○ Mombasa ▽ Rusinga

PC = Principle Component

Table 7: Analysis of variance for comparing locations using band widths of malic enzyme (ME)

Source	df	WB2 MS	WB3 MS	WB4 MS	WB5 MS	WB6 MS
Location	5	18.79	12.45	2.74	0.37	13.96
Error	12	40.70	16.45	5.81	1.24	36.83
F Value	-	1.11	1.82	1.13	0.71	0.91
Significance level	-	0.4059	0.1839	0.3943	0.6274	0.5063
Corrected Total	17	59.50	28.91	8.56	1.61	50.79

** represent significant values.

Locational Means					
	a	b	a	a	a
Busia	5.667	2.700	1.000	0.667	3.067
	a	b,a	a	a	a
Embu	7.500	3.500	1.067	0.600	0.683
	a	a	a	a	a
Ishiara	7.233	5.067	2.067	1.030	0.500
		a	a	a	a
Mombasa	5.433	3.167	1.067	0.867	0.833
	a	a	a	a	a
Nairobi	8.100	2.500	1.067	0.867	0.767
	a	b,a	a	a	a
Rusinga	7.733	3.500	0.933	0.850	0.833

*Mean comparison was by Duncan's multiple range test.
Means with the same alphabet,within a particular treatment are not significantly different.

Table 8: Analysis of variance for comparing locations using distances between bands of Malic enzyme (ME).

Source	df	LB12 MS	LB23 MS	LB34 MS	LB45	LB56
Location	5	34.570	2.790	2.650	0.100	0.110
Error	12	8.000	1.830	1.690	1.000	0.370
F Value	-	10.37	3.65	3.76	2.47	0.74
Significance level	-	0.0005 **	0.0306 **	0.0280 **	0.0920	0.6060
Corrected total	17	42.57	4.63	4.34	0.200	0.480

** represent significant values

Locational Means

Busia	a 29.500	b 4.500	c 1.670	b 0.833	(
Embu	a 28.333	b,a 5.167	b,c,c 1.400	b,a 1.000	a 0.767
Ishiara	a 28.500	b,a 5.167	b,c 1.600	b,a 0.900	1.000
Mombasa	a 28.500	a 5.333	b 1.600	b,a 1.000	a 0.833
Nairobi	a 25.330	a 5.667	a 2.330	a 1.067	a 0.867
Rusinga	a 26.670	a 5.667	b,a 2.000	b,a 0.967	a 0.933

*Mean comparison was by Duncan's , multiple range test (a=0.05) Means with the same alphabet, within a particular treatment are not significantly different.

correlation coefficient. These results indicate that there is no locational differences between populations.

4.2.2 Glucose phosphate isomerase

For glucose phosphate isomerase (Plate 2 and Fig 11). Three clearly discernable bands and a single faint band were produced in each of the CGM populations. The red spider mite shows a different banding pattern. Qualitative differences were not evident from the expressions of bands, this consistency was observed in all replicates.

Results of the measurements of distances between bands and of band widths are shown in Appendix 8. Analyses were carried out using the same statistical methods as for malic enzyme. Table 9 gives the results of correlation coefficient half matrix between the variables. There exists a high degree of intercorrelation between the variables, both in the length between bands and in band width.

Table 10 shows the normalized eigenvectors of the correlation matrix. These represent the three best components of the variables. Component 1 accounts for 31.93% of the

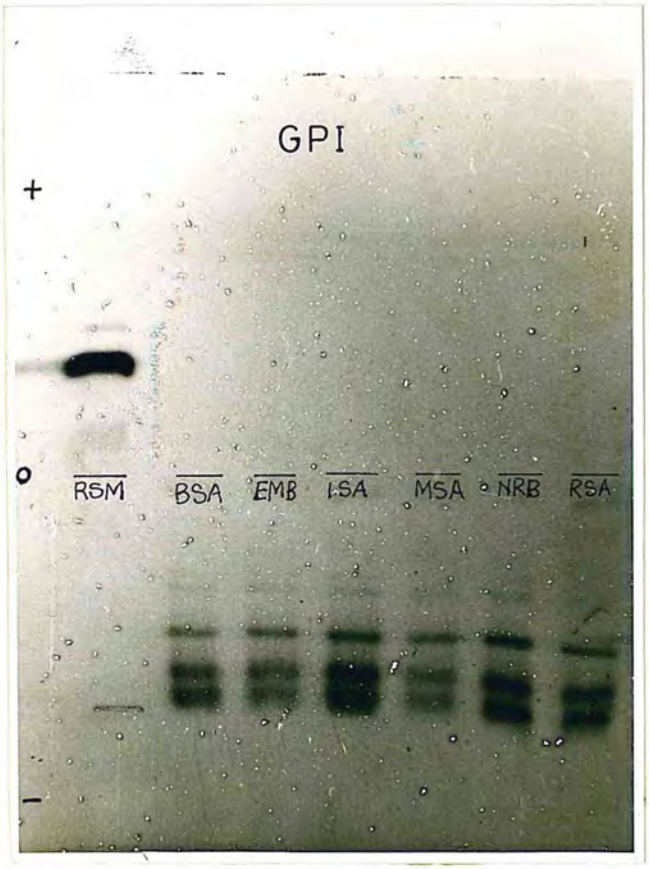


PLATE 2: Photograph of glucose phosphate isomerase bands.

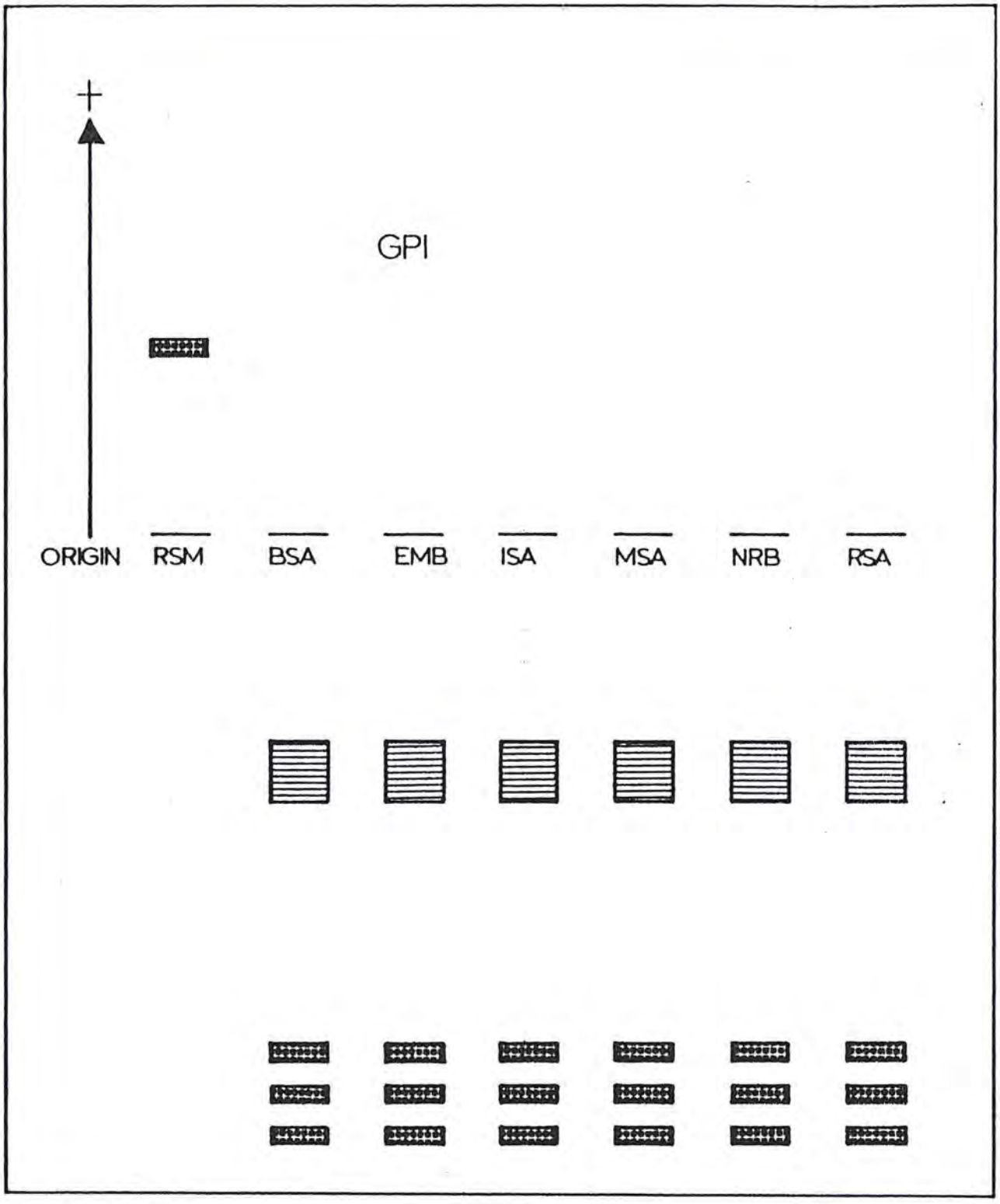


Fig. 11 A schematic representation of zymograms from the IEF of glucose phosphate isomerase (GPI)

RSM = Red Spider mite, BSA = Busia, EMB = Embu, ISA = Ishiara
MSA = Mombasa, NRB - Nairobi, RSA = Rusinga

TABLE 9 : Coefficient of correlation half matrix for variables of Glucose phosphate isomerase

	LB12	LB23	LB34	LB45	WB2	WB3	WB4	WB5
LB12	1.00	-0.12	-0.46	-0.15	0.44	-0.03	0.08	-0.65
LB23		1.00	0.07	-0.41	0.34	0.27	0.10	0.19
LB34			1.00	-0.12	-0.26	0.33	-0.20	0.33
LB45				1.00	0.09	0.14	0.15	0.11
WB2					1.00	0.54	0.38	-0.48
WB3						1.00	0.37	0.12
WB4							1.00	0.15
WB5								1.00

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands)

"WB" denotes the band width (e.g. WB1 is the width of the first band from the application point)

TABLE 10: Normalised eigenvectors (Weightings) and variance explained by the best 3 principal components for the 8 variables of glucose phosphate isomerase.

	Principal Components		
	1	2	3
LB12	0.715	-0.374	-0.430
LB23	0.205	0.227	0.857
LB34	-0.658	-0.048	0.248
LB45	0.161	0.465	-0.600
WB2	0.846	0.085	0.275
WB3	0.515	0.607	0.405
WB4	0.393	0.650	0.002
WB5	-0.636	0.679	0.080
cummulative % of variance	31.930	20.880	19.830

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands)

"WB" denotes the band width (e.g. WB1 is the width of the first band from the application point)

variation. This is much higher than the other two components which contribute 20.88% and 19.83% respectively. Figs 12 - 14 show plots of the principal components whose values cluster, showing evidence of a close correlation in the variables between and within populations.

Table 11 and 12 show the ANOVA and DMRT for GPI bands respectively. Significant differences are shown by distances between bands, except LB12. None of the values for band widths show any significant differences in locational mean. Comparison by DMRT indicate a high degree of similarity within the CGM populations. Except LB23, all other populations do not show significant differences. Among the band width, WB2 and WB3 have closely related means while WB4 and WB5 show variations. Results with GPI again indicate intercorrelation of CGM in the six locations.

