STUDIES ON THE MODE OF TRANSMISSION OF RICE YELLOW MOTTLE SOBEMOVIRUS

BY

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ZARIA, NIGERIA

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DECLARATION

I hereby declare that this Ph.D Thesis entitled "Studies on the mode of transmission of rice yellow mottle sobemovirus" has been written by me and it is an original record of my research work. It has not been presented anywhere before for a higher degree.

. M. Abo, MSc

14/9/98

Date

The above declaration is confirmed.

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CERTIFICATION

The thesis entitled "Studies on the mode of transmission of rice yellow mottle sobemovirus" by Emmanuel Myimaorga Abo meets the regulation governing the award of the degree of Doctor of Philosophy of the Ahmadu Bello University, Zaria, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION.

This work is affectionately dedicated to my parents, the late Mr. Abo Tule Shie and Madam Mbafan Abo Tule Shie.

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ABSTRACT

The investigation on the mode of transmission of rice yellow mottle sobemovirus (RYMSV) was carried out in the screenhouse and field in Cote d'Ivore, West Africa from 1995 to 1997. The study was based on visual assessment utilising standard evaluation scale (SES) for rice and enzyme linked immunosorbent assay test of rice leaves, other organs and components that have been exposed to RYMSV infections through natural and artificial inoculations with the virus. RYMSV was found by ELISA test to be located in the husks/hull (testa) of a rice seed. The virus sap extracted from husks with distilled water could not infect seedlings of Bouake 189, a susceptible check variety. RYMSV was not transmitted through rice seeds but through plant debris and to a lesser extent by empty spikelets and surface contaminants on rice seeds. It was also transmitted when rice roots were intertwined, leaf contact from closely spaced plants, rice stubble and contaminated hands. Ratooning and trimming of rice roots increased the incidence of RYSMV. The virus was lost more rapidly in the spikelets and husks than dry leaves stored at room temperature ($28\pm2^{o}C$). It was almost equally eliminated in the leaves, spikelets and husks stored in the dry and waterlogged soils.

Two Chrysomelid beetles, *Trichispa sericea* (Hispinae), *Chaetocnema pulla* (Halticinae), a Coccinellid beetle, *Chnootriba similis (Epilachna similis*, and a Tettigonidae, *Conocephalus longipennis* transmitted it. *T. sericea* and *C. similis* transmitted RYMSV for 1-3 days in a semi-persistent manner while *C. pulla* transmitted the virus for 5-6 days in a persistent manner. The *T. sericea, C. pulla,* and *C. similis* were more efficient vectors of RYMSV than the *C. longipennis*. RYMSV was detected

by ELISA in the faecal droplets of *Oxya hyla* (Acrididae) a non vector whereas it was not detected in the faeces of the vector, *C. longipennis*. The virus sap extracted from faecal droplets of the two insects with distilled water was not infectious to seedlings of Bouake 189.

The virus was not transmitted by any of the nematodes screened and neither by the nematode transmitting viruses such as *Xiphinema* and *Paratrichodorus* spp.

Oryza sativa, *O. glaberrima*, *O. barthii* are systemic hosts and *O. longistaminata* is a local lesion host of RYMSV. A localised infection of *Echinochloa crus-pavonis*, *E. pyrimadilis* and *Eragrosti ciliaris* were observed. *Chenopodium amaranticolor*, *Nicotianna tabaccum*, and *N. benthamania*, widely used as indicators for most plant viruses, were not susceptible to RYMSV. The virus did not infect the dicotyledons, other cultivated cereals and grasses screened. From field surveys only rice *(O. sativa.)* and *E. crus-pavonis* were infected in nature. *T. sericea* overwintered on *Leersia hexandra* and ratooned/volunteer rice in the field from which the insect moved into newly transplanted rice. But *L. hexandra* was not susceptible to RYMSV both in screening test and in nature. Thus, infected ratooned/volunteer rice that persisted through out the year due to the availability of irrigation water served as the main source of RYMSV inoculum in the field from which *T. Sericea* acquired and transmitted the virus to newly transplanted rice.

TABLE OF CONTENTS

		PAGE
TITLE	.....	i
DECLARATION	.....	ii
CERTIFICATION	.....	iii
DEDICATION	.....	iv
ACKNOWLEDGEMENTS	.....	v
ABSTRACT	.....	viii
TABLE OF CONTENTS	.....	x
LIST OF FIGURES	.....	xvi
LIST OF TABLES	.....	xvii
LIST OF PLATES	.....	xx
LIST OF APPENDICES	.....	xxii
CHAPTER ONE		
1.0. INTRODUCTION	.....	1
1.1 Origin, Distribution and Uses of Rice	.....	1
1.2 Origin and Distribution of Rice Yellow Mottle Virus in Rice	...	4
CHAPTER TWO		
2.0 LITERATURE REVIEW	.....	8
2.1 Pests and Diseases of Rice	.....	8
2.2 Rice yellow mottle virus: Variability, Symptomatology, and Infectivity	.....	9

2.2.1 Characteristics of Rice Yellow Mottle Virus..... 9

2.2.2 Mechanical Transmission and Symptomatology of Rice Yellow Mottle Virus 11

2.3 Ecological Distribution of Rice Yellow Mottle Virus and Enviromental Factors Affecting Infection 12

2.4 Evaluation of Rice Germplasm for Disease Reaction, Disease Incidence and Yield Loss Assessment 13

2.4.1 Varietal Evaluations 13

2.4.2 Incidence and Yield Loss Assessment..... 15

2.5 Transmission of Plant Viruses 16

2.5.1 Transmission of Plant Viruses Through Seed..... 16

2.5.2 Transmission of Plant Viruses by Insects..... 18

2.5.3 The Reservoir Hosts of Plant Viruses..... 23

2.5.4 Transmission of Plant Viruses by Soil Nematodes..... 25

2.5.5 Transmission of Plant Viruses Through Other Sources..... 27

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS..... 29

3.1 Field Surveys and Sample Collection..... 29

3.2 Soil Sterilization and Screenhouse Facility..... 30

3.3 Source of Plant Materials and Virus Isolate 30

3.4 Identification of Samples..... 30

3.5 Visual Assessment Procedure..... 31

3.6 Antisera, Conjugates, Substrates and Microtitre Plates..... 31

3.7 Buffers and Leaf Extracts..... 31

3.7.1 ACP-ELISA Protocol 32

CHAPTER FOUR

4.0 RYMV INFECTIVITY, LONGEVITY, AND INDEXING IN RICE 34

4.1 INTRODUCTION..... 34

4.2 MATERIALS AND METHODS..... 36

4.2.1 Transmission of RYMV Through Rice Seed from Infected Plants 36

4.2.2 RYMV Indexing in Whole Rice Seed, Components and Empty Rice Spikelets by ELISA..... 37

4.2.3 The Influence of Time of Infection and Position of Inoculated Leaf on Translocation of RYMV in Rice Seed..... 40

4.2.4 Virus Contamination, Plant Infection, and Infectivity of Virus Extract from Rice Husks..... 40

4.2.5 The Role of intertwinning of Rice Roots in The Transmission of RYMV 42

4.2.6 The Role of Rice Stubble in The Transmission of RYMV 44

4.2.7 The Role of Leaf and Body Contacts in The Transmission of RYMV 46

4.2.8 The role of Direct Seeding and Transplanting of Rice in The Transmission of RYMV..... 47

4.2.9 The Longevity of RYMV Infected Plant Materials in Storage..... 47

4.3 RESULTS..... 48

4.4 DISCUSSION..... 69

CHAPTER FIVE

5.0 SCREENING OF SOME RICE INSECTS FOR THEIR ABILITY TO TRANSMIT RICE YELLOW MOTTLE SOBEMOVIRUS 74

5.1 INTRODUCTION..... 74

5.2 MATERIALS AND METHODS..... 75

5.2.1 *Screening of Some Rice Insects for Their Ability to* Transmit Rice Yellow Mottle Virus..... 75

5.2.2 Detection and Infectivity Test with Faecal Droplets of Grasshoppers 78

5.2.3 Investigation on The Mode of Transmission and Retention Period of RYMV by *Trichispa sericea* Guer., *Chaetocnema pulla* Chap. and *Chnootriba similis* Thunberg..... 78

5.3 RESULTS..... 79

5.4 DISCUSSION..... 90

CHAPTER SIX

6.0 THE REACTION OF SOME WEEDS AND CULTIVATED CEREALS TO RICE YELLOW MOTTLE VIRUS IN SCREENING TEST AND IN NATURE..... 92

6.1 INTRODUCTION..... 92

6.2 MATERIALS AND METHODS..... 94

6.2.1 Screening of some weeds and cultivated cereals for susceptibility to RYMV..... 94

6.2.2 Investigation on The Transmission of RYMV to *Leersia hexandra* Sw. by *Trichispa sericea* Guerin in Direct Feeding Test..... 95

6.2.3 Field Surveys..... 96

6.3 RESULTS 96

6.4 DISCUSSION..... 108

CHAPTER SEVEN

7.0 SCREENING OF SOIL NEMATODES FOR THEIR ABILITY TO TRANSMIT RICE YELLOW MOTTLE SOBEMOVIRUS 110

7.1 INTRODUCTION..... 110

7.2 MATERIALS AND METHODS..... 111

7.3 RESULTS..... 112

7.4 DISCUSSION..... 115

CHAPTER EIGHT

8.0 SUMMARY AND CONCLUSIONS..... 116

REFERENCES..... 120

APPENDICES..... 142

BIOGRAPHY..... 146

LIST OF FIGURES

FIGURES		PAGE
1.	Areas of Domestication of *Oryza glaberrima* Steud. and Dispersal Route of *O. sativa* L.....	2
2.	Distribution of RYMV in Africa.....	6
3.	Some of the Several Chrysomelid Beetles Reported as Vectors of RYMV in East Africa and Madagascar.....	21
4.	Several Species of *Chaetocnema* (Galerucinae) Reported to be Vectors of RYMV in East Africa.....	22
5.	Different Organs and Components of a Rice Seed used in RYMV Indexing by ELISA.....	38
6.	Insect Vectors of Rice Yellow Mottle Virus in Cote d'Ivoire, West Africa.....	80

LIST OF TABLES

TABLE		PAGE
1.	Determination of Infection by ELISA in Leaf Samples of Screen-house Reared Plants Raised from Seeds of RYMV Infected Rice Plants.....	49
2.	Determination of Infection by ELISA in Leaf Samples of Screenhouse Reared Plants Raised from Seeds of Plants Inoculated with RYMV at Different Plant Ages	50
3.	Determination of the presence of RYMV in Whole Rice Seeds of Naturally RYMV Infected Plants Collected from the Field	51
4.	Determination of the presence of RYMV in Empty Rice Spikelets of Naturally RYMV Infected Plants Collected from the Field	52
5.	Determination of the presence of RYMV in the Organs of Germinating Rice Seeds of Naturally RYMV Infected Plants	53
6.	Determination of the presence of RYMV in the Rice Components of Naturally RYMV Infected Plants	54
7.	The Effect of Time of RYMV Infection on the Detection of the Virus in the Seeds of Rice	55
8.	The Effect of Position of Inoculated Leaves with RYMV on the Detection of the Virus in Seeds of Rice.....	56
9.	The Infectivity of Rice Seeds, Leaf Debris, and Empty Rice Spikelets from Non-infected and RYMV Infected Rice Plants.....	58

10. Determination of Infectivity of Rice seeds Treated with Virus Extract 59

11. The Effect of Intertwinning of Rice Roots in the Main and Ratoon Crop Stages on the Transmission of RYMV 60

12. The Effects of Leaf and Body Contacts on the Transmission of Rice Yellow Mottle Virus..... 61

13. The Incidence of RYMV Due to Direct Seeding and Transplanting of Rice on the Virus Infected Stubble Incorporated in the Soil..... 62

14. The Longevity of RYMV in Plant Materials of Bouake 189 Preserved at Room Temperature..... 64

15. The Longevity of RYMV in Plant Materials of Bouake 189 Incorporated into Sterile Dry Soil..... 65

16. The Longevity of RYMV in Plant Materials of Bouake 189 Incorporated into Non-Sterile Dry Soil..... 66

17. The Longevity of RYMV in Plant Materials of Bouake 189 Incorporated into Sterile Waterlogged Soil..... 67

18. The Longevity of RYMV in Plant Materials of Bouake 189 Incorporated into Non-Sterile Waterlogged Soil..... 68

19. The Transmission and Non-transmission of RYMV by Some Rice Insects in Screening Tests..... 81

20. Mode of Transmission of RYMV by *Trichispa sericea* Guerin., *Chaetocnema pulla* Chap. and *Chnootriba similis* Thunberg (*Epilachna similis* Mulsant)..... 88

21. Retention Period of RYMV in *Trichispa sericea* Guerin, *Chaetocnema pulla* Chapius and *Chnootriba similis* Thunberg in Daily Transfer Test on Healthy Seedlings of Bouake 189..... 89

22. Sensitivity Status of Some Weeds and Wild Rice Species to Rice Yellow Mottle Virus in Mechanical Inoculation Test 98

23. Sensitivity Status of Some Cultivated Cereals to Rice Yellow Mottle Virus in Mechanical Inoculation Test 101

24. Non-susceptibility Reaction of *Leersia hexandra* Sw. to RYMV by Drect Feding of Viruliferous *Trichispa sericea* Guerin and Mechanical inoculation..... 102

25. The Result of ELISA Test on Weeds and Rice Samples Collected from Irrigated Rice Fields Endemic to RYMV at Sakkassou, Cote d'Ivoire 105

26. Reaction of Bouake 189 Seedlings Transplanted in Soil Containing Nematodes Collected Around RYMV Infected Rice Plants 113

27. Reaction of Bouake 189 to Viruliferous Nematodes Previously Raised in Pure Culture of RYMV Infected Rice Plants..... 114

28. Reaction of Bouake 189 to Nematodes Extracted from the Root Zones of RYMV Infected Rice Plants..... 114

LIST OF PLATES

PLATE		PAGE
I.	The Inoculated Rice Plant (Left) and the Uninoculated Healthy Plants (Right) with Roots Fused Together	43
II.	The Artificially Inoculated Rice Plants Exhibiting Typical and Classical Symptoms of RYMV in the Screenhouse.....	45
III.	The RYMV Infected Rice Plants (Yellow Symptoms) in the Circled Patch at the Foreground are a Consequence of the RYMV Infected Stubble Incorporated into the Soil	45
IV.	Typical Whitish Feeding Streaks of the Adults of *Trichispa Sericea* Guerin on all the Rice Leaves	82
V.	The Typical Devastating Feeding Damage of the Adults and Larvae of *Trichispa sericea* Guerin on the Rice Foliage in the Screenhouse	83
VI.	*Chnootriba similis* Thunberg and its Typical Feeding Damage (Whitish Streaks/Chlorosis) on Rice Leaves	86
VII.	*Chnootriba similis* Thunberg and Whitish Streaks/Chlorosis with Drying up of Rice Leaves Occasioned by its Feeding Habit	87

VIII. *Echinochloa colona* Link Grown in the Same Spot with RYMV Infected Rice Plant for Infectivity Test..... 103

IX. *Echinochloa crus-pavonis* Schultes Growing in Nature in the Same Vicinity and in Close Contact with RYMV Infected Rice Plants 104

X. Fallow Rice Field Populated With *Leersia hexandra* Sw (*Oryza* Tribe) attacked by the Adults and Larvae of *Trichispa sericea* Guerin at Sakassou, Cote d'Ivoire 107

XI. Volunteer/Regrowth Rice Field Attacked by *Trichispa sericea* Guerin at Sakassou, Cote d'Ivoire..... 107

LIST OF APPENDICES

APPENDIX		PAGE
1.	Scoring System for RYMV.....	142
2.	Rice Yellow Mottle Virus (RYMV) Scale for Field Test at Growth Stages from 4 Weeks and Above.....	143
3.	Dr Sven Erik Albrechsten's Letter to Dr Abdoul Aziz Sy on Seed Test and Analysis for RYMV Transmission....	144
4.	The Life Cycle of the Hispidae (Chrysomelidae Hispinae)	145

CHAPTER ONE

1.0 INTRODUCTION.

1.1 Origin, Distribution and Uses of Rice

Rice (*Oryza* species), the principal staple food for millions of people in most Asian countries, parts of Africa and Latin America, feeds more people in the world than any other crop (Ng *et al.*, 1988; Herdt and Palapac, 1983). But feeding the world's growing population will depend on increased production of rice. Today rice is produced in about 10% of all cropland (144 million ha.) in over 110 countries (Andriese, 1986; Webster, 1992) including in varying degrees every country in West Africa (WARDA, 1993).

The origin of rice domestication in West Africa still remains speculative (Ng, *et al.*, 1988). According to Poteres (1976), African rice originated or was first domesticated in the Inland delta area of the Niger river in about 1500 B.C. by the indigenous inhabitants of the area and then westward to the Senegal Valley and Casamance (Figure 1). The *Oryza* species, *O. glaberrima* steud., *O. sativa* L., *O. longistaminata* Chev and Roehr., *O. barthii* Chev., and *O. Punctata* Kotsky and Steud, are found in West African rice ecology (Rossel *et al.*, 1982a; John *et al.*, 1984; Imeokparia, 1996). *O. glaberrima* Steud is said to be indigenous to West Africa (Purseglove, 1972) and has been in cultivation for the past 3500 years whereas *O. sativa* L. was introduced into the region in about 1890 (Poteres, 1956; Mustapha, 1994; WARDA, 1996). Also in East Africa, the following *Oryza* species are found: *O. sativa* L., *O. Barthii* Chev., *O. eichingeri* Peter, *O. punctata* Kotsky and Steud., *O. longistaminata* Chev and Roehr as well as the Oryzeae, *Leersia* spp., and *Maltebrunia schliebenii* (Pilg) C.E. Hubbard (Bakker, 1974). It is also reported that rice seeds were probably brought to the East African Coast by the Swahili and Arab slave traders before the advent of the white man to the Munias areas of northern



Fig. 1. Areas of Domestication of *Oryza glaberrima* Steud and Dispersal route of *Oryza sativa* L.
Source: Ng *et al.*, 1988

Kavirando about 60 km North West of Kismu (Oka *et al.*, 1977). Also another account has it that rice was introduced in Central Kavirondo near the Kavirondo Gulf by officers of the former British Administration in the early days of the Colonial period (Wilson, 1944). In 1934, this area was expanded and reached by 1938 an estimated 300ha (Bakker, 1974).

The indigenous domesticated *Oryza* species (*O. glaberrima* Steud) is grown mainly in West Africa but the Asian domesticated species, *O. sativa* L., is also widely grown in Africa (Ng *et. al.*, 1988). *O. Sativa* L. was probably introduced into East Africa some 2000 years ago when the sea trade between East African Ports and India was flourishing (Harlan and Stemler, 1976). It is also probable that Asian rice was carried to East Africa at a later date (by the 10th century AD) by Polynesians using the Southern Indian Ocean Sea route from Indonesia to Madagascar and the east coast of Africa (Chang, 1976; Carpenter, 1978). Also according to Nayar (1973), *O. sativa* L. was first brought to Madagascar from Indonesia and then to East and West Africa. Thus, farmers from East Africa bought the imported rice and intensified their cultivation (Wilson, 1944; Bakker, 1974). Thereafter a campaign organized by the government resulted in an increase of the planted area from 1000 to 4000 ha. in 1943 (Wilson, 1944).