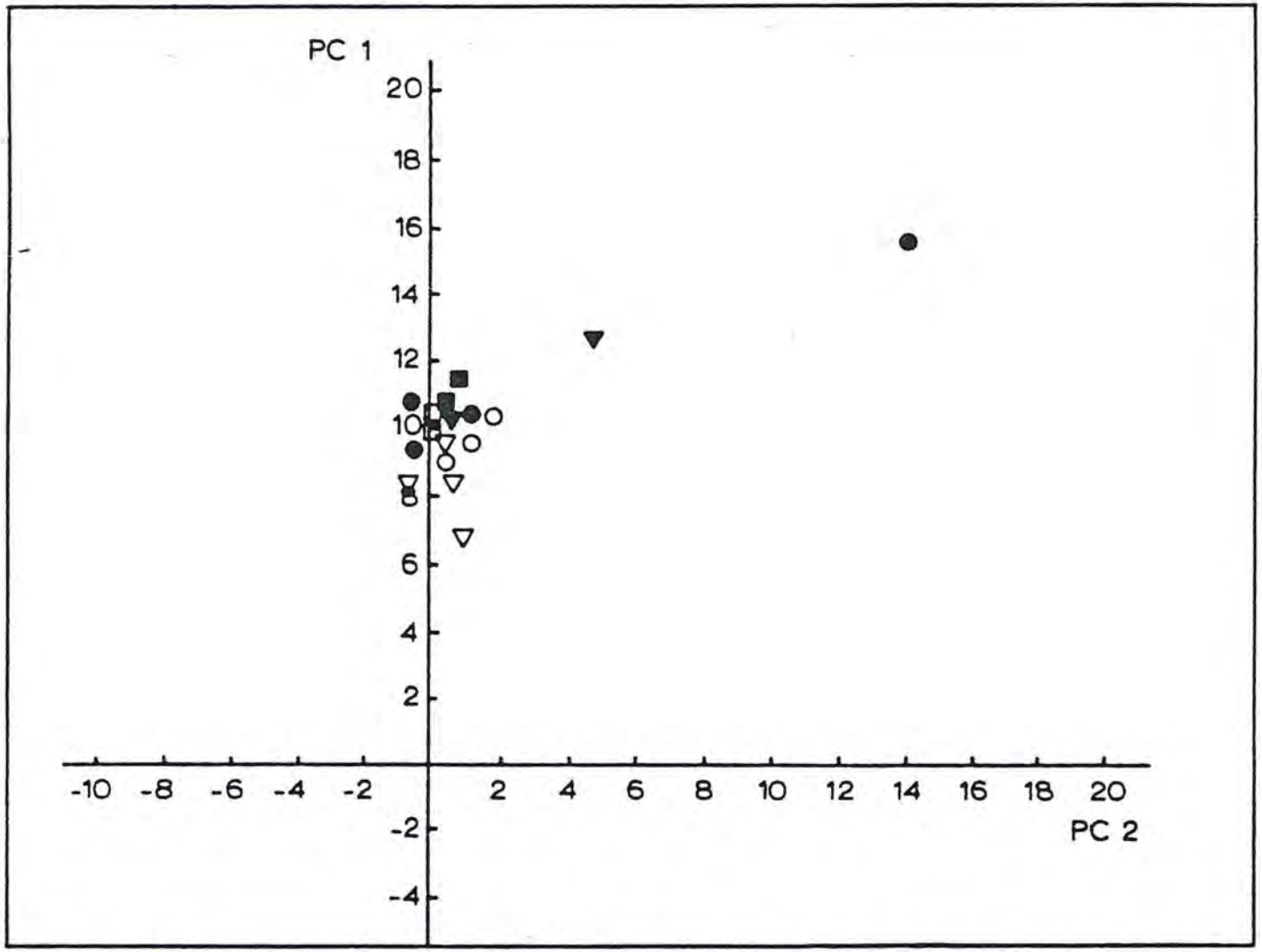


Fig. 12. First and second principle component plot comparisons of six populations of Mononychellus spp. using Glucose Phosphase Isomerase (GPI)

● Busia ▼ Ishiara □ Nairobi

■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

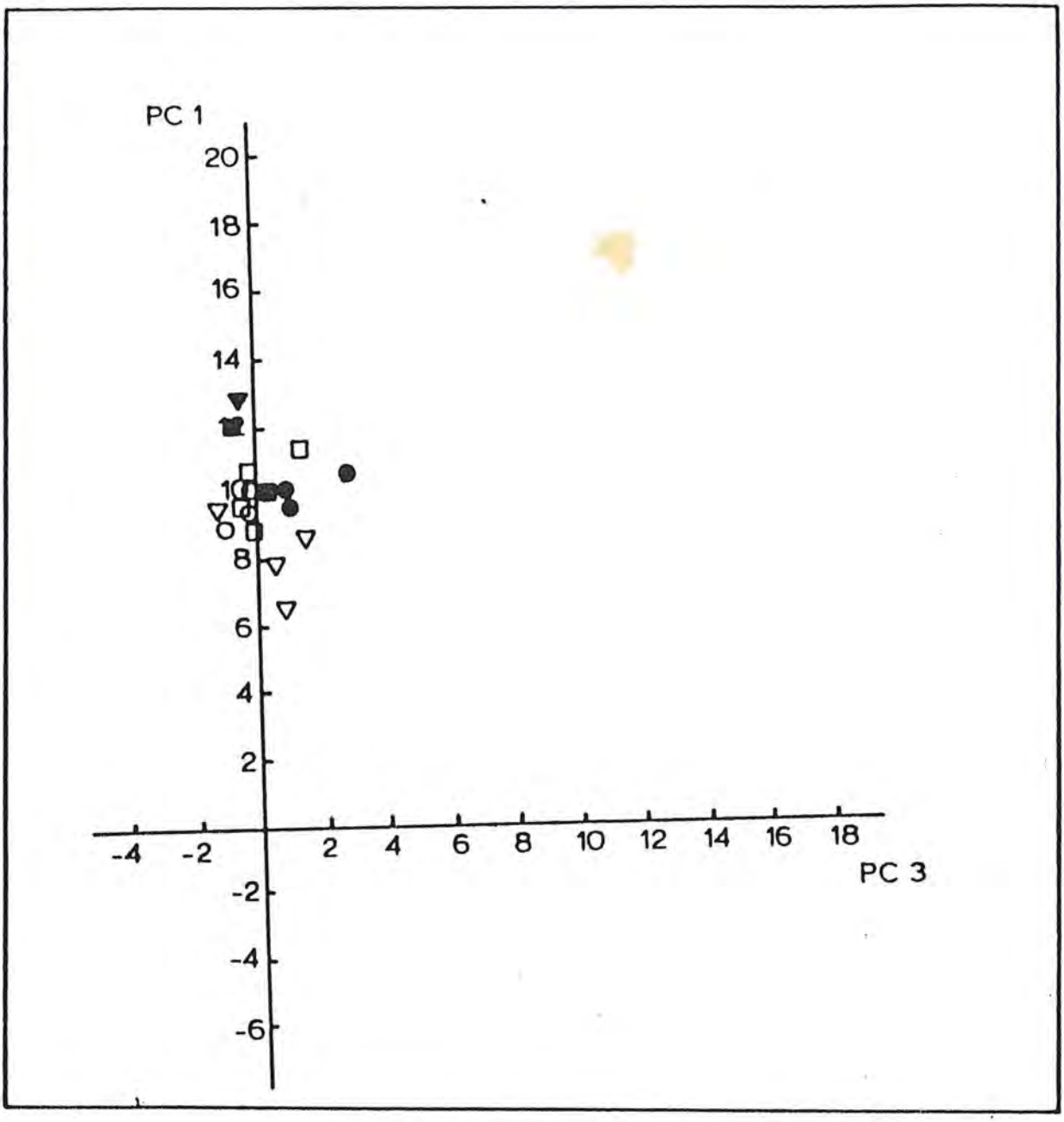


Fig. 13 First and third principle component plot comparisons of six populations of Mononychellus spp. using Glucose Phosphate Isomerase (GPI)

● Busia ▼ Ishiara □ Nairobi

■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

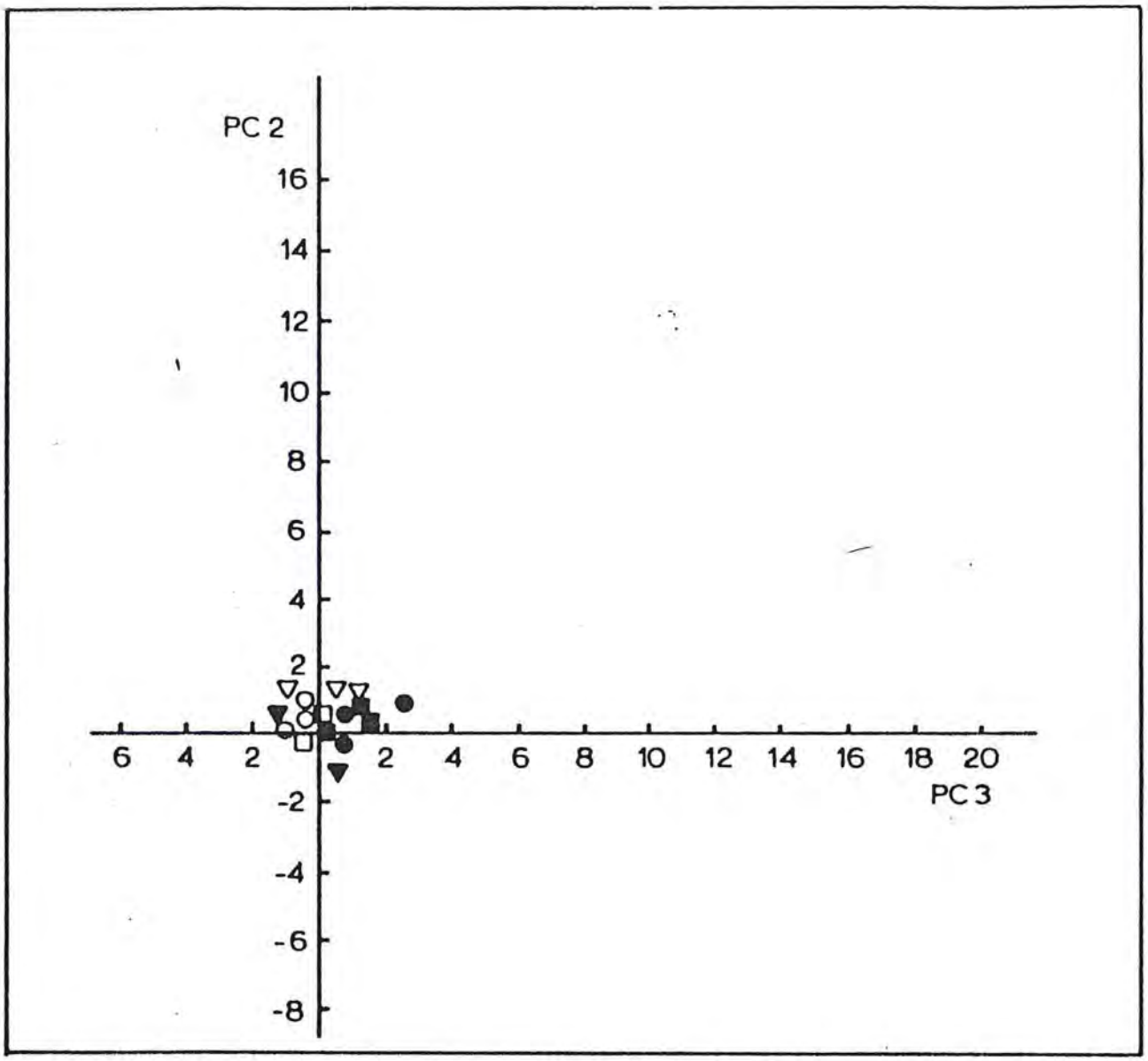


Fig. 14 Second and third principle component plot comparisons of six populations of Mononychellus, using Glucose Phosphate Isomerase (GPI)

● Busia ▼ Ishiara □ Nairobi
■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

TABLE 11: Analysis of variance for comparing locations using distances between bands of glucose phosphate isomerase (GPI)

Source	Degrees of freedom	LB12 Ms	LB23 Ms	LB34 Ms	LB45 MS
Location	5	0.4530	0.0070**	0.0001**	0.0330**
Error	12	1.8800	3.82	0.92	0.4900

**represent significant values.

Locational means				
Busia	a 11.267	a 5.167	b 2.100	a 1.133
Embu	a 11.267	ba 4.833	b 2.033	a 1.567
Ishiara	a 11.167	d 3.167	b 2.000	a 1.733
Mombasa	a 11.000	dc 3.500	b 2.000	a 1.733
Nairobi	a 10.000	bdc 4.000	b 2.167	a 1.600
Rusinga	a 10.630	abc 4.267	a 4.167	a 1.533

*Mean comparison was by Duncan's multiple range test (a=0.05). Means with the same alphabet within a particular treatment are not significantly different.