These Asian rice cultigens were subsequently introduced to West Africa either by the Portuguese in about 1500 AD via the Sea Coast (Poteres, 1950) or by traders or Muslim missionaries travelling across the Sahara from North Africa to across Central Africa in the 9th to 10th Centuries AD (Nayar, 1973; Carpenter, 1978). The Asian rice *(O. sativa* L.) a good colonizer has established well in many African ecologies soon after it was introduced in West Africa almost replacing the African rice (Williams, 1978; Ng, 1979; Ng *et al.*, 1988; Mustapha, 1994).

Milled raw and parboiled rice is consumed mainly as boiled rice (Kongseree, 1979; Juliano,

1985a). Waxy rices are used in sweets, dessrts and salad dressing (Juliano, 1985a). In the United States and the Philippines rices are used in baby foods, breakfast cereals, canned foods, fermented rice cakes, and yeast-leavened rice desserts (Juliano, 1979,1985a). Rice bran is used for oil extraction and food (Juliano, 1985b), pharmaceuticals (Talwalker *et al.*, 1965), brewing (Kiyoshi and Sohtaro, 1985), and other miscellaneous products (Juliano, 1985c). Rice hull and straw are sources of energy (Beagle, 1978) and animal feed (Ranjhan and Beagle, 1978). Industrial utilisation of straw and hull has been modest because of the uncertainty about the raw material (Juliano, 1985d). Rice straw has also been used as a source of nutrient for wetland rice (Ponnamperuma, 1984).

1.2 Origin and Distribution of Rice Yellow Mottle Virus in Rice

The RYMV was first noticed in 1966 at Otonglo near Kisumu along the shore of the Kavirondo Gulf of lake Victoria where the disease had probably been present for a number of years (Bakker, 1974). It was noticed in West Africa in 1975 and called 'Pale yellow mottle virus' (Raymundo and Buddenhagen, 1976). The name 'rice yellow mottle' and the causal agent 'rice yellow mottle virus (RYMV)' were coined by Bakker (1970), the Pioneer worker on the disease.

Bakker (1970, 1974) assumed that RYMV was present around lake Victoria on grass hosts before rice cultivation was introduced and that it then spread to rice. The virus is environmentally stable and is transmitted mechanically from rice to rice (Bakker, 1970; Fauquet and Thouvenel, 1977) and by Chrysomelid beetles in Kenya, East Africa (Bakker, 1971, 1974). Bakker (1974) indicated that the rice introductions from Asia such as Sindano, IR 22, Basmati 217, IR 154-61-1-1, IR 5, IR 8, IR 20, IR 24, among others were highly susceptible to the virus in East Africa. Also all of these varieties and their close relatives introduced to West Africa

(Raymundo and Buddenhagen, 1976; Fauquet and Thouvennel, 1977; Rossel *et al.*, 1982a,b; IITA, 1984, 1986; Awoderu *et al.*, 1987; Thottapilly and Rossel, 1993) and Madagascar (Reckhaus and Andriamasintseheno, 1995) are similarly susceptible to the virus.

With these evidences, the apparent absence of RYMV from other continents and its detection in a wild rhizomatous species (*O. longistaminata*) in Mali and Niger (John *et al.*, 1984; Sy, 1994), Senegal (Mbodj *et al.*, 1984) and Burkina Faso (Konate, 1995) are all consistent with the view that the virus is an indigenous one to which local rices have become adapted (Fomba, 1988). As with hoja blanca virus in Central America, the apparent equilibrium established between host and pathogen seems to have been disrupted by the introduction of exotic varieties and an intensification of cropping practices (Thresh, 1989), and without extensive adaptive testing in these new environments (Rossel, 1986).

Therefore, increasing incidence and importance of RYMV in the West and East Africa (Figure 2) are attributed to the cultivation of new highly susceptible exotic rice varieties mostly from Asia and the availability of water through irrigation which allows for sequential planting and maintenance of higher crop intensity without dry season gaps (IITA, 1981, 1982; Rossel *et al.*, 1982b). The area originally affected in Kenya was part of a new irrigation project which had led to an increase in rice cultivation due to the availability of water for sequential planting throughout the year (Bakker, 1974; Thresh, 1991). Traditionally in Africa rice is grown under rainfed conditions in upland environments, although cultivation in lowlands and swamps is practised in certain areas (Rossel, 1986). It was under these conditions that RYMV was first reported on rice in East Africa (Bakker, 1970), and West Africa (Raymundo and Buddenhagen, 1976; Rossel *et al.*, 1982b). Furthermore, according to Raymundo *et al.* (1979), prior to 1975 no virus disease of rice was known in West Africa.

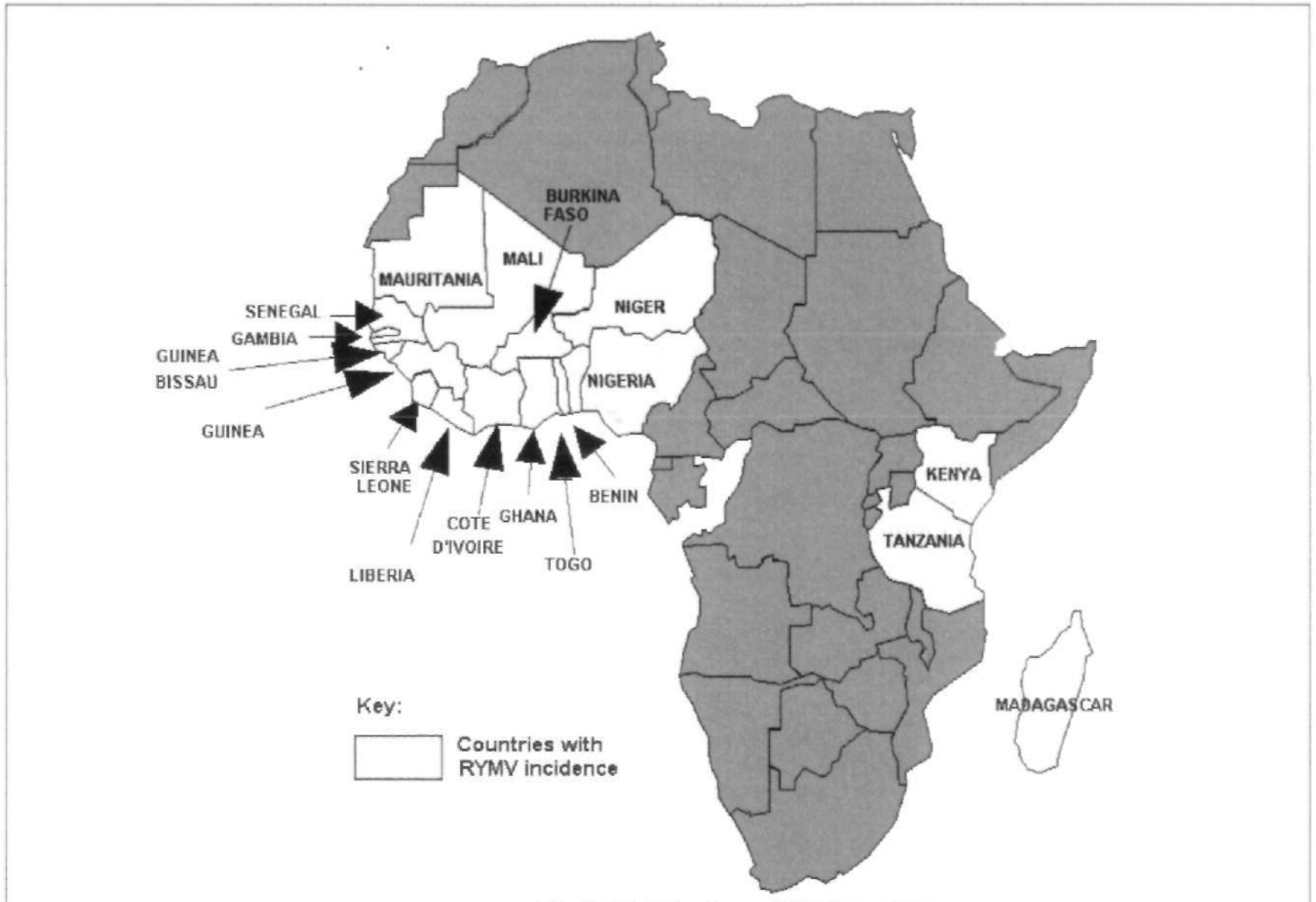


Fig 2. Distribution of RYMV in Africa.

Sources: Bakker (1970), Raymundo & Buddenhagen (1976), Fauquet & Thouvenel (1977)
IITA (1978), Rossel et al., (1982a), Awoderu (1991a,b), and Reckhaus & Randrianangaly (1990).

Studies conducted at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria on the reactions of hundreds of accessions of *O. glaberrima* Steud and *O. sativa* L. to rice yellow mottle virus (RYMV) have shown clearly that *O. glaberrima* Steud has a higher level of resistance than *O. sativa* L. (Alam, 1988; Ng, 1988). Out of the 75 accessions of *O. glaberrima* Steud inoculated with the African indigenous RYMV at IITA, 45 were resistant, and some immune to the virus (Ng *et al.*, 1988).

Thus, in view of the increasing incidence (John *et al.*, 1984; Awoderu, 1991a, b; Sy, 1994) and importance of RYMV on rice production (Fomba, 1986, 1988) in West Africa in particular (Abo *et al.*, 1998) it is vital and necessary to identify the vectors of the virus and the mode of transmission in order to develop management strategies in the affected areas to prevent further spread of the disease. The objectives of this study are as follows:

1. To investigate the role of rice seeds, empty rice spikelets, stubble, roots fusion, leaves and other sources from RYMV infected plants in the transmission of the virus.
2. To determine the insect vectors of RYMV and their mode of transmission.
3. Screen and identify weeds and cultivated plant species that are reservoirs of RYMV.
4. Screen soil nematodes for the their ability to transmit RYMV.

The management of virus diseases will be enhanced by knowing the vectors and their mode of transmission. Thus, meeting the objectives of this study will contribute greatly to further direct the development and adoption of an integrated approach for the sustainable management of RYMV and consequently enhance the production of rice in the West African sub-region which is now being threatened by this devastating disease.

CHAPTER TWO

2.0 LITERATURE REVIEW.

2.1 Pests and Diseases of Rice

The production of rice is limited by insect pests, nematodes, bacteria, fungi, virus, weeds and environmental stresses (Alam *et al.*, 1984; John *et al.*,1986; IRTP, 1989; Heinrichs, 1994; Heinrichs *et al.*, 1995a). Rice production is also affected by birds, rodents and other vertebrate pests (Alam *et al.*, 1984; Thresh, 1989). However, insects, diseases and weeds are the major constraints that limit rice production in Asia and Africa (Alam *et al.*, 1984; Arrandeau and Harahap, 1986; Loevinsohn, 1994). Barr *et al.* (1975) estimated that the yield loss attributable to insects, diseases, and weeds in Africa was 33.7% of potential production of which insects and diseases account for 14.4% and 5.8% respectively.

Insect pests attack rice from the seedlings stage to maturity and feed on all parts of the plant-the roots, stems, leaves, and grains (Alam *et al.*, 1984; Heinrichs and Miller, 1991). Within Africa the severity of rice insect pests differs considerably in different climatic zones and ecosystems (Agyen-Sampong, 1982a,b; Alam and Akibo Betts, 1984; Alam *et al.*, 1984).

About 70 major and minor diseases of rice have been reported in Asia and Africa (Ou, 1985). However, in Africa the more common rice diseases are blast, brown spot, leaf scald, sheath blight, sheath rot, glume discolouration, bacterial leaf blight and rice yellow mottle virus (Zan *et al.*, 1981; John *et al.*, 1984; Ou, 1985). While the rest of the rice diseases are reported in most rice growing countries, RYMV disease is found only in Africa (Alam *et al.*, 1984; Rossel and Thottappilly, 1985; Rossel, 1986).

There are many virus or virus-like diseases of rice (Ou, 1985; Abo & Sy, 1998) but five are of particular importance in tropical and sub-tropical areas (Thresh, 1989). These include

rice hoja blanca virus (Galvez, 1959), rice tungro virus (Rivera and Ou, 1965), rice grassy stunt virus (Ling, 1975), rice ragged stunt virus (Ling *et al.*, 1978), and rice yellow mottle virus (Bakker, 1974, 1975). Although in Africa five viruses, rice necrosis stripe, maize streak, African cereal streak, rice crinkle, and rice yellow mottle are known to affect rice (Harder & Bakker, 1973; Raymundo *et al.*, 1979; Rossel & Thottappilly, 1985; Thottappilly, 1992; Abo & Sy, 1998), the latter is the only economically important virus disease at the moment (Rossel and Thottappilly, 1985).

2.2 Rice Yellow Mottle Virus: Variability, Symptomatology, and Infectivity.

2.2.1. Characteristics of rice yellow mottle virus.

RYMV is a single stranded positive sense RNA and a distinctive spherical virus measuring about 28±3nm in diameter (Bakker, 1974; Rossel *et al.*, 1982a; Rossel and Thottappilly, 1985). The percentage of nucleic acid was estimated to be about 18% RNA while the molecular weight of coat protein was estimated at 28±1kd (Fauquet and Thouvenel, 1987). The percentage nucleic acid in the infective virus particle was estimated to be 23.6±0.05% (Bakker, 1974). According to Brugidou *et al.* (1995), the genomic RNA consists of 4,550 nucleotides that is not polyadenylated. It also consists of 4 open reading frames (ORFs). The ORF1 (nt. 80 to 553) encodes a protein called P1 of 17.8 KDa and presumed to be the movement protein of the virus whereas the ORF2 (nt. 608 to 3607) encodes for a polyprotein of 110KDa and contains a putative linked protein (VPg), helicase, protealse, and polymerase. ORF3 (nt. 2092 to 2467) encodes a small protein of 13.7KDa of an unknown function. The ORF4 (nt. 3447 to 4116) codes for the coat protein (CP) of 26KDa (Ayassi *et al.*, 1994; Brugidou *et al.*, 1995).

The virus is a member of the sobemovirus group (Seghal, 1981; Hull, 1988). Sobemoviruses are characterized by being mechanically transmissible except blueberry shoestring virus (BBSSV), having relatively narrow host ranges, and having isometric particles that sediment between 110 and 120S and form one band in $CSC1_2$ (some form several bands in CS_2SO_4), and having a single viral coat protein species of molecular weight of about 30,000 (30K) (Hull, 1988). The genome is a single species of single stranded RNA (ssRNA) of molecular weight of about $1.4x10^6$ daltons (about 4500 nucleotides) and has a plus or messenger sense polarity (Hull, 1988). Thus, the cryptograms of RYMV is R1:1.4/23:S/S:S/C1 (Gibbs, 1968) and the base composition of the RNA is G29; A21; C25; U24 (Bakker, 1974, 1975). Furthermore, most lack immunological reactions with other sobemoviruses (Thottappilly *et al.*, 1992). The natural vectors of most sobemoviruses are insects (Hull, 1988). The sobemoviruses such as southern bean mosaic virus (SBMV), cocksfoot mottle virus (CFMV), cocksfoot mild mottle virus (CFMMV), panicum mosaic virus (PMV), turnip rossette virus (TROSV), maize chlorotic mosaic virus (MCMC) and RYMV are transmitted efficiently by Chrysomelid beetles (Walters, 1969; A'Brook and Benigno, 1972; Bakker, 1971, 1974, 1975; Nault *et al.*, 1978; Gordon *et al.*, 1984;). All the known sobemoviruses have isometric particles of about 25-30nm in diameter (Hull, 1988). Most of the sobemoviruses occur at relatively high concentrations in infected plants and the particles are found in the cytoplasm of most cells (Bakker, 1974; Mohammed and Mossop, 1981; Hull, 1988) and in the nuclei (Mohammed and Mossop, 1981). RYMV and barley yellow dwarf virus are the only two known viruses that have host ranges restricted to the monocotyledons and that support replication and encapsidation or low molecular weight RNAs (Seghal *et al.*, 1993).

2.2.2 Mechanical Transmission and Sympmatology of Rice Yellow Mottle Virus.

RYMV is characterized by stunting, mottle and yellowing symptoms of varying intensities depending on genotype. These symptoms could be mistaken for iron or nitrogen deficiency (Thottappilly and Rossel, 1993). Some infected varieties flower early (Sy, 1994) while others show delayede flowering (Bakker, 1974; Fomba, 1988). Infected rice plants are found in most cases near the bunds but soon thereafter whole fields may be affected (Bakker, 1975).

Bakker (1974) reported yellowish leaves on 'Sindano', mild yellowish green on 'Basmati 217' or orange on 'IR 8', and in each case the youngest leaves showed mottling. Raymundo and Buddenhaggen (1976) stated that infected plants had pale yellow mottle leaves, stunted, reduced tillering, non-synchronous flowering, poor panicle exertion, spikelet discolouration and sterility, erect panicles, compact appearance of spikelets, brown to dark-brown discolouration of grains, a consequence of secondary infection by fungi, and crinkling of new growth. Others developed conspicuous bronze or orange pigmentation followed by rolling of leaf margins and subsequent leaf desiccation. They also stated that some varieties continue to produce relatively green leaves with faint, hardly discernible streaking and mottling but with conspicuous stunting, others with narrow and very chlorotic leaves and severe stunting, and yet others showed pronounced chlorosis but minimal stunting.

Fauquet and Thouvenel (1977) reported that the disease appeared as a yellow and green mottle at the leaf tip. In an infested field, yellow-orange discolouration of leaves was seen on plant scattered throughout the field but such plants were more prevalent at field margins. They also stated that symptoms occurred at any stage from transplanting to booting.

Symptom expression may be strongly influenced by light intensity, day length, humidity,

temperature, growth stage or plant, among other factors (Bakker, 1974). According to Bakker (1974) mechanical inoculation of 'Sindano' at the 3-4 leaf stage showed at high temperatures (30°C or more) the first systemic symptoms after 4-5 days, at 25°C the symptoms were noticed after 6 days, and at 20°C after 7-8 days. Below 20°C, the yellow dots enlarged more slowly parrallel to the veins than at 25°C, while at higher temperatures the period in which yellow dots were seen was very short. Inoculation of 'Sindano' at increasing age delayed symptom appearance and the symptoms were more pronounced and necrotic in the glasshouse than in the field (Bakker, 1974).

Sap derived from young infected rice leaves (2-3 weeks old) is still infective at a dilution of 10^{-11}. However, the dilution end-point varies with the origin of the inoculum (Fauquet and Thouvenel, 1977, 1987). The virus sap remains infective for 71 days (Bakker, 1970, 1974) and at 4°C for 84 days (Fauquet and Thouvenel, 1987). However, the inoculum progressively looses its capacity for infection from 55°C to 70°C, although in crude sap the virus remains viable for at least 34 days at 27°C (Fauquet and Thouvenel, 1987). It can replicate at a surprisingly rapid rate (Bakker, 1974; Fauquet *et al.*, 1995). It can also be transmitted through guttation fluid and in the irrigation water of heavily infected fields (Bakker, 1974; Ou, 1985).

2.3. Ecological Distribution of Rice Yellow Mottle Virus and Enviromental Factors Affecting Infection.

RYMV was first detected in Kenya in irrigated fields (Bakker, 1970). It was later reported in Sierra Leone under hydromorphic and swamp conditions (Raymundo and Buddenhagen, 1976; Raymundo and Konteh, 1980; Fomba, 1988), in Nigeria in irrigated and

farmers' fields (IITA, 1978; Rossel *et al.*, 1982a,b), in irrigated rice in Cote d'Ivoire (Fauquet and Thouvenel, 1977; Awoderu *et al.*, 1987; Yoboue, 1989), in lowland and irrigated rice fields in Niger, Burkina Faso, Senegal and in the deep water polders of Mali (John *et al.*, 1984), in lowland irrigated rice areas in Madagascar (Reckhaus and Randrianangaly, 1990). It was reported in Tanzania including the islands of Zanzibar and Pemba (Rossel *et al.*, 1982b; Ali and Abubakar, 1995). Infact the disease has now become a major limiting factor to rice production for lowland and irrigated ecosystems in Africa since it is now found in virtually all rice-growing countries in West Africa (Abo *et al.*, 1998). Disease outbreaks have been reported mainly from irrigated, lowland production systems. However, it does occur sporadically in highly susceptible exotic varieties under rainfed, upland production systems (Raymundo *et al.*, 1979; Raymundo and Konteh, 1980; Awoderu *et al.*, 1987; Taylor, 1989; Sy and Akator, 1993).