TABLE 12: Analysis of variance for comparing locations using band widths of glucose phosphate isomerase (GPI)

Source	Degrees of freedom	WB2 Ms	WB3 Ms	WB4 Ms	WB5 MS
Location	5	0.02	0.60	0.51	0.084
Error	12	1.08	2.66	18.16	1.00

**represent significant values .

Locational means				
Busia	b 2.400	a 2.330	a 2.833	ba 2.667
Embu	a 3.067	a 2.667	a 2.667	b 2.330
Ishiara	b 2.500	a 2.500	4.167	ba 2.667
Mombasa	b 2.167	a 2.330	a 2.500	a 3.000
Nairobi	b 2.167	a 2.167	a 2.500	ba 2.500
Rusinga	b 2.167	a 2.000	a 2.330	a 3.000

*Means with the same alphabet, within a given treatment are not significantly different.

*Mean comparison was by Duncan's multiple range test.(a=0.05)

4.2.3 Malate dehydrogenase

Malate dehydrogenase bands (Plate 3 and fig 15) were slow to form, but once they appeared, remained consistent for several hours. MDH banding pattern was more complex than ME and GPI. A single, rapidly migrating anodic band appeared in all the CGM populations. Data of the measurements of distance between bands and those of the band widths are shown in appendix 9. Table 13 shows the coefficient of correlation values between the variables. MDH values are much lower than ME and GPI.

Table 14 shows the two principal components which between them account for 55.12% of the variation. Plotted values of the principal components are shown in Fig. 16 Uniformity between populations is portrayed by clustering of the points. Table 15 and 16 show results of the ANOVA and DMRT of locational means for all populations to be very close

.

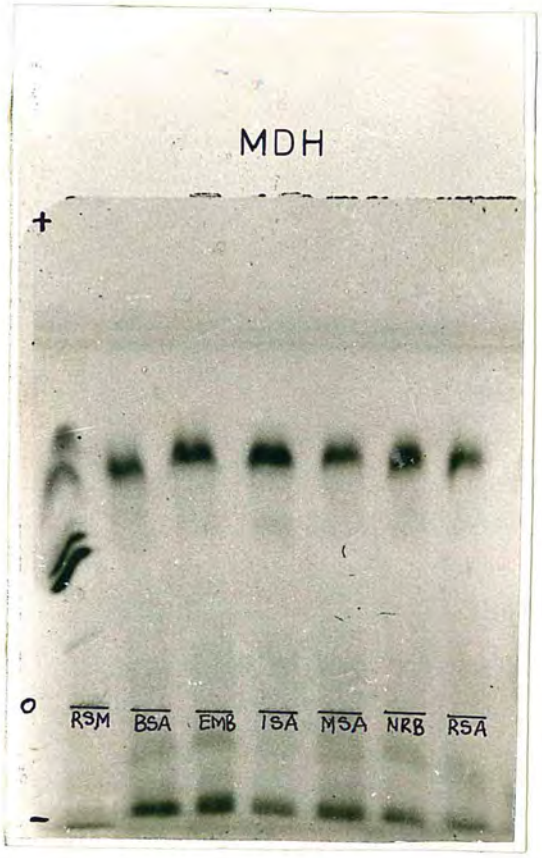


PLATE 3: Photograph of malate dehydrogenase bands.

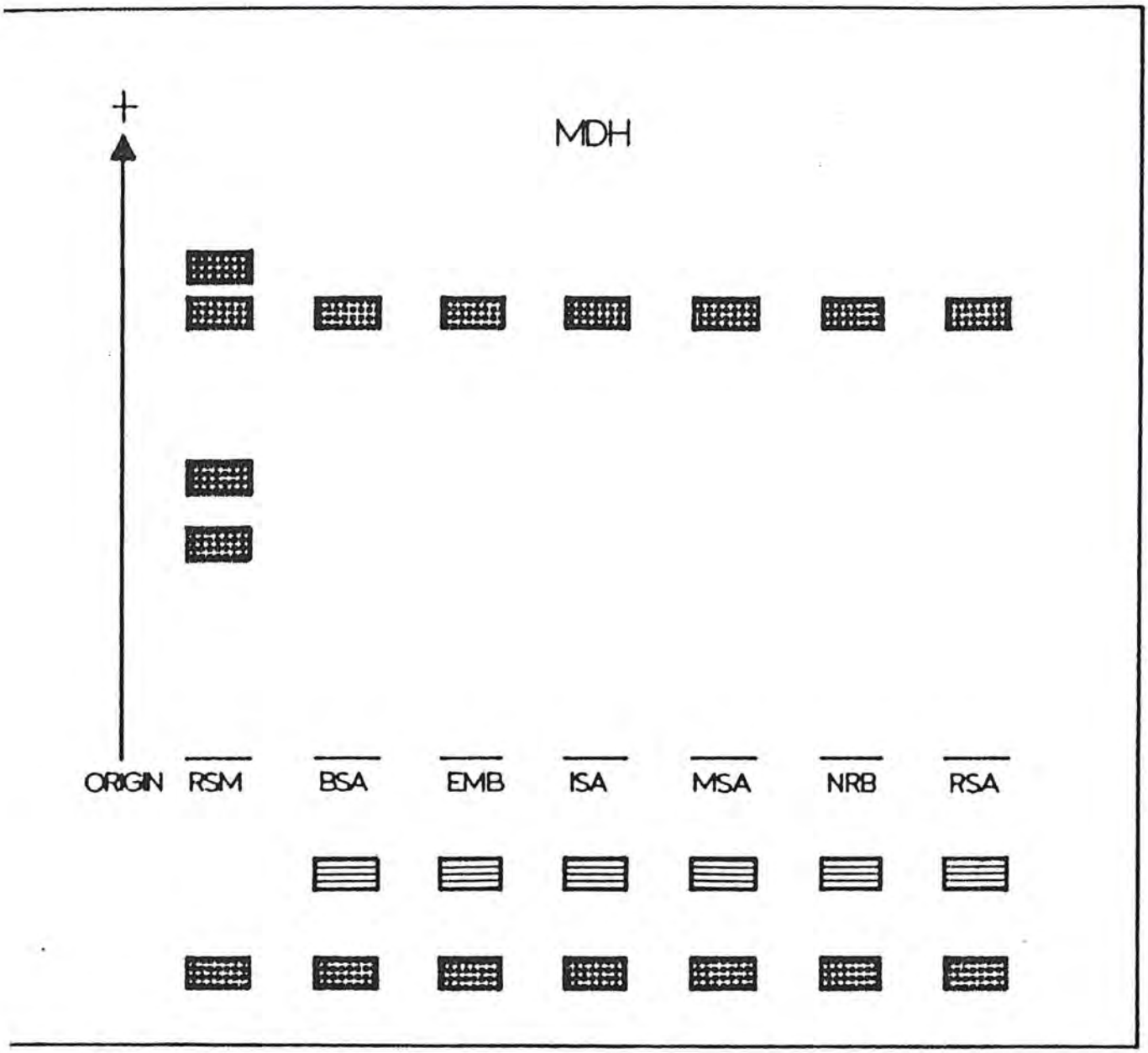


Fig 15 A schematic presentation of zymograms from IEF of malate dehydrogenase (MDH)

RSM = Red Spider Mite, BSA = Busia, EMB = Embu, ISA = Ishiara
MSA = Mombasa, NRB = Nairobi, RSA = Rusinga.

TABLE 13: Coefficient of correlation half matrix for variables of Malate dehydrogenase (MDH)

	LB12	LB23	LB14	WB2	WB3	WB4
LB12	1.000	-0.140	0.177	-0.230	-0.400	0.010
LB23		1.000	-0.120	-0.030	0.220	-0.180
LB14			1.000	0.210	0.560	0.069
WB2				1.000	0.130	0.300
WB3					1.000	0.270
WB4						1.000

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands)

"WB" denotes the band width (e.g. WB1 is the width of the first band from the application point)

TABLE 14: Normalised eigenvectors (Weightings) and variance explained by the best 2 principal components for the 6 variables of malate dehydrogenase.

	Principal Components	
	1	2
LB12	-0.595	0.370
LB23	0.093	-0.767
LB14	0.706	0.056
WB2	0.519	0.361
WB3	0.837	-0.231
WB4	0.409	0.629
Cummulative % variance	33.310	55.120

* Note: "LB" denotes distance between bands (e.g LB12 is the distance between the first and second bands)

"WB" denotes the band width (e.g. 'WB1 is the width of the first band from the application point)

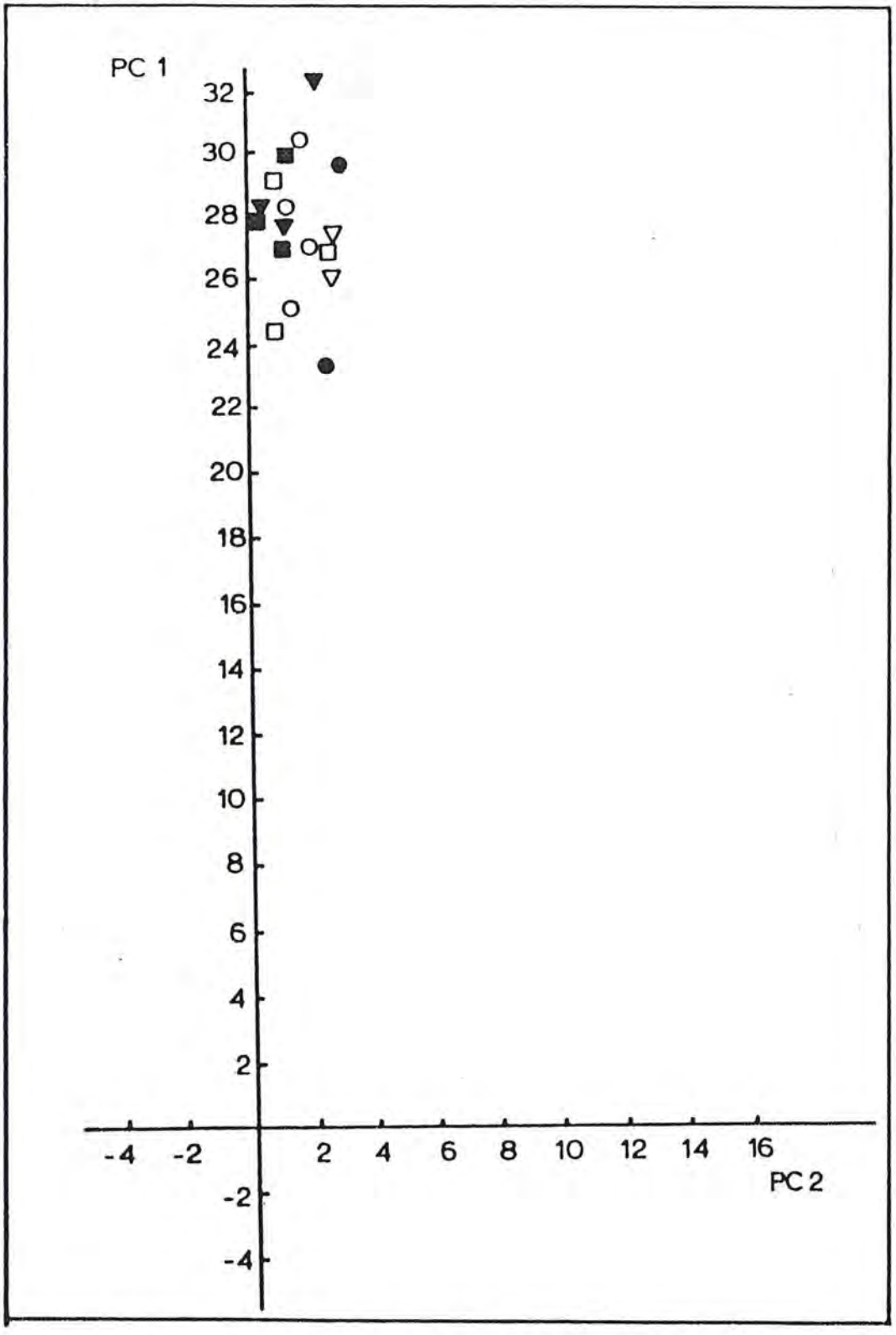


Fig. 16 First and second principle component plot comparisons of six populations of Mononychellus using Malate Dehydrogenase (MDH)

● Busia ▼ Ishiara □ Nairobi

■ Embu o Mombasa ▽ Rusinga

PC = Principle Component

TABLE 15 : Analysis of variance for comparing locations using distances between bands of Malate dehydrogenase (MDH).