2.4 Evaluation of Rice Germplasm for Disease Reaction, Disease Incidence and Yield Loss Assessment

2.4.1 Varietal Evaluations.

The Screening of *Oryza* germplasm in Nigeria has shown the existence of potential sources of resistance to RYMV disease (IITA, 1976, 1984; Ng *et al.*, 1980; Attere and Fatokun, 1983; Paul, 1991; Singh, 1995). Indigenous African *Oryza* species such as *O. glaberrima*, *O.longistaminata* and *O. barthii* as well as other traditional African upland rice varieties such as OS 6 are either highly tolerant, resistant or immune (Raymundo and Konteh, 1980; IITA, 1980, 1984; Rossel *et al.*, 1982b). So far immunity to RYMV has only been reported in *Oryza barthii* and *O. longistaminata* accessions (IITA, 1984; Rossel and Thottappilly, 1985). Studies conducted at IITA on the reaction of hundreds of *O. glaberrima* Steud to some indigenous

African insects and RYMV have shown that *O. glaberrima* Steud has a high level of tolerance than *O. sativa* L (Alam, 1988; Ng, 1988). Screening for RYMV resistance which started in the late 70's at Rokupr, Sierra Leone and IITA, Ibadan, Nigeria showed the following upland tropical Japonica cultivars to be resistant: OS 6, LAC 23, Moroberekan, Iguape Cateto, 63-83, IAC 25, IRAT 78, IRAT 13, NSO/2, Gbongosy, Taninahum, Marlie and many other traditional farmers' landraces (Raymundo *et al.*, 1979; Raymundo & Konteh, 1980). Most of these resistant lines also provide horizontal resistance to rice blast (Fagade *et al.*, 1988; Fomba, 1988). On the other hand Rossel *et al.* (1982a, b) reported infection of ADT 32, ITA 212, ARC 1175, FARO 15, Americana, and other traditional rice varieties on farmers' lowland fields in Nigeria. RYMV was detected on rice cultivars IRAT 13 and Gambiaka in the hydromorphic areas of Bongouanou province in Cote d'Ivoire in 1984 and during a survey in 1985, the disease was noticed on UPL Ri5, IR 52 and IR 10110-23-1 at Tombokro in Cote d'Ivoire (Awoderu *et al.*, 1987). Mbodj *et al.* (1984) reported a severe infection of RYMV on the rice cultivar ROK 5 in 1983 in Djibelor Casamance region of Senegal. The disease was also recorded on Yeguitti, Kuatik Putih Tinngi, Tat No, Padi Mentul, Nang Ra, and CP4 in a tidal mangrove swamp at Koba, Guinea (WARDA, 1982, 1983). Fomba (1984) observed RYMV infection on local varieties Pa Matches and Pa Black in Sierra Leone. Varietal reactions in field and greenhouse tests confirmed high level of susceptibility to RYMV in BG 90-2, Bouake 189, Jaya, IR 1529-680-3, IR 5, IR 8, IR 20, ITA 212, Gambiaka, ITA 306, ITA 123, FARO 15, IR 46, ROK 5, among others (Sy and Akator, 1993). Thus, in most of the RYMV affected areas one or more than one susceptible varieties are grown extensively, for example, Sindano, Basmati 217, IR 5, IR 8, IR 20, IR 22 in East Africa, BG 90-2 in mali, Bouake 189 in Cote d'Ivoire, ITA 212 and

FARO 29 (BG 90-2) in Northern Nigeria, ITA 123 in Burkina Faso, Jaya in Senegal, IR8, IR 20 in Madagascar and IR 1529-680-3 in Niger republics (Bakker, 1974; Singh, 1995).

2.4.2. Incidence and Yield Loss Assessment.

Under natural infection by RYMV disease Bakker (1974), Rossel *et al.*, (1982a), and Taylor *et al.*, (1990) obtained a yield reduction of 5- 82% in farmers' fields. During a special survey of the WARDA-constituted Rice Integrated Pest Management Task Force (IPM-TF) to Mali in November 1994 losses on BG 90-2 in the Office du Niger region, Mali were estimated to be as high as 68% under farmers' conditions (Sy, 1994). The screening of entries of the African Rice Yellow Mottle Virus Evaluation and Screening Set (ARYMVESS) and WARDA breeding lines indicated a disease incidence ranging from 1.2% to 16.9% at Mbe and 2.7% to 16.6% at Gagnoa, Cote d'Ivoire (Sy *et al.*, 1991). Fomba (1986) reported yield reduction of 19.6% in Angkatta and 95-98% in ROK 5 at Rokupr, Sierra Leone. Reckhaus and Adamou (1986) and Anonymous (1988) recorded yield reductions from 58-68% in Niger Republic. Observations on a trial conducted by the Institute des Savannes (IDESSA) at Gagnoa, Cote d'Ivoire showed that the incidence of RYMV infections was 75% in IM 16 variety and 80% in Jaya variety (Sy *et al.*, 1991). A study of yield reductions from inoculated and naturally infected rice plants showed yield reduction rate of 84% for Bouake 189, 67% for BG 90-2, and 4% for Moroberekan (Sy *et al.*, 1993).

A survey of the incidence of RYMV carried out in four rice growing environments and vegetation zones between 1983 and 1986 in West Africa showed that the incidence was 75% in the Sahel, 40% in the Sudan Savanna, 18% in the Guinea Savanna, and 7.5% in the tropical

rainforest (Awoderu, 1991a).

2.5. Transmission of Plant Viruses:

2.5.1. Transmission of Plant Viruses through Seeds.

It has been reported that the earlier the plant is infected, the higher the percentage of seed that will transmit the virus (Owusu *et al.*, 1968). Most seed-transmitted viruses are transmitted through pollen of infected plants (Frosheiser, 1974). Whether or not a particular virus is seed transmitted in a particular host, the efficiency of the transmission may depend on the rate of loss of virus from embryos that were initially almost all infected. According to Gunnery and Datta (1987) inhibitors of virus infection have been found in seed extracts. They isolated a low molecular weight (MW) RNA from barley embryos that specifically inhibited the initiation of protein synthesis and suggested that such molecules might play a role in inhibiting virus synthesis and seed transmission in some species.

Athow and Laviolette (1962) found that position of seed in the pod and location of pod on the plant did not affect the proportion of soybean seeds that were infected with tobacco ringspot virus (TRSV).

Seeds harvested from 30 pumpkin (*Cucumis melo*) plants infected with zucchini yellow mosaic virus (ZYMV) failed to show that ZYMV is seed transmitted in pumpkins (Davies and Mizuki, 1980; Providenti and Robinson, 1987; Gleason and Provvidenti, 1990). Lettuce plants infected with lettuce mosaic potyvirus may have a seed transmissin rate of 3-15% (Couch, 1955), while 1% transmission rate was reported for andean potato latent virus in Potato (Jones and Fribourg, 1977). In a few cases such as TRSV in soybean (Athow and Bancroft, 1959)

andsStrawberry latent ringspot nepovirus in Celery (Walkey and Whittingham-Jones, 1970), 100% virus transmission in seed from infected plants was reported. In others, seed transmission may vary, for example 28-94% in squash mosaic virus in Muskmelon (Rader *et al.*, 1947), and 50-100% in barley stripe mosaic virus in barley (Eslick and Afanasiev, 1955; Caroll, 1972). Also seed transmission of alfalfa mosaic virus (AMV) (Frosheiser, 1974) and peanut mottle virus in peanut vary considerably for different strains (Adams and Huhn, 1977). Hanada and Harrison (1977) reported that seed transmission in *Setaria media* was markedly dependent on some virus function carried by RNA1. Other viruses such as dodder latent mosaic may be seed transmitted in one host but not in others (Lister, 1960).

Different varieties of the same host species often vary in the rate at which seed transmission of a particular virus occurs (Couch, 1955; Caroll and Chapman, 1970). There is also variation between plants of a given variety (Matthews, 1991) and geographical location (Shoyinka *et al.*, 1978; Rossel & Thottappilly, 1985). Three sobemoviruses are seed borne (Hull, 1988). The southern bean mosaic virus (SBMV) is transmitted in cowpea (Shoyinka *et al.*, 1978; Givord, 1981). There is some controversy as to whether the virus is in the embryo (Hull, 1988) but there is evidence for infection of germinated seedlings from contaminated seed coats (Lamptey & Hamilton, 1974; Uyemoto and Grogan, 1977). Sowbane mosaic virus (SOMV) is transmitted up to 83% in *Chenopodium spp* (Dias and Watermoth, 1967). It has been reported that the virus is carried internally (Bennett and Costa, 1961) but in which tissue was not ascertained (Hull, 1988). The St. Augustine strain (SAS) of panicum mosaic virus (PMV) is seed transmitted in *Setaria italica* (Niblett and Toler, 1977).

If a virus is transmitted through seed, such transmission may be an important source of

infection since it introduces the virus into the crop at very early stage, allowing infection to be spread to other plants while they are still young (Tomlinson, 1962; Kimble *et al.*, 1975). Thus, the blackeye cowpea mosaic virus (BlCMV) is considered to be the most important seedborne virus in the South-eastern U.S.A (Harrison & Gudauskas, 1968).