Source	Degrees of freedom	WB2 Ms	WB3 Ms	WB4 Ms
Location	5	0.705 NS	0.250 NS	0.030 **
Error	12	5.660	5.120	3.000

**represent significant values .

Locational means			
Busia	a 3.500	a 3.330	a 4.500
Embu	a 4.167	ba 3.067	b 3.330
Ishiara	a 3.833	a 3.330	b 3.500
Mombasa	a 3.500	ba 2.660	b 3.330
Nairobi	a 3.330	ba 2.833	b 3.000
Rusinga	a 3.830	b 2.000	b 3.167

*Mean comparison was by Duncan's multiple range test (a=0.05)
Means with same alphabet within a particular treatment are not significantly different.

TABLE 16 : Analysis of variance for comparing locations using band width of Malate dehydrogenase (MDH).

Source	Degrees of freedom	LB12 Ms	LB323 Ms	LB14 Ms
Location	5	0.250 NS	0.810 NS	0.480
Error	12	4.320	5.160	45.830

*represent significant values.

Locational means			
Busia	a 4.330	a 5.500	b 32.167
Embu	a 3.000	a 6.000	a 33.667
Ishiara	a 2.900	a 6.000	a 34.667
Mombasa	a 3.000	a 6.000	a 35.000
Nairobi	a 3.167	a 5.500	a 32.833
Rusinga	a 4.600	a 5.500	a 34.330

*Mean comparison was by Duncan's multiple range test (a=0.05)

Means with the same alphabet within a particular treatment are not significantly different.

4.3 Cuticular hydrocarbon analysis

Cassava green mites produced sufficient quantities of cuticular hydrocarbon extracts which gave high resolution and consistent chromatograms. Fig 17 and 18 show the chromatograms from CGM collected from Mombasa and Nairobi which represent the long and short setae forms respectively. Fig 19 shows the profiles for the red spider mite. Retention times, absolute areas and relative areas for the prominent peaks for all samples are presented in appendices 10 - 12.

The prominent peaks represent abundant hydrocarbon components while the minor peaks represent the less abundant components. Profiles of both the Mombasa and Nairobi populations had abundant hydrocarbons. A total of six prominent peaks eluted at similar retention times in both the populations. The pattern in RSM was different from that in the two CGM populations, particularly with increasing retention time. These profiles express close relationship between the short and long setae forms in the CGM populations that were studied.

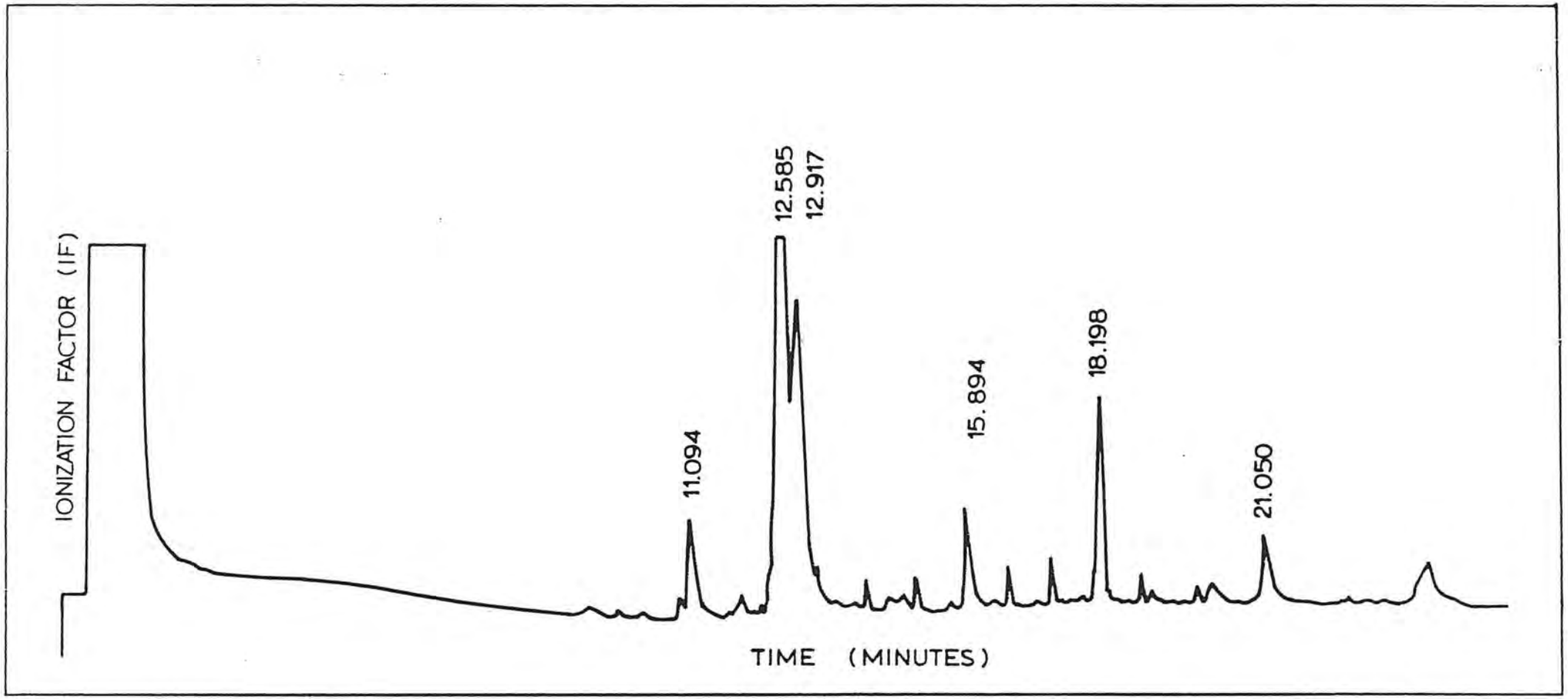


Fig. 17 Gas chromatogram of cuticular hydrocarbons isolated from Nairobi population of cassava green mite Mononychellus spp.

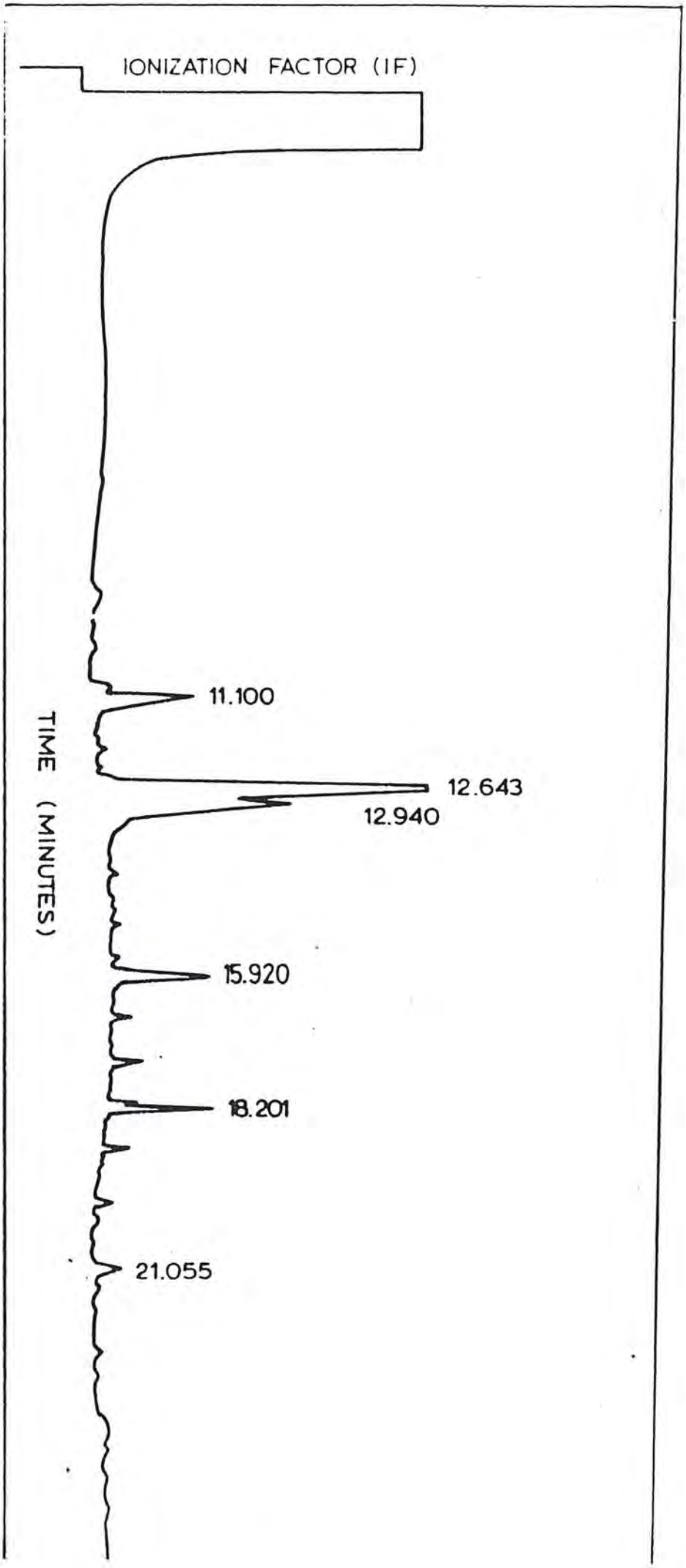


Fig. 18 Gas chromatogram of cuticular hydrocarbons isolated from Mombasa, population of cassava green mites Mononychellus spp.

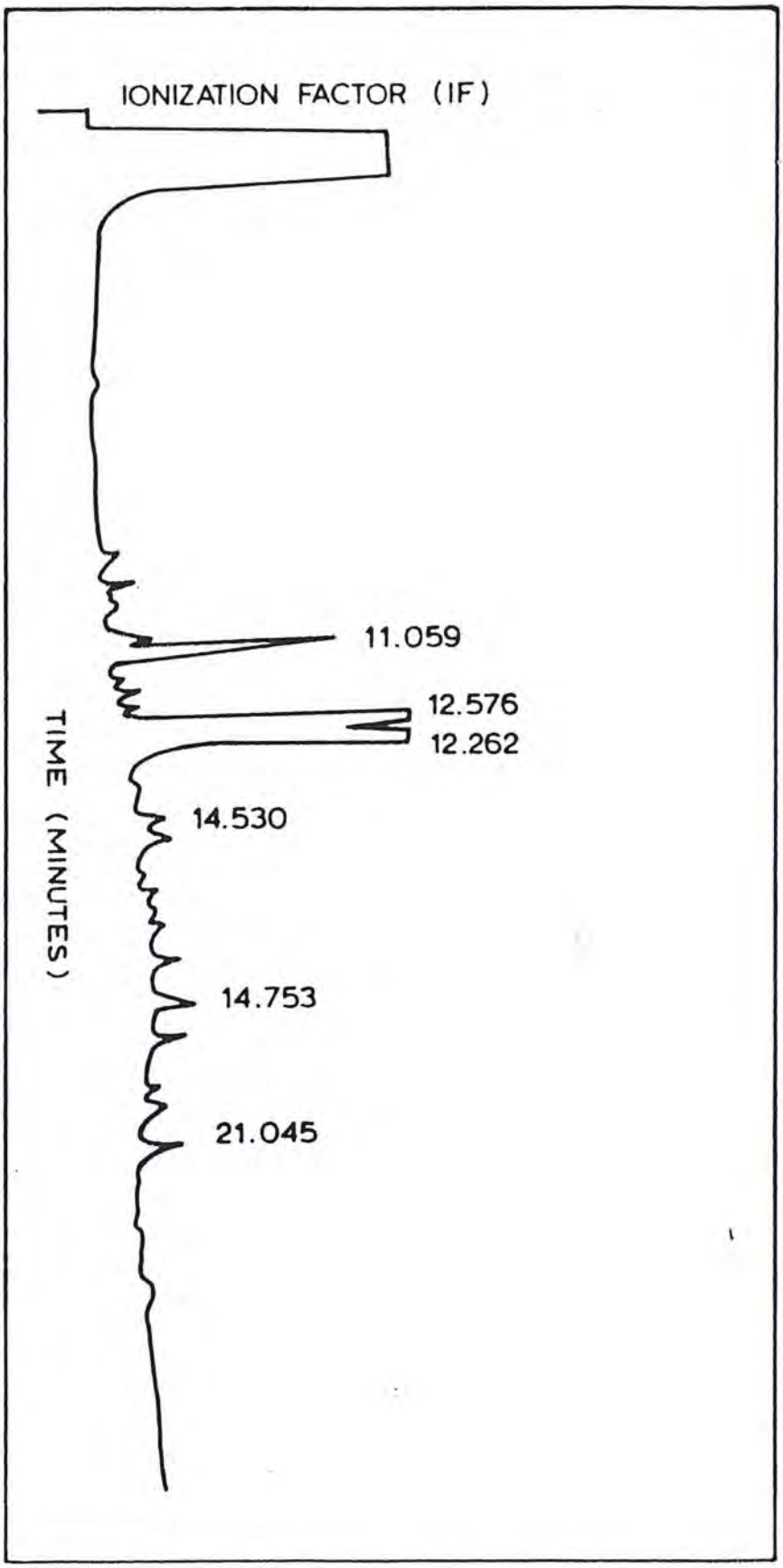


Fig. 19 Gas chromatogram of cuticular hydrocarbons isolated from red spider mite Tetranychus urticae

5. DISCUSSION

5.1 Morphological examination.

Studies on Morphology were done to correlate whatever biochemical findings with morphological work, which has been the traditional criteria for classifying CGM. Factors that make morphological features unreliable for characterizing CGM were revealed in the study. This showed why the descriptions given by Doreste (1981), based on setal lengths, created confusions for workers who tried to describe the species much later, such as Flechtmann and Baker (1982).

Among the factors responsible for this confusion in the description of CGM is the transient nature of the slide preparation of the specimens. In the preparation of CGM for observation, specimens are flattened out between slides, and little idea is obtained of the actual physical state of the mite, and the relative positions of the setae. This is particularly important in groups which are differentiated on the basis of the setal length such as the CGM. It would be most ideal to make such preparations in cavity slides.

Furthermore microscopic slides tend to degenerate and are likely to break after only a short time making comparisons of present and past descriptions difficult. For instance, Bondar's original slide used in describing *Tetranychus tanajoa* (Bondar) in 1938 was apparently lost and not available to later authors for comparative studies (Gutierrez, 1987). Another drawback in morphological analysis observed in this study was the shrinking of specimen as a result of preservation in alcohol. This could be detected by the reduced distances between the dorsal integumentary setae. Sometimes the bodies of older females were found to be swollen. In such cases integumentary setae became further apart. Rogo *et al.* (1987) and Bob-Manuel (1987) observed similar occurrences in CGM. Biochemical analysis could perhaps be the most meaningful method of characterizing CGM.

Other than the experimentally induced changes in CGM morphology, characters such as the chaetotaxy used in this study and employed in previous characterization, often differ from one specimen to another. Description could for instance be based on a species that is clearly musculinized, which is a frequent phenomenon in Tetranychids (Helle and Sabelis, 1985). At times

the number of ordinary setae is not the same in the individual specimens. This lack of consistency in morphological features does not allow for effective application of setae in species distinction.

Measurements and indices of morphological features must always be taken with a lot of caution during species definition. Variations in morphology observed in setal lengths are typical of Tetranychids (Boudreaux, 1963). The same author reported variations in *Tetranychus telarius* (L.) which was mistakenly considered as the closely related *Tetranychus cinnabarinus* (Biosduval). Only after experimental demonstration of reproductive isolation followed by the establishment of decisive morphological characters such as the male genetalia, was the correct identity of this mite known.

Suggestions by previous workers (Rogo *et al.*, 1987; Bob-Manuel, 1987) that a cross-section of taxonomic characters be used to identify CGM has been well considered in this study. As noted by Boudreaux (1963) the study of tetranychid systematics should be extensive enough to replace the examination of morphological characters alone.

5.2 Isoenzyme analysis.

In the isoenzymes where resolution was quite distinct, interpopulation similarities in bands were observed, these are clear indications of the closeness in species relationship of CGM. Harris and Hopkins (1976) stated that the more enzymes that are electrophoretically identical between samples, such as is observed in this study the greater the likelihood that samples are from related organisms, especially if the enzyme bands are repeatable and consistent. Biometric analysis further revealed the statistical significance of the close relationship between CGM populations. Scatter diagrams for the three most active enzymes showed a high degree of cluster which is normally expected of inter-correlated characters in a Principle Component Analysis (PCA) (Rolf, 1967).

Plots of GPI and MDH were particurlarly effective indicators of the close relationship among CGM populations. This corresponds with the high rate of activities normally exhibited by the two isoenzymes. Mehrotra (1961) and later Kotter (1978) demonstrated the presence of large quantities of MDH in *T. urticae* necessary for the

glycolytic pathway, the pentose and tricarboxylic cycles in metabolism. Ward *et al.* (1982) in separating three species of tetranychid mites, ***Tetranychus urticae, T. turkestani*** and ***T pacificus*** reported MDH as a particularly powerful diagnostic character. Rogo *et al* (1988) in separating ***Phlebotomus pedifer*** and ***Phlebotomus elgonensis*** reported ME, MDH and GPI as having distinct and reproducible bands which are useful in taxonomic differentiation. Biometric analysis carried out in this study further revealed the statistical significance of the close relationship between CGM populations. Scatter diagrams for the three most active enzymes showed a high degree of cluster which is normally expected of inter-correlated characters in a Principle Component Analysis (PCA) (Rohlf, 1967).

In those enzymes in which clear discernable bands were not seen, the causes of reduced activity or inactivity could have been due to over dilution of extracts or in some cases a complete lack of a particular enzyme in the mite. Other possible reasons for the lack of activities could be similar to the work reported by Blank (1979), who studied enzyme activity in *T. urticae* and showed that a considerable amount of enzymes with cholesterase activity failed to penetrate the

electrophoretic gel. This would indicate that in spider mites, such enzymes are strongly bound to membranes or other proteinaceous material and could not be easily extracted, and therefore, no banding patterns were observed.

There are certain pitfalls in the use of enzyme electrophoresis for taxonomic characterization which could have resulted in minor variations of the banding patterns. For instance, only a single mite specimen should have been homogenized for electrophoresis, but for practical purposes, such as the small size of the mite and therefore, a low protein content, this was not possible. Furthermore biochemical studies cannot be carried out in the field where there is no electricity. For this reason samples have to be specially stored, making the whole exercise very expensive.

Although changes resulting from storage were not observed unlike in setal length measurements, enzyme patterns may degenerate or alter upon storage especially if specimens are not stored in liquid Nitrogen. In which case valid comparisons may not be possible, since very weak bands appear. Faint bands also occur when organisms have enzymes which are broad-spectrum in their activity. This tends to interfere with the

staining of a particular enzyme which is being investigated (Dolan *et al.* 1988).

This study used female specimens for the analysis. In the preliminary studies, the banding pattern of both males and females were shown to be the same, only that males produced faint bands. No biasness was therefore introduced by using females alone. Likewise Ward *et al.* (1982) and Rogo *et al.* (1988) in differentiating *Phlebotomine* species reported that the main components of enzymes normally detected for male and females in IEF runs are the same.

The whole concept of using females was to prove Doreste's (1981) criteria of using the long and short setae forms of females to warrant species differentiation in CGM, as incorrect. These biochemical investigation have proved beyond reasonable doubt that Doreste (1981) was not correct in naming two species of CGM, ***M. progresivus*** and ***M. tanajoa*** basing his description on setal lengths of females. His classification sparked off controversy about the precise identity of CGM in Africa. Workers trying to use the same criteria of setal lengths employed by Doreste (1981) have therefore come up with conflicting reports. Flechtmann (1982) reported that there was ***M. progresivus*** and ***M. tanajoa*** in Gabon.

Macfarlane (1984) also reported the two species, *M. tanajoa* and *M. progresivus*, but added that M.tanajoa were fewer in number. Gutierrez (1987), contrary to these reports, stated that there is only one species in Africa and called it *M. progresivus*. Rogo *et al.* (1987) reported that only one species is present in Africa, but called the species *M. tanajoa*. Similarly Bob-Manuel (1987) using the ontogeny of the dorsal central body setae found only one species and called it *M. tanajoa*. Murega (pers comm.) working in the same region using hybridization methods to describe the species, reported one species, but maintained that the species in Africa is *M. Progresivus*. Yaninek and Herren (1988) stated that until otherwise proved, only one species exists in Africa and that this should be reffered to by the original name given to CGM in Africa, *M. tanajoa*

From these biochemical studies we can correctly say there is only one species, and that Doreste's report of *M. progresivus*, as yet another species in Africa differentiated from *M. tanajoa* on the basis of females setal lengths, is incorrect. The males he described to be *M.*

progresivus were infact those of *M. tanajoa* and what he described as *M. tanajoa* males were *Mononychellus estradai*. In otherwords *M. progresivus* does not exist. It is evident that even if the long and short setae forms are in the process of becoming separate species, the biochemical analysis shows that they have not yet acquired the attributes of distinct species status and should be treated as one.

The occurence of long and short setae forms of CGM could be a result of enviromental factors. Bob-Manuel (1987) reported that setal measurements of Mombasa mites which had predominantly long setae forms, varied from other populations of CGM in Kenya due to differences in climatic conditions. Such differences influence mites phenotypically. The changes resulting from these environmental conditions can mislead workers in morphological taxonomy to believe that more than one species is involved, particurlaly a worker looking at the extreme ends of the setal lengths (short and Long). Isoelectric focusing results in this study have provided a source of additional taxonomic characters for classifying CGM

5.3 Cuticular hydrocarbon analysis.

Cuticular hydrocarbon analysis of cassava green mites were complimentary to the isoenzyme investigation. Chromatographic patterns of Nairobi and Mombasa mites that represent the short and long setae forms respectively were shown to be identical. This confirms isoenzyme investigation which indicated similarity in the CGM populations.

It is interesting to note that although IEF and GC are two different methods of studying biochemical characters, they showed results indicating close species relationship among CGM populations. The occurrence of minor peaks on the gas chromatograms can partially be explained by the contrasting eco-climatic conditions in the mite collection sites. Varying weather conditions could induce changes in the cuticles of the mites. For example Lees (1952), and Van de vrie *et al.* (1972) noted that humidity and temperature exert strong influence on spider mites. The loss or gain of water from the atmosphere is important for organisms the size of CGM because of their large surface area/volume ratio. High relative humidity may prevent loss of moisture. Mites in areas of high humidity tend to have thin cuticle. Likewise

in low humidity areas the cuticles are thicker (Baudreaux, 1958). For instance, Arnold and Reigner (1975) reported that hydrocarbons are of major importance in a wide range of ecological roles and their quantity or at times quality are correlated with the need to conserve water. He gave the example of ***Ptenonacys carlifonica*** (Newport). Members of this species which inhabit hot areas had more surface lipids than those which are found in cool habitats. Qualitative and quantitative changes in cuticular hydrocarbons related to environmental conditions have also been reported in the flesh flies, ***Sarcophaga bullata*** (Haag) (Armorld and Reignier, 1975) ***Sarcophaga ritcheri*** (Lok ***et al.*** 1975), and in the beetles, ***Tenebrio molitor*** (L.) and ***Tenebrio obscurus*** (F.) (Lockey, 1984).

Despite their potentials as tools for studying taxonomic characters, it should be noted that use of GC as a taxonomic tool requires improved quality in handling and extraction of the specimens. In this particular study, the small size of CGM was a limiting factor in the sense that individual specimen have low absolute quantities of Cuticular hydrocarbons which could not easily be investigated with the kind of equipment available. Studies on the quantities of

cuticular hydrocarbons have been carried out by Morthes and Sietz (1982) who found the average cuticular thickness in *Tetranychus urticae* to be only 1.5 µm. Other authors who investigated cuticular hydrocarbon thickness include (Gibbs and Morrison, 1959; Hernesberg *et al.*, 1965; Albert and Storch, 1976). All are in general agreement concerning the thin layer of cuticular hydrocarbons.