2.5.2. Transmission of Plant Viruses by Insects.

Japanese workers were the first to experimentally prove the role of insects in the spread of a plant virus about the beginning of the twentieth century (Ishikara, 1928). They demonstrated the transmission of rice dwarf disease by the leafhoppers [*Inazuma* (*Deltocephalus*) *dorsalis* Motschulsky (Ishihara, 1964) and *Nephotettix cincticeps* Uhler] from 1895 to 1908 (Takata, 1896; Maramorosch, 1969). A wealth of information on many different types and species of insects has meanwhile accumulated, and arthropods are now known to be highly important vectors or natural vehicles of spread of plant viruses (Matthews, 1991). Most insects transmitting viruses belong to the order Homoptera which include aphids (Alegbejo, 1986; Piron, 1986), leaf and planthoppers (Harrison, 1985; Nault & Madden, 1988), white flies (Etessami *et al.*, 1988; Alegbejo, 1996), mealybugs (Bigger, 1981), bugs (Gibbs & Randles, 1988) of which 66% of the arthropod borne viruses are transmitted by aphids (Matthews, 1991). Additional insects that transmit viruses include thrips (Kaiser *et al.*, 1982) and beetles (Bakker, 1974,1975; Fulton *et al.*, 1987).

The biological relationship between plant viruses and their insect vectors are not identical (Watson & Roberts, 1940). Based on the method of transmission and virus vector interaction, virus diseases are grouped as persistent, semi-persistent, or non-persistent (Sylvester, 1956; Day

& Venable, 1961). Kennedy *et al.* (1962) introduced new terms which are less empirical and which give some information of the location of the virus in the insect and the route followed by the virus. They suggested "stylet borne" instead of "non-persistent", and "Circulative propagation" in lieu of "persistent". One criterion of whether a virus is stylet borne or circulative is transtadial passage. Viruses that have transovarial passage must multiply in their vectors (Hsieh, 1969). Some virus groups that are transmitted in a non-persistent manner include: potyvirus, carlavirus, caulimovirus, cucumovirus, alfalfa mosaic virus and fabavirus (Hull 1988; Matthews, 1991). Classic cases of semi-persistent viruses are beet yellow closterovirus and citrus tristeza virus (Raccah *et al.*, 1976). However, cauliflower mosaic caulimovirus (CaMV) is transmitted bimodally (Non-persistently & Semi-persistently) (Lim & Hogedorn, 1977). Persistent or circulative transmission is the movement of ingested virus to the salivary glands (Nault & Ammar, 1989). The Circulative viruses may or may not replicate in their vectors (Matthews, 1991).

Virus transmission by insects with biting mouthparts immediately after acquisition feeding is commonly explained by an effective dose of virus on contaminated mouthparts or in faeces entering partly damaged cells (Walters, 1969; Tinsley, 1973). Viruses retained by beetle vectors for prolonged periods of time have long been thought to be transmitted through regurgitation of fluid during feeding (Walters, 1969). This explanation was based on the fact that beetles which have no salivary glands have been claimed to regurgitate fluids during feeding. According to Selman (1973) the presence or absence of salivary glands is not responsible for the ability or otherwise of biting insects to transmit viruses, although he did not exclude that differences in the secretions of the glands is the determining factor. In addition viruses have been found in the

hemolymph of Chrysomelid beetles and could be retained for a long time, so acting as virus reservoir from which the virus can be transmitted at intervals (Selman, 1973). The finding that some plant viruses move into the hemolymph of Chrysomelid beetles feeding on infected plants, and that injection of virus into the haemocoel causes beetles to become viruliferous has led to the generalization that beetle transmissible viruses have a circulative type of vector transmission. Therefore, the differences between the infectivity of different species of Chrysomelids feeding in the same host indicate that the determining factor in the mode of transmission remains to be discovered. Ability, efficiency and manner of virus transmission by arthropods are determined by their mouthparts structure and ways of feeding (McLean & Kinsey, 1984). Insects like beetles (Coleoptera) and caterpillars (Lepidoptera) have biting and chewing mouthparts which eat away pieces of leaf and wound the plant in a such course fashion that cells that have been touched are ruined. It has been estimated that regurgitant fluid is a key factor in determining whether a virus will or will not have beetle vectors (Gergerich *et al.*, 1983; Monis *et al.*, 1986). The viruses transmitted by beetles belong to the tymovirus, comovirus, bromovirus, and sobemovirus groups (Fulton *et al.*, 1987; Matthews, 1991).

Adult beetles belonging to the family Chrysomelidae such as *Sesselia pussila (Galerucinae), Chaetocnema pulla* Chapius (Halticinae), *Trichispa sericea* Guerin. (Hispinae), *Dicladispa (Chrysispa) viridicyanea* Kraatz (Hispinae), *D. bayoni* Gest. (Hispinae) and a host of others have been found to transmit RYMV to rice (Figures 3 and 4). The first vector found to transmit RYMV was *Sesselia pussila* Gerstaker (Chrysomelidae, Galerucinae) (Bakker, 1971). *Trichispa Sericea* Guerin and *Dicladispa gestroi* Chapman are considered the most serious insect pest problem of rice in Madagascar where RYMV incidence is alarming (Reckhaus &

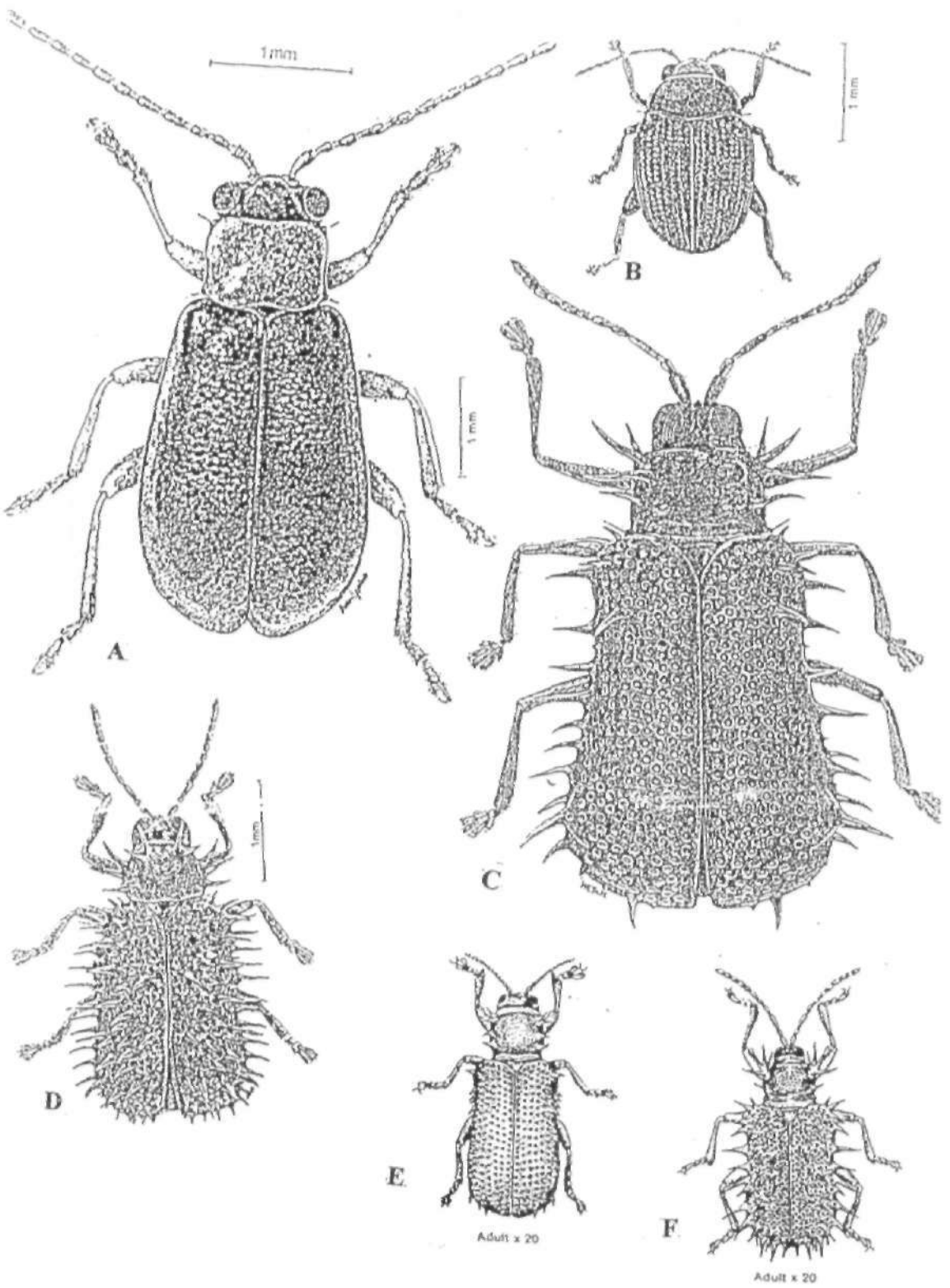


Fig.3 . Some of the several Chrysomelid beetles reported as vectors of RYMV in East Africa. A= *Sesselia pussilla* (Galerucinae), B= *Chaetocnema pulla* Chapius (Halticinae), C= *Dicladispa (Chrysispa) viridicyanea* Kraatz (Hispinae), D = *Dicladispa bayoni* Gest (Hispinae), E = *Trichispa sericea* Guerin (Hispinae), F = *Hispa* (*Dicladispa*) *gestroi* Chapius (Hispinae) in Madagascar (Reckhaus & Andriamasintseheno, 1995).
Figures are adapted from Bakker (1974) and Breniere (1983).