The thin layer of cuticular hydrocarbons meant that as many as ten adult pure-line bred females of CGM had to be used in the analysis of each population of CGM. Furthermore cuticular hydrocarbon analysis requires expensive chemical equipment (Howard *et al.*, 1988). This makes it difficult to conduct GC studies in the field. Fortunately, hydrocarbons are relatively stable molecules. They store well and can be readily extracted under field conditions and preserved for analysis at a later date (Howard, 1982). Inspite of being a good taxonomic tool, such drawbacks as mentioned above cannot allow the use of GC alone in characterizing CGM. However, these preliminary studies of cuticular hydrocarbons lend confidence in establishing the precise identity of CGM.

6. SUMMARY

1. The study assessed the taxonomic status of cassava green mite (CGM) using the traditional morphological characters and the biochemical characters.

2. Morphological analysis indicated that CGM populations have a gradient of setal lengths ranging from short, intermediate to long forms.

3. The lack of a clear cut difference between the short and long setal lengths makes this character unreliable for species distinction.

4. Morphological analysis carried out in this study confirmed previous reports by authors who had worked on the taxonomy of the cassava green mite, and who suggested that other taxonomic characters be applied in resolving the species identitity of CGM.

5. Thirteen isoenzymes were studied on mites collected from six cassava growing areas of Kenya. Isoelectric focusing (IEF) method was used in separating these isoenzymes.

6. Enzyme bands appeared in all the six populations of CGM and in the Red spider mite (RSM) which were used as controls.

7. Not all bands were clear enough for use in taxonomic classification. Only the clear and consistent bands were used for taxonomic purposes.

8. Malate dehydrogenase (MDH), malic enzymes (ME) and glucose phosphate isomerase (GPI), were powerful indicators of species relationship, judged from the clarity and consistency of the bands.

9. Multivariate analysis of the band parameters revealed no statistically significant variation in the results.

10. In particular, PCA plots of MDH and GPI showed high degrees of clusters. However, ME showed minor variations.

11. Analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) showed significant differences in the population means.

12. Further analysis compared the cuticular hydrocarbon components of the long and short setae forms of CGM using gas chromatography (GC).

13. Cuticular hydrocarbon components showed identical peaks for both the short and long setae forms of CGM.

14. Minor peaks appeared in profiles of both the long and short setae forms of CGM, but these were not consistent for either populations and could not be considered to express species differences.

15. The occurance of minor peaks on the chromatograms was explained in terms of differences in environmental conditions which alters the nature of the cuticle

16. The chromatographic peaks show that cuticular hydrocarbons of cassava green mites are closely related.

17. Based on investigations in this study, cassava green mites in Kenya do not have any major biochemical variants. This implies that the mites cannot be distinguished into two separate species.

18. The methods of characterization considered in this study emphasize the existence of only a single species of cassava green mite.

19. There is need for biochemical studies to be conducted extensively on CGM from various parts of Africa where cassava is grown. Further studies should involve specimens collected from the type region.

20. Until distinct species are demonstrated, cassava green mite in Africa should be called *Monoychellus tanajoa* (Bondar), which is the name originally given to the species in Africa.

CONCLUSION

In correlating the various findings obtained in this study, they collectively emphasize the existence of a single species of cassava green mite. However, data recorded in this work cannot be taken as entirely adequate. Further research will be required in both isoenzymes and cuticular hydrocarbons of CGM. These studies should involve investigations of CGM from other regions of Africa. Comparative biochemical investigations should be conducted with CGM from the type region. Additional GC investigation should involve isolation and the identification of cuticular components using a mass-spectrometer. Lastly, with the biochemical proof of only one species of CGM, workers of Biological control can now evolve more appropriate strategies for its control.

LITERATURE CITED

Akinlosotu, T.A. and K. Leuschner (1981). Outbreak of two new cassava pests *Mononychellus tanajoa* and *Phenacoccus manihoti* in South western Nigeria. Trop. Pest Manag. 27: 247-250.

Alberti and Storch (1976). Ultrastuktiv untersuchungen am mannlichem Genitaltrakt und an spermeem von *Tetranychus urticae* (Tetranychidae: Acari). Zoomorphologie 83: 283-296.

Allen, R.C. Harley R.A. and Talamo, R.C. (1974). A new method for the determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. Am. J. Clin. Pathol. 62:732-739.

Allsops, B.A. and W.C. Gibson (1983). Isoelectric focussing in Agarose. A highly discriminatory method for the detection of enzyme heterogeneity. Ann. Trop. Med. and Parasitol. 77: 169-173.

Arnold,.M.T. and Reigner F.E (1975). A development study the cuticular hydrocarbons of *Sarcophaga bullata*. J. insect physiology 21,1827-1833.

Ayala, J.F. R. Jeffrey, L. Martin, A. Celso Santiago Perez-Salas (1971). Enzyme variability in Drosophila in the *Drosophila willistoni* group IV. Genic variation in natural populations of *D. willistoni*. Genetics 70: 113-139.

Ayalla, J.F. and J.R. Powell (1972). Allozymes as diagnostic characters of sibling species of *Drosophila*. Proc. Nat. Acad. Scl. (USA). 69(5): 1094-1096.

Baker, E.W. (1979). Spider Mites revisited : a review. Systematic entomology laboratory. Rec. Advan. in Acarol. 21: 387-391.

Baker, J.E. and Fatland (1979). Developmental changes in cuticular lipids of the Black carpet Beetle *Attagenus megatoma* Insect biochem 9: 335 -339.

Baker, J.E., R.D. Sukkestaad and C. Fatland (1979). Cuticular Lipids of larvae and adults of the cigarette beetle, *Lasioderma serricorne*. Insect Biochem. 9: 603-611.

Bark, V.E. and M. Firth (1976). Typing of common phospho-glucomutase variants using Isoelectric focusing, a new interpretation of the phosphoglucomutase System. J. Forens. Soc. 16, 115.

Baudreaux, H.B. (1958). The effect of of relative humidity on egg hatching and survival in various spider mites. J. Insect Physiol. 2 (1) 65-72.

Bennett, F.O. and M. Yaseen (1980). Distribution Biology and Population dynamics in the tropics. Proceedings of a workshop held in Rwanda, Kigali 23-27 Nov. (1980).

Bianchi, U and G. Chesa (1970). Alloenzimi ad attivita xantin diedrogenasic in Anopheles artroparvus. Bull. Zool.37: 477.

Blank, R.M. (1979). Studies on the non-specific esterase and acetylcholinesterase isoenzymes electrophoretically separated from the mites *Sancassania berlesei* (Tyroglyphidae) and *Tetranychus urticae* (Tetranychidae). N.Z. Journ. of Agric. Res. 22: 497-506

Bob-Manuel, R. (1987). Study of the Cassava green mite Spp. (Acari: Tehranychidae) in Africa. M.Phil. Thesis. International centre of insect physiology and ecology, Nairobi.

Bondar, G. (1938). Notas entromologicas da Bahia. Rev. Ent. 9 (3-98):441-449.

Boudreax, H. B. (1963). Biological aspects of some phytophagous mite. Ann. Rev. Entomol. 8: 137-154.

Brazil, (1978). Electrophoretic variation of the enzyme phosphoglucomutase in different strains of Leishmania. Ann. Trop: Med. and Parasitol. 72(2): 113-119

Bryne, D.H. Bellotti A.C. and Guerrero J.M.(1983). The Cassava mite. Tropical Pest Mang.29,378-394.

Cardwell-Grafton, E. Eash J.A. and Granett J. (1988). Isoenzyme differentiation of *Tetranychus pacificus* from *T. urticae* and *T turestani* (Acari:Tetranychidae) in laboratory and field populations. J.Econ. Ent.81(3)770-775

Carlson, D.A. (1982). Chemical taxonomy; Analysis of cuticular hydrocarbons for identification of *Simulium*, *Anopheles* and *Glossina*. Proceedings of a symposium of the application of the Biochemical and molecular biology techniques to problems of parasites and vector identification. Geneva, Switzerland. 8-10 Nov. 1982.

Carlson, D.A. (1986). Identification of mosquitoe of *Anopheles gambiae* Species complex A and B by analysis of cuticular components. Science 207: 1039.

Carlson, D.A. and Brenner R.J.(1988). Hydrocarbon-based discrimination of three North American Blatella cockroach specis (Orthorptera: Blatellidae). using gas chromatography. Ann. Ent.Soc.Am. 81(5): 711-723.

Centro Interncional de Agricultura Tropica, CIAT (1986). Cassava newsletter vol 10 No.1

Cicolani, B., A.B.P. Bullini and L. Bullini (1981). Morphological aspects of some phytophagous mites. Ann. Rev. Entomol. 8:137-154.

Compere, H. (1961). The red scale and its enemies. Higardia 31,173-278.

Cox, D. and J. Willis (1987). Post translational modification of cuticular proteins of *Hyalophora cecropia* from different anatomical regions and metamorphic stages. Insect biochem. 17: 469-484.

Daly, J. and Fisk J. (1988). Identification of Heliothes eggs using electrophoresis. International symposium on electrophoretic studies of agricultural pests, Rothamstead England. 6-8 April 1988.

Danks, H.V. (1988). Systematics in support of entomology. Ann. Rev. entomol 33: 271-296.

Dolan, S. and C.C. Flemming (1988). Isoenzyme in the identification and systematics of Terrestrial slugs of the *Arion hortonsis* Complex. Biochem Syst. and ecol. 16: 195-198.

Donnadieu, A.L. (1875). A higher classifiation of the spider mites. Soc. Linn. (n.ser.22 (1875), 34-163.

Doreste, E. (1981). Acaros del genoro *Mononychellus* wainstain (Acari; Tetranychidae) asociados con la Yuccas (*Manihot* spp.) en Venezuela. Bull. ent. Venez. 10: 119-130.

Doreste, E. (1981). Spider Mites as important pests of cassava (*Manihot esculenta*) Acarology 2: 761-769.

Doreste, S.E. and Aponte L.O. (1978). Egecto de los ateques del comlejo de acaros Tetranychidae, en los rendimentos del cultivo de la yuca. Revt. Fac. Agron. Cent. Venez. 10, 105-119.

Ewing, H.E (1913). The taxonomic value of characters of the male genitalia arpature in the genus Tetranychus Dufour. Expt. Applic, 25: 207-303

Flechtmann, C. H. W. and Baker E.W (1982). The cassava mite complex: III New distribution records, mainly in Colombia and Africa. References to other plants - Anas ESC. Sup. Agric. 'Luiz Queroz' 39,809-813.

Flechtmann, C.H.W. and E.W. Baker (1970). A preliminary report on the Tetranychidae (Acarina) of Brazil. Ann. of the Entomol. Soc. Am.. 63: 156-163.

Flechtmann, C.H.W.and Baker, E.W. (1975). Plant feeding mites of Thailand, Plant protection service. Technical Bulletin No.35

Goto, H.E. (1982). Animal taxonomy. Arnold, London.58 pp

Grafton-Cardwel, E. (1988). Isoenzyme differentiation of *T. urticae* and *T. turkestani* (Acari: Tetranychidae) in laboratory and field populations. J. Econ. Entomol 81: 770-775.

Greathead, D.J. (1970). A review of biological control in the Ethiopian region. Tech. Comm. CIBC 5, 162pp

Gutierrez, A.P.(1987). An overview of a systems model of cassava and cassava pests in Africa. Insect Sci. Appl. 8: 919-924.

Gutierrez, J. (1987). The Cassava Green Mite in Africa; one or two species; (Acari Tretranychidae). Expt. and Appl. Acarol. 3: 163-168.

Harris, H. and Hopkins D.A.(1976). Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam

Haverty, M I. Page L.Nelson and G.J Blomquist (1988). Cuticular hyrocarbons of the dampswood termite zootermopsis, inter and intra colony variations and potential as taxonomic characters. J. Chem Ecol. 14: (3) 1035-1058.

Helle, W. and Overmeer W.P.J.(1985). Rearing techniques. World crop pests. Vol.1A Elsevier, Amsterdam pp 331-335.

Helle, W. and Sabelis M.M. (Editors) (1985) spider mites. Their biology , Natural enemies and control. World crop pest Vol 1B Elsevier, Amsterdam 458pp.

Hernesberg, I.J. J.R. Adams and G.E. Cantwell (1965) Fine structure of the integument of the two-spotted spider mite *Tetranychus telarius* (Acarina: Tetranychidae). Ann. Entomol. Soc. Am. 58:532-535.

Herren, H.R. and Bennett (1984). Cassava pests, their spread and control. Proceedings of CAB'S First Scientific conference , Arusha Tanzania 12-18 Feb, 1984.-455pp.

Hienze, J. and A. Buschinger (1988). Electrophoretic variability of esterases in the ant tribe Leptothoracini. Biochem. System. Ecol. 16: 217-221.

Houke E.J. and Hardy (1978). Electrophoretic characteristics of non-specific esterases of the mosquitoe *Culex tansalis*; conventional and isoelectric focused acrylamide gels. Comp. Biochem. Physiol. 61: 291-295.

Howard, R.W. Thorne L.T. Lewing S.C.and McDaniel. (1988). Cuticular hydrocarbons as chemotaxonomic characters for *Nasutitermes corniger* (Motschulski) and *N.ephratae* (Holmgren) (Isoptera: Termitidae). Ann.Entomol. Soc. Am. 81 (3) 395-399.

Howard, R.W., C.A. McDaniel, D.R. Nelson and G. Blomquist (1982). Cuticular hydrocarbons of *Reticulitermes virginicus* (Banks) and their role as potential species and cast-recognition cues. Journ. Chem. Ecol. 8(9): 1227-1239.

Howard, W. R. (1982). Chemical Ecology and Biochemistry of insect hydrocarbons. Ann. Rev. ent. 27: 149-172.

IITA (1984). Africa-wide biological control of cassava mealybug and green spider mites. Information series No 16.

IITA (1986). Cassava Green Mite and the Africa-wide Biological control programme at IITA. 1986 IITA report.

Jackson, L.L. (1971). Cuticular lipids of insects IV. Hydrocarbons of the cockroaches Periplaneta japonica and Periplaneta americana. Compared to the cockroach hydrocarbons. Comp. Biocem. Physiol.41B: 331-336.

Jackson, L.L. (1971). Cuticular lipids of insects. IV Hydrocarbons of the cockroaches *Periplaneta japonium* and *Periplaneta americana* compared to other cockroach hydrocarbons. Comp. Biochem.Physiol. 41B: 331-336.

Jennings, D.C.(1970). Cassava in Africa. Field crop abstract vol 23 No.3

Jeppson, L.R. ,H.H. Keifer and E.W. Baker.. (1975). Mites injurious to economic plants. University of California Berkerly and Los angeles C.A.613.

Kottler, C (1978). Ein bietrag zur stoffwechessel-Physiologie von *Tetranychus* urticae Koch. (Acari:Tertranychidae). Z.Agnew Ent. 86:337-348.

Krutzer R.D. (1979). Etterasa isozyma in the moquitoe Culex (melanoconious) Erratious Mosquitoe News 39, 500-505.

Kumar, R. (1984). Insect pest control, with special reference to African agriculture. Edward Arnold, London. pp 286.

Le Pelley, R.H. (1943). The biological control of a mealybug on coffee and other crops in Kenya. Emp. J. Expt. Agri. 11:78-88.

Lees, G. (1952). The role of cuticular growth in the feeding process of ticks. Proc. Zool. Soc. Lond. 121: 759-772

Lockey, H.K. (1980). Insect cuticular hydrocarbons. Comp. Biochem. 65B: 457-462.

Lockey, K.H. (1984). Hydrocarbons of *Metriopus pepressus* (HAAG) and *Renatiella scrobipennis* (HAAG) (Coleoptera: Tenebrionidae). Insect biochem. *14: 65-7*

Lok, J. B. Cupp E.W. and Blomquist G.J (1975). Cuticular lipids of imported fireants *Solenopsis invicta* and *richteri*. Insect Biochem. 5, 821-829.

Lyon, W.F. (1973). A plant-feeding Mite *Mononychellus tanajoa* (Bondar) (Acarina: Tetranychidae). New to the African continent, threatens cassava (*Manihot esculenta)* Crantz in Uganda, East Afr. PANS 19: 36-37.

MacFarlen, D. (1984). Key to spider mites (Tetranychidae) recorded on cassava in Africa. Proc. of a Regional Training Workshop in E. Africa. 30April- 4th May 1984., 31-35.

May, B., Bauer, L.S. Vadas, L.R. and Granett, J. (1977). Biochemical genetic variation in the family Simulidae: electrophoretic identification of the human - biter in the isormorphic *Simulium jenningsi* group. Ann.Ent. Soc. Am. 70 (5) : 637-640.

Megevand, B., J.S. Yaninek and D.D. Friese (1987). Classical biological control of the Cassava Mite. Insect Sci. Appl. 8: 871-874.

Mehrotra, K.N. (1961). Carbohydrate metabolism in the two spotted spider mite *Tetranychus telarius* L. Hexose

monophosphate cycle. Comp. Biochem. Physiol.3:184-198.

Mehrotra, K.N. (1961). Carbohydrate metabolism in the two spotted spider mites *Tetranychus telarius* L. Comp. Biochem. Physiol. 3:184-198.

Menken S.B.J. (1988). Electrophretic studies on population, host races and sibling species in some families of Lepidoptera and Diptera. International symposium on electrophoretic studies of agricultural pests, Rothamstead, England. 6-8 April, 1988.

Meredith, S.E.O. (1982). Filariasis: Simulium needs and progress in the application of biochemical Techniques to the problem of vector identification. Proceedings of a symposium on application of biochemical and molecular biology techniques to problems of parasite and vector identification held in Geneva, Switzerland, Nov. 1982.

Meredith, S.E.O. and Townson H. (1981). Enzymes for the species identification of simulium damnosum complex from West Africa. Tropenmedizin und Parasitologie, 32:123-129.

Miles, S. J.(1979). A biochemical key to the adult members of **Anopheles gambiae** group. J. Med. Entomol.15: 297-9.

Miles, S.J and Ward (1978). Enzyme variation in the **Anopheles gambiae** Giles group of species. (Diptera: Culicidae. Bulletin of entomological Research. 68, 85-95.

Mothes,.U. and Sietz. K.A.(1982) Five structural alterations in bean plant leaves by feeding injury of Tetranychus urticae Koch (Acari: Tetranychidae). Acarologia 23:(149-159).

Murray (1877). 'Economic entomology aptera'. Chapman and Hall. London 1-433.

Nachman, G (1984). Estimates of mean population density and spartial distribution of the *Tetranychus urticae* and *Phytoseilus permisilis* based upon the proportion of empty units. J. Appl. Ecol. 21: 903-914.

Ndayiragiye, P. (1984). Cassava green mite in Burundi. Proceedings of a Regional Training Workshop in E.Africa.

Nelson, D.R., D.A. Carlson and C.L. Fatland (1988). Cuticular hydrocarbons of Tsetse flies. II *Glossina*

fuscipes, G. palpalis palpalis G. P. gambiensis, G. tachnoides and *G. brevipalpis* . Journal of chemical ecology 14: 6-13.

Nelson, D.R., J.W. Dillwith and G.J. Blomquist (1981). Cuticular hydrocarbons of house fly *Musca domestica*. Insect Biochem. 11: 189-197.

Nokoe, S. and L.M. Rogo (1988). A discriminant function for the short and long setae forms of mononychellus (Acari: Tetranychidae). Insect sci.applic.vol.9: 1-4.

Nyiira (1972). Report of investigation of cassava mite *Mononychellus tanajoa* Bondar. Kawanda research station, Kampala Uganda 1972 113p.

Pashley D.P. (1987). Host associated differentiation in armyworms (Lepidoptera: Noctuidae). International syposium on electrophoretic studies of agricultural pest, Rothamstead, England. 6-8 April, 1988

Peschke, K. (1987). Cuticular hydrocarbons regulate male recognition, male aggression and female choice of the Rove beettle, *Aleochara curtula*. Journ. Chem. Ecol. 13: 1993-2008.

Petersen, J.L. (1982). Application of biochemical and

molecular biology techniques to problems of parasite and vector identification. Geneva 8-10 Nov. 1982.

Petersen, J.L. (1982). Technique for isoenzyme analysis of live mosquitoes (Diptera culicidae). Entomol. 75: 719-720.

Pickett, C.H., F.E. Gilstrap, and L.F. Bouse (1987). Release of predatory mites (Acari: Phytoseideae) by aircraft for biological control of spider mites (acari:tetranychidae) infesting corn. J. Econ Ent. 80: 906 910.

Pritchard, A.E. and E. W. Baker (1955). A revision of the spider mite family, Tetranychidae. Pacific coast Entomol. Soc. San. Fransisco. (Memoir Ser. 2) 472 pp

Rogo, L.M., C.H.W. Flechtmann and E. Doreste (1987). A preliminary study of the taxonomic status of Cassava Green Spider Mite Complex *Mononychellus* (Acari: Tetranychidae). Insect Sci. Applic 8:11-13.

Rogo, L.M., C.P.M Khamala and M.J. Mutinga. (1988). Biochemical identification of *Phlebotomus (Larrousius) Pedifer* and *Phlebotomous (Larrousius) elgonensis*. Biochem. Syst. Ecol. Vol.16 nos 7/8 pp 655-659.

Rohlf, F.J. (1967). Correlated characters in numerical taxonomy. Syst. Zool. 16:109-126.

Rosen, D. and DeBach (1973). Systematics, Morphology and Biological control. Entomophaga 18: 215-222.

Saul, S.H., M.J.Sinsko, P.R.Gimstad and C.B. Graig (1977). Identification of siblings *Aedes triseriatus* and *A. hendersoni* by electrophoresis. J. Med. Entomol.. 13: 705-8.

Saul, S.H.and P.R.Gimstad (1977). Identification of Culex species by electrophoresis. Amer. J.Trop. Med. Hyg.26:1009- 1009-12

Sauti, R.F.N (1981). Problems of cassava production in Malawi. Proceedings of the first triannual root crops symposium of the international society of tropical root crops - Africa 8-12 Sept. 1980, Ibadan, Nigeria.

Schelinger, E.I. and Doutt, R.L. (1964). Systematics in relation to biocontrol. Biocontrol of insect pests and weeds.pp 247-280, Chapmann and Hall, London.

Shaw, C.R and Prassad R. (1970). Starch gel electrophoresis of enzymes - A compilation of recipes. Biochem. Genet. 4: 297-320.

Shukla, P.T. (1976). Preliminary report on the Green Mite (*Mononychellus tanajoa*). Resistance to Tanzanian local cassava varieties. E. Afr. Agr. For. J. 42: 55-59.

Singh and cunninghum (1981). Morphological and genetic differentiation in aphids (Aphididae). Can entomol. 113, 539-550.

Sula, J. and Weyda (1983). Esterase polymorphism in several populations of the two spotted spider mite *Tetranychus urticae* Koch. Experimentia 39: 78-79.

Synder T.P. (1982). Electrophoretic characterization of blackflies in *Simulium venustum* and *verecundum* species complexes. (Diptera: Simulidae). Canadian entomologist 114:503-507.

Townson, H., S.E.O. Meredith and K. Thomas (1977). Studies of enzymes in Aedes scutellaris group. Trans Royal Soc. Trop.Med. Hyg.71:110.

Tuttle, D.M., and E.W. Baker (1968). Spider mites (Tetranychidae: Acarina of the state of Coara Brazil. Int. Journ. Acarol. 3: 1-8.

Unruh, T.R. and R.D. Goeden (1987). Electrophoresis helps

to identify which race of the introduced weevil. *Rhinocyllus* conicus (*Coleoptera: Curculionidae*) was transfered to two Southern Californian thistles. Environm. Entomol. 16: 979-983.

Van der vrie, M. J.A McMurty and C.B. Huffacker (1972). Ecology of Tetranychid mites and their natural enemies . *A review*. Hilgardia 41:343-437.

Wainstein, B.A. (1960). Tetranychoid mites of the Khazakhastan. J.Sci. Res. Inst. Plant. Prot.5: 1-156

Wainstein, B.A. (1971). **Mononychellus**, A new name for **Mononychus** (Acariformes: Tetranychidae). Zool. zh. 50:589.

Ward, P.S., A. Boussy and D.E. Swincer (1982). Electrophoretic detection of enzyme polymorphism and differentiation in 3 species of spider mites (Acari: Tetranychidae). Ann. Entomol Soc. Am. 75: 595-598.

Wodagenen, A. (1985). Cassava and cassava pests in Africa. FAO plant protection Bull. 33.

Yaninek, J.S. and H.R. Herren (1988). Introduction and spread of Cassava Green Mite, *Mononychellus tanajoa* (Bondar) (Acari: Tetranychidae) an exotic pest in Africa and the

search for appropriate control methods. Bull. ent. Res. 78: 1-13.