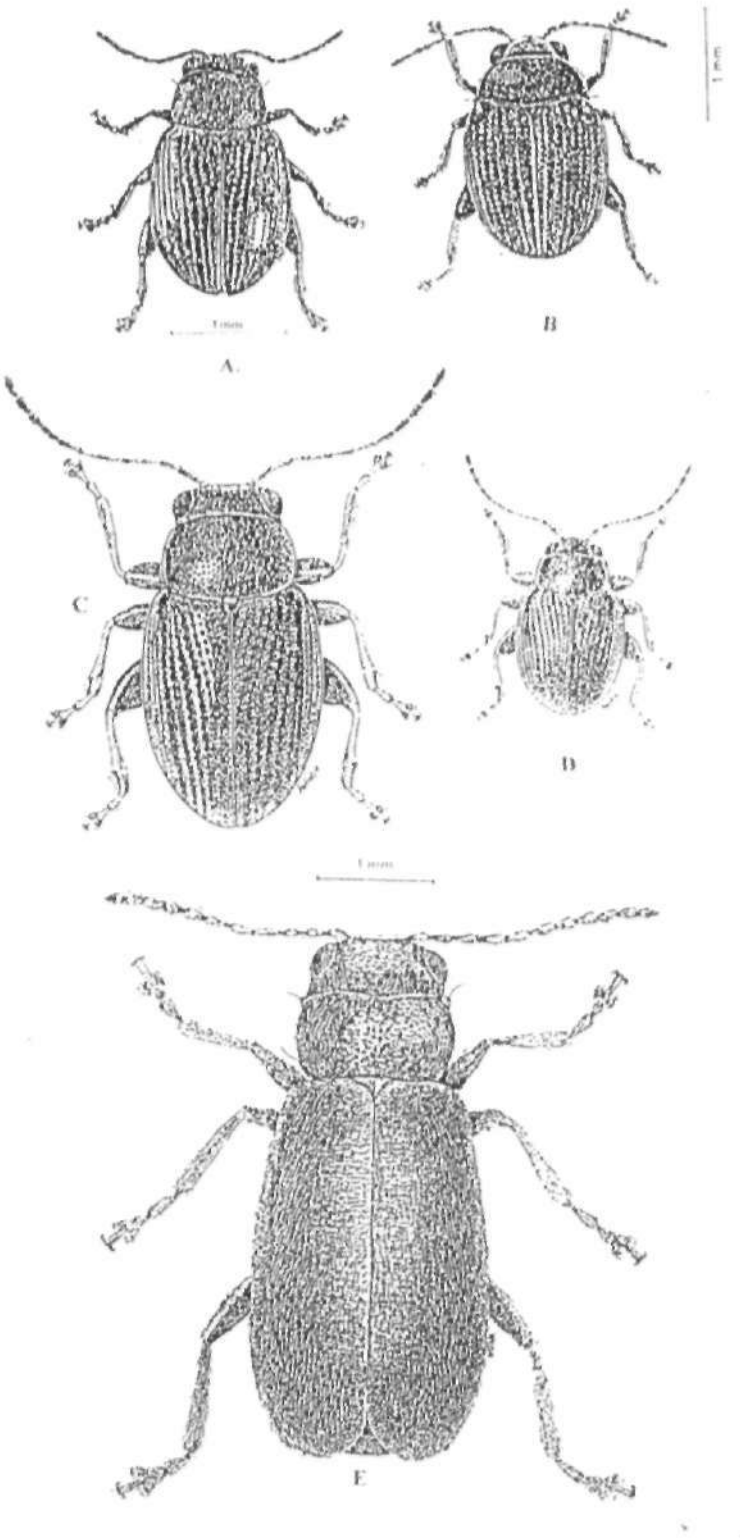


Fig. 4 Several species of *Chaetocnema* (Halticinae) and a betle of the genus near *Apophyila* (Galerucinae) reported to be vectors of RYMV in East Africa. A = *Chaetocnema abyssinica* Jac., B = *Chaetocnema pulla* Chapius, C = *Chaetocnema kenyensis* Bryant, D = *Chaetocnema pussilla* Lab., and E = a beetle of the genus near *Apophyila.*
Source: Bakker *(1974).*

Randrianangaly, 1990). There are complaints from farmers of serious *Trichispa* outbreaks with devastating effect on young rice crops in Niger, Mali, and Cote d'Ivoire. It has also been reported in Cameroun, Senegal, and Togo (Breniere, 1983). *C. pulla* Chapius (*C. zeae* Bryant) has been reported attacking maize and millets in Sierra Leone (Hargreaves, 1937). Doval *et al.*, (1975) reported the damage caused to wheat by *C. basalis* Baly in India. *C. basalis* Baly, *C. obesula* Lec., and *C. gregaria* Weise have been reported infecting rice crops in Cuba, Indonesia, and Madagascar (Grist and Lever, 1969).

Fomba (1990) reported the incidence of *Chaetocnema* spp in all rice fields infected with RYMV. *Chaetocnema* spp had earlier been suspected as a possible vector of RYMV in Sierra Leone (Raymundo, 1980).

2.5.3. The Reservoir Hosts of Plant Viruses.

Although the major rice pests as perceived by farmers are weeds, vertebrates (birds and rodents), and insects (Adesina *et al.*, 1994), weeds are the most widely reported biological constraint to rice production (Akobundu and Fagade, 1978; Arrandeau & Harahap, 1986; Rouw, 1993). Insects have been reported to survive fallow periods by feeding on other hosts including weeds (Loevinsohn, 1994). During the fallow period of several years between rice crops in a given field in traditional farming systems in West Africa the rice insects will either hibernate or feed on weeds during the first dry season after the rice harvest, then continue on weeds during the next rainy season or move to newly planted rice fields in the general vicinity (Heinrichs *et al.*, 1995a). Litsinger (1984) and Afun *et al.* (1995) reported that weedy rice fields often have high populations of insect pests. Williams (1981) suggested that the presence of weeds increased activity of predators in some crops by producing shelters, modifying crop microclimates and

altering crop background to enhance predator colonization. However, this relationship is also a source in which plant viruses are transmitted and spread in the field.

The role of weeds in plant virus transmission has been well elucidated (Duffus, 1971; Alegbejo, 1987; Matthews, 1991). Thus, Van Hoof (1959) reported hoja blanca virus disease transmission from *Echinochloa colonum* (L.) Link to *Echinochloa colonum* Link but not from *E. Colonum* Link to rice with the leaf-hopper *Sogata cubana* Crawf. Gavez *et al.*(1961a) transmitted the virus with *S. cubana* from rice to *E. colonum* Link and from *Echinochloa spp* to *Echinochloa spp* but not from rice to rice or from *Echinochloa spp* to rice. Acuna and Ramos (1958), Galvez *et al.* (1961a), and Elias *et al.* (1962) effected the passage of the virus from rice to *Echinochloa colonum* (L.) Link using *S. orizicola*. Galvez *et al.* (1961a, b), and Lamey *et al.* (1964) have transmitted the virus with *S. orizicola* from rice to oats (*Avena sativa* L.), wheat (*Triticum aestivum* L.), *Leptochloa* spp., and *Digitaria* spp.

Many weeds important in rice culture are not native to the production area but have been introduced as impurities in rice seeds (Delouche, 1988; Fujisaka *et al.*, 1993). Cung (1981) reported that the most common contaminant in rice seed in Vietnam was *E. crus-galli*. In a similar manner Huelma *et al.*, 1996 found that *Echinochloa spp* were the most frequent in rice shipments to and from the International Rice Research Institute (IRRI), the Philippines. Rao and Moody (1990) observed that *E. glabrescens, E. oryzoides, Fimbristylis miliacea, and Scripus supinus*, were common contaminants in farmers' rice seeds. Plant viruses require one or more host plant species for multiplication, survival, and spread. Weeds, wild rice, hedgerows, ornamental trees and shrubs may also act as virus reservoirs (Duffus, 1971; Matthews, 1991). However, the actual importance of alternative hosts for neighbouring crops will depend most

particularly on the presence of active invertebrate vectors. For example, the presence of okra mosaic virus in three malvaceous weeds in Nigeria was associated with the active infective beetle vectors (Atiri, 1984), while the presence of pepper veinal mottle virus in a solanaceous weed in northern Nigeria was associated with active infective aphid vectors (Alegbejo, 1987). In general the role of weeds in the development of the disease is complex because it is possible that one plant species might be a host for the virus, but at the same time it might not be preferred by insect vectors (Daniel *et al.*, 1996). It is also likely that a plant may be an alternate host to an insect for feeding and breeding purposes but yet will not be susceptible to the virus transmitted by the insect (Bakker, 1974).

2.5.4. Transmission of Plant Viruses by Soil Nematodes.

Certain nematodes predispose plants to attack by other pathogens (Powell, 1979; Wallace, 1983). Some nematode species in the order Dorylaimida are biological vectors of several plant viruses (Hussey and McGuire, 1987). Free living parasitic nematodes first detected in 1958 as virus vectors are now known to play a major role in soil transmission of grapevine fanleaf nepovirus by the dagger nematode, *Xiphinema index* (Hewitt *et al.*, 1958). This phenomenon, first described by Hewitt *et al.* (1958), incited an intensive search for nematode vectors of soil borne viruses (Lamberti *et al.*, 1975). The nematode species that are vectors of plant viruses are ectoparasites of annual and perennial plants (Hussey and McGuire, 1987; Weischer, 1993). Nematodes feeding as ectoparasites (e.g. *Xiphinema* spp., *Longidorus* spp., *Macroposthonia* spp) remain outside the root and use their stylets to feed on internode cells (Harris and Maramorosch, 1980; Hussey and McGuire, 1987). However, nematode transmitted viruses belong to two

different groups, nepo-and tobra viruses (Trudgill *et al.*, 1983; Ploeg *et al.*, 1989). *Xiphinema* and *Longidorus* spp transmit nepoviruses, and trichodorid nematodes (*Trichodorus* and *Paratrichodorus* spp) transmit tobraviruses (Lamberti *et al.*, 1975). These viruses are transmitted by viruliferous nematodes via the roots of virus susceptible host plants growing in the soil containing nematodes. Initial sources of virus acquisition by nematodes may be weeds, infected transplants, or plants grown from virus infected seeds (Hussey & McGuire, 1987).