Yaninek, J.S. H.R. Herren and A.P. Gutierrez (1987). The biological basis of the seasonal outbreak of CGM on Africa. Insect Sci. Applic. 8: 861-865.

APPENDICES 1 - 6.: Measurements (µm) on adult female Cassava Green Mite of each generation from six locations in Kenya.

Appendix 1: Busia

		Setal Length in (um)		
Generation	Repl.	D1	D2	D3
1	1	27.80	44.48	61.44
	2	27.80	44.48	44.48
	3	28.08	44.48	44.48
2	1	28.08	44.48	61.72
	2	27.80	44.76	44.48
	3	27.80	44.48	44.48
3	1	27.52	44.48	44.48
	2	27.80	44.76	60.88
	3	28.08	44.48	44.48
4	1	27.80	44.48	44.48
	2	27.80	44.78	44.48
	3	28.08	44.48	44.48
5	1	27.80	44.48	44.48
	2	27.80	44.76	61.44
	3	28.08	44.48	61.44

Appendix 2: Embu

		Setal Length in (µm)		
Generation	Repl.	D1	D2	D3
1	1	19.46	25.30	30.58
	2	20.16	25.57	31.14
	3	20.16	25.58	30.36
2	1	19.46	25.02	30.86
	2	20.16	25.58	31.14
	3	20.57	25.58	30.86
3	1	18.90	25.30	31.14
	2	20.16	25.30	31.14
	3	20.57	25.58	31.00
4	1	19.46	25.02	30.58
	2	20.16	25.58	31.14
	3	20.57	25.58	30.86
5	1	16.46	25.30	30.58
	2	20.29	25.58	31.14
	3	20.57	25.58	31.14

Appendix 3: Ishiara

		Setal Length in (µm)		
Generation	Repl.	D1	D2	D3
1	1	27.74	33.92	41.98
	2	25.29	33.36	42.25
	3	25.58	32.80	41.98
2	1	25.58	33.92	41.70
	2	25.29	33.92	42.25
	3	25.58	33.36	41.98
3	1	25.02	33.63	41.98
	2	25.29	33.92	41.98
	3	25.58	33.36	41.98
4	1	25.58	33.92	41.70
	2	25.29	34.05	42.25
	3	25.58	33.36	41.98
5	1	25.02	33.92	41.70
	2	25.58	34.05	42.25
	3	25.58	33.36	41.98

Appendix 4: Mombasa

		Setal Length in (µm)		
Generation	Repl.	D1	D2	D3
1	1	24.46	36.14	61.16
	2	25.02	26.41	50.04
	3	25.02	44.48	58.39
2	1	25.02	34.75	55.60
	2	26.41	30.58	50.04
	3	23.63	44.48	53.93
3	1	25.02	30.58	61.44
	2	25.02	33.36	50.04
	3	25.02	45.04	52.41
4	1	25.02	30.86	61.16
	2	25.02	33.36	50.04
	3	25.02	45.04	53.39
5	1	26.13	30.58	61.72
	2	25.02	33.92	50.32
	3	25.20	45.36	53.93

Appendix 5: Nairobi

		Setal Length in (µm)		
Generation	Repl.	D1	D2	D3
1	1	20.85	25.30	38.92
	2	20.57	25.02	22.24
	3	20.85	25.02	32.80
2	1	19.46	25.58	38.36
	2	20.02	25.02	33.64
	3	20.57	25.02	31.14
3	1	20.02	26.13	38.36
	2	20.02	26.41	22.26
	3	20.57	26.13	31.14
4	1	20.29	26.13	38.64
	2	20.02	25.58	22.24
	3	20.57	26.13	30.86
5	1	20.57	25.30	38.92
	2	20.02	25.58	33.92
	3	20.85	26.13	32.80

Appendix 6: Rusinga

		Setal Length in (µm)		
Generation	Repl.	D1	D2	D3
1	1	41.98	52.82	61.72
	2	41.70	53.10	62.27
	3	41.70	52.82	62.40
2	1	41.70	52.82	61.72
	2	41.70	53.10	62.27
	3	41.70	52.82	62.40
3	1	41.98	53.10	63.00
	2	41.70	53.10	62.27
	3	41.70	52.82	62.40
4	1	41.98	52.82	61.72
	2	41.98	53.10	62.27
	3	41.70	52.82	62.40
5	1	41.70	53.10	61.72
	2	41.70	52.82	62.27
	3	41.70	52.82	62.27

Appendix 7.

Location	Repl.	LB12	LB23	LB34	LB45	LB56	WB2	WB3	WB4	WB5	WB6
Busia	1	29.00	4.50	1.50	1.00	1.00	7.00	3.80	1.00	1.00	0.60
	2	30.00	5.00	1.00	0.80	0.90	6.50	3.50	1.00	0.80	1.00
	3	29.50	4.00	1.00	0.70	1.00	6.00	3.50	0.80	1.00	0.60
Embu	1	28.50	5.00	1.60	1.00	0.80	7.50	3.50	1.20	0.60	0.45
	2	28.50	5.00	1.00	1.00	0.70	7.00	3.80	0.80	0.50	0.80
	3	28.00	5.50	1.00	1.00	0.80	7.50	3.50	1.00	0.60	0.80
Ishiara	1	29.00	5.00	1.80	0.80	1.00	7.50	4.00	1.50	1.00	0.50
	2	28.00	5.50	1.50	0.90	1.00	7.20	4.00	1.20	0.60	0.50
	3	28.50	5.00	1.50	1.00	1.00	7.00	4.00	1.00	1.00	0.50
Mombasa	1	27.00	5.00	2.00	1.00	1.00	0.80	3.50	1.20	1.00	0.50
	2	29.50	6.00	1.80	1.00	1.00	7.50	3.00	1.00	1.00	0.80
	3	29.00	5.00	1.80	1.00	1.00	0.80	3.00	1.00	1.00	1.80
Nairobi	1	26.00	6.00	3.00	1.00	0.80	8.50	3.50	1.00	0.80	0.60
	2	25.50	5.50	2.00	1.00	0.80	7.80	3.00	1.20	0.80	0.90
	3	25.00	5.50	2.00	1.20	1.00	8.00	3.00	1.00	1.00	0.80
Rusinga	1	28.00	6.00	2.00	1.00	1.00	7.00	4.00	0.80	0.75	0.70
	2	26.00	5.50	2.00	0.90	1.00	8.00	3.00	1.20	0.80	0.90
	3	26.00	5.50	2.00	1.00	0.80	8.20	3.50	1.00	0.80	0.90

Appendix 8.

Location	Repl.	LB12	LB23	LB34	LB45	WB2	WB3	WB4	WB5
Busia	1	10.50	6.00	2.00	1.00	2.20	3.00	3.00	3.00
	2	11.50	5.00	1.80	1.20	2.50	2.00	2.50	2.50
	3	10.50	4.50	2.50	1.20	2.50	3.00	2.50	2.80
Embu	1	11.00	5.50	1.80	2.00	3.00	3.00	2.50	2.50
	2	11.80	4.50	1.80	1.50	3.20	2.00	3.00	2.00
	3	11.00	4.50	2.50	1.20	3.00	3.00	2.50	3.00
Ishiara	1	11.50	3.00	2.00	1.80	2.50	2.50	2.50	2.50
	2	11.00	3.00	2.00	1.60	2.00	2.00	2.50	2.50
	3	11.00	3.50	2.00	1.80	3.00	3.00	2.50	3.00
Mombasa	1	11.00	4.00	2.00	1.80	2.00	2.00	3.00	3.00
	2	11.00	3.00	2.00	1.60	2.00	2.00	2.00	3.00
	3	11.00	3.50	2.00	1.80	2.50	3.00	2.50	3.00
Nairobi	1	10.80	4.00	2.50	1.60	2.00	2.00	2.50	2.50
	2	11.00	3.50	2.00	1.40	2.00	1.80	2.00	2.50
	3	10.90	4.50	2.00	1.80	2.50	2.50	2.50	2.50
Rusinga	1	10.90	5.00	4.50	1.50	2.50	2.00	2.00	2.50
	2	10.00	3.80	4.00	1.60	2.00	2.00	2.00	3.50
	3	11.00	4.00	4.00	1.50	2.00	2.00	3.00	3.00

Appendix 9.

Location	Repl.	LB12	LB23	LB34	WB2	WB3	WB4
Busia	1	4.00	6.00	31.00	3.50	2.00	4.50
	2	6.00	6.00	30.50	3.00	2.50	4.00
	3	3.00	4.50	35.00	4.00	3.00	5.00
Embu	1	3.00	6.00	34.00	3.00	2.50	3.00
	2	2.50	5.50	32.50	5.00	2.20	3.00
	3	3.50	6.50	35.00	4.50	4.00	4.00
Ishiara	1	2.20	6.50	34.50	3.50	3.00	3.00
	2	4.00	6.00	33.00	3.50	3.00	3.00
	3	2.50	5.50	37.00	4.50	4.00	4.50
Mombasa	1	3.00	6.00	35.00	3.00	2.50	3.00
	2	3.50	5.50	34.00	3.50	2.50	3.50
	3	3.00	6.50	36.00	4.00	3.00	3.50
Nairobi	1	3.00	6.00	30.00	3.00	2.00	3.00
	2	4.00	5.00	33.50	4.00	2.50	3.00
	3	2.50	6.00	35.00	3.00	4.00	3.00
Rusinga	1	5.00	4.50	35.00	3.00	2.00	3.00
	2	6.00	6.00	35.50	4.00	2.00	3.00
	3	2.80	6.00	32.00	4.50	2.00	3.50

Appendix 10: Relative time (RT) and % area of peaks from Cassava Mites (CGM)

LOCATION	REPL	RELATIVE TIME (RT)	AREA	% AREA
NAIROBI	1	11.0800	16664	11.3900
		12.6140	5445	37.2000
		12.9010	29303	20.0280
		15.8560	47670	3.2580
		18.1950	4280	2.9254
		21.0450	3132	2.1400
	2	11.1000	11494	9.6820
		12.6430	48538	40.8900
		12.9400	33790	28.4600
		15.9200	41160	3.4600
		18.2010	4943	4.1600
		21.0550	3368	2.8300
	3	11.0680	19498	10.3500
		12.5740	5036	50.3600
		12.9020	2215	22.1540
		15.9220	6392	0.6392
		18.2050	1303	1.3000
		21.0470	2739	2.7300

Appendix 11: Relative time (RT) and area of peaks from Cassava Mites (CGM)

LOCATION	REPL	RELATIVE TIME (RT)	AREA	% AREA
MOMBASA	1	11.1020	10,656.00	15.4570
		12.6500	32.45	47.0700
		12.9000	79.29	11.5000
		15.9400	18.75	2.7200
		18.9500	6,482.00	9.4000
		21.0500	40.32	5.8500
	2	11.0940	9,888.00	5.0740
		11.5920	79,523.00	40.8000
		12.9170	45,582.00	23.3900
		15.8940	8,881.00	4.5500
		18.1980	11,557.00	5.9300
		21.0500	8,007.00	4.1000
	3	11.0890	16,593.00	8.0200
		12.6180	64,734.00	31.2900
		12.6800	33,477.00	16.1800
		12.9280	57,265.00	27.6800
		18.2180	5,122.00	2.4700
		21.0800	2,223.00	1.0800

Appendix 12: Relative time (RT) and % area of peaks from Red Spider Mites

LOCATION	REPL	RETENTION TIME (RT)	AREA	% AREA
RSM	1	9.8030	2,909.00	0.8500
		11.0590	16,148.00	4.7500
		12.5760	1,832.00	53.8700
		12.8620	94,851.00	27.8800
		14.7530	6,463.00	1.8900
		21.0450	6,479.00	1.9000
	2	9.8160	1,318.00	1.0700
		11.1100	6,376.00	5.1700
		12.6280	51,642.00	41.9000
		12.9300	32,046.00	26.0000
		14.7990	2,160.00	1.7500
		15.9160	2,135.00	1.7300
	3	11.0990	113,911.00	4.0600
		12.6380	39,993.00	11.7000
		12.9180	41,381.00	12.1000
		15.8750	21,544.00	6.3000
		16.6330	9,580.00	2.8000
		17.3880	15,694.00	4.59