Nematodes are very efficient vectors and single individuals of adult and juvenile stages can transmit a virus (Taylor & Robertson, 1977). Single viruliferous nematodes may transmit a virus to a high percentage of host plants in laboratory or greenhouse tests and theoretically in the field (Hussey & McGuire, 1987).

Field spread of nepoviruses and tobraviruses, especially in perennial hosts takes the typical pattern of small, slowly enlarging areas of virus infected plants where vector nematodes occur (Harrison, 1977; McGuire & Wickizer, 1981). Nematodes recovered from soil in these areas have been demonstrated to be viruliferous (Hussey & McGuire, 1987). These nematodes only move short distances during their life time (Harrison and Winslow, 1961; Cooper and Harrison, 1973). Mechanical transport of nematodes down rows during cultivation can result in infection of plants along the row (McElroy, 1977). Also infected mother plants transplanted into vector-infested soil will cause transmission and spread of the virus to adjacent plants (Thresh & Pitcher, 1978; Mountain *et al.*, 1983). The presence of nematodes and virus in the same plant in most cases results in a synergistic effect that aggravates the plant damage considerably (Powell, 1979; Wallace, 1983; Weischer, 1993).

Many genera of nematodes are associated with rice (Babalola, 1980; Diomande, 1981;

Fomba, 1984; Ou, 1985; Fademi, 1987; Bridge et al., 1990; Fademi et al., 1995). some of these nematodes have been found to be associated with poor rice growth and yield (Babalola, 1983; Plowright et al., 1990; Sawadogo and Thio, 1995; Coyne et al., 1996). It is however, noteworthy that none of these nmatodes has so far been implicated in the transmission of viruses of rice (Bakker, 1974; Raymundo *et al.*, 1979).

This study is designed to screen some nematodes associated with rice for their ability to transmit RYMV.

2.5.5. Transmission of Plant Viruses through Other Sources.

The mechanical transmission of viruses through sap is one way in which plants are deliberately infected artificially for experimental plant virology. Potato virus X (PVX) can be readily transmitted by leaf contact (Clinch *et al.*, 1938), contaminated implements or machines (Ryder and Gerhardson, 1978) and by workers or animals that have been in contact with diseased plants (Todd, 1958). In tobacco mosaic tobamovirus (TMV) on Tomato and Tobacco, virus infected sap adhering to tools, hands, clothing of workers can transmit the virus (Matthews, 1991) and by tobacco smokers (Wetter, 1975). Some viruses have been shown to be transmitted by cutting tools (Wutscher and Shull, 1975; Tsuboi *et al.*, 1995).

Vegetative propagation by budding, cutting or grafting (Dimick *et al.*, 1971) and by the use of bulbs, tubers, corms or runners are ways in which viruses present in the mother plant are transmitted to the progeny (Agrios, 1988; Mathews, 1991).

Virus transmission through infected pollen has been reported in raspberry (Murant *et al.*, 1974) and Sowbane (Francki and Miles, 1985). The pollen may be carried by wind, humans,

animals, or honey bees (Converse and Lister, 1969). Cross pollination experiments have shown that seed transmission of TRSV in soybean was by infection of the gametophytes (Yang and Hamilton, 1974). Dodder (*Cuscuta* spp) can transmit viruses from plant to plant (Desjardins *et al.*, 1969).

Soil inhabiting parasitic fungi such as *Olpidium brassicae* (Hiruki, 1987), *O. cucurbitacearum* (Dias, 1970), *Polymyxa graminis* (Ratna *et al;* 1991), and *Spongospora subterranea* (Jones and Harrison, 1969) transmit several viruses through numerous zoospores released in the soil at appropriate conditions.

Mites from the family Eriophyidae have been shown to transmit several viruses (Orlob, 1966) including ryegrass mosaic virus (Heard and Chapman, 1986), wheat mosaic virus and wheat streak mosaic virus (Slykuis, 1967). Their main method of spread from plant to plant is by wind (Slykhuis, 1955). Also mites from this family and the family Tarsonemidae have been implicated in the transmission of RYMV in Kenya (Bakker, 1974). RYMV has also been demonstrated to be transmitted by farm implements such as the sickle used in harvesting rice and through the cultural practice of trimming rice roots when transplanting (Reckhaus and Andriamasintsoheno, 1995; Tsuboi *et al.*, 1995).

Abiotic transmission of viruses to plant roots from virus contaminated soil without fungi or arthropods being involved has been reported e.g. contaminated soil coming in contact with leaves (Allen, 1981), Tomato bushy stunt virus (TBSV) (Kleinhempel and Kegler, 1982), Carnation ringspot virus (CRSV) (Brown and Trudgill, 1984) and SBMV (Teakle, 1986).

Infection of a plant by *Agrobacterium tumefaciens* mediated genes has been demonstrated (Grimsley *et al.*, 1987,). This novel experimental method for virus transmission involves insertion of DNA copies of viral genomes into the T1 plasmid of *A. tumefaciens* (Grimsley *et al.*, 1987).

CHAPTER THREE

3.0 GENERAL MATERIALS AND METHODS.

The materials and methods which were used throughout the study are highlighted here, while details of other procedures are given in the appropriate chapters. The research on the mechanism of RYMV transmission was carried out at WARDA headquarters in Cote d'Ivoire with the technical supervision and assistance of many units (Pathology, Entomology, Weed Science, and Nematology) involved.

3.1. Field Surveys and Sample Collections.

Rice seeds and spikelets were harvested from RYMV infected plants from the fields that had high incidence and severity of the disease in Mali (Niono, Selingue), Cote d'Ivoire (Mbe, Sakassou, Gagnoa), and Niger (SAY 1). The samples were transported in jute bags to the laboratory for virus indexing in seeds and biological analysis. These samples were rated by visual inspection as highly or moderately susceptible based on the incidence and severity in the fields affected. All together, 27 seed and spikelet samples were collected and analysed (21 from the field and 6 from the screenhouse). Additional samples were harvested from screenhouse reared plants from rice varieties artificially inoculated at different growth stages and similarly analysed as presented in the relevant sections. The seeds and spikelets were separated with a winnowing machine and stored at room temperature until the analysis lasted. Field surveys were carried out once every month from January to July, 1997 at the Rice Irrigation Project, Sakassou, Cote d'Ivoire to sample for insects and weed hosts of RYMV.

3.2. Soil Sterilization and Screenhouse Facility.

Sterilization of soil was carried out for 30 minutes at 120°C using an autoclave. The Rossel type of screenhouses situated in the lowland environment were used during this work. However, entomological studies were carried out in cages placed in a similar screenhouse in the upland environment.

3.3. Source of Plant Materials and Virus Isolate.

Most rice seeds were obtained from the Plant Pathology and Breeding units at WARDA. One *O. longistaminata* and one *O. barthii* accessions were obtained from Senegal. Weed seeds were provided by the Weed Science Unit of WARDA, while some were collected from the fields and raised from splits.

The virus isolate used throughout this study was obtained from infected rice plants in the field at M'be, Cote d'Ivoire. The virus was maintained through serial transfer by artificial (sap) inoculation on young seedlings of Bouaké 189. This variety also served as a susceptible check in the study.

3.4. Identification of Samples.

The insects and weed species were identified by the Entomology and Weed Science Units at WARDA, respectively. The handling, extraction, and identification of nematodes were carried out at the Nematology Unit of WARDA.

3.5. Visual Assessment Procedure.

Visual assessment of foliar symptoms for RYMV based on Standard Evaluation System (SES) for Rice (IRRI, 1988) in combination with ELISA were used for accurate assessment. The SES is based on a scale of 1-9 (Appendix 2).

3.6. Antisera, Conjugates, Substrates and Microtitre Plates.

The antiserum to RYMV (IITA Standard isolate) was generously donated by Dr George Thottappilly, Biotechnology Unit, IITA, Ibadan, Nigeria. The Anti-Rabbit IgG (Whole molecule) alkaline phosphatase conjugate and the substrate tablets (4-nitrophenyl phosphate) were purchased from Sigma Immuno Chemicals, New York, USA. The Polystyrene microtitre plates were purchased from Corning Glass Works Company, St. Louis, USA.

3.7. ELISA Buffers and Leaf Extract.

Buffers: The buffers used were Coating buffer (0.015M sodium carbonate, 0.035M sodium bicarbonate, PH9.6); 2% Polyvinyl pyrollidone (PVP)-this helps to reduce the adverse effects of polyphenolics and tannins in plant extracts. Phosphate buffered saline-Tween (PBS-T)) (0.137M sodium chloride, 0.002M potassium phosphate, 0.008M sodium phosphate, 0.003M potassium chloride and 0.05% (v/v) Tween 20); Conjugate buffer (PBS-T, 0.2% ovalbumin, 2%PVP per litre to inhibit non-specific binding of the conjugate); Blocking solution containing PBS-T and 3% of 99% fat free milk (Marvel). Note that 0.003M Sodium azide (NaN_3) was normally added to PBS-T and all buffers for preservation.

Leaf Extracts: Leaves were homogenized with a roller press and in some cases with mortar and

pestle. For each test sample, 0.2g was weighed and ground in 2ml (1:10) coating buffer with 2% PVP. The extracts were always stored in eppendorf tubes in the deep freezer(0°c) when not analysed immediately.

3.7.1. ACP-ELISA Protocol.

1. To every duplicate well of microtitre plate 100 μl of a test sample were pipetted in and incubated overnight in the refrigerator at 4°C.
2. The plant sap was washed thrice at intervals of 3 minutes each with PBS-T and tapped dry with tissues or soft cloth. All the uncoated sites were blocked with 150 μl of 3% non-fat milk (Marvel) in PBS-T and incubated at 37°C for 30 minutes.
3. The milk was decanted without washing and 100 μl of trapping antibody diluted 1:1000 with coating buffer was pippeted into each well of microtitre plate and incubated at 37°c for 2hrs.
4. The solution containing trapped antibody was thereafter washed thrice with PBST at intervals of 3 minutes each and tapped dry. All the untrapped sites were blocked with 150 μl of 3% non-fat milk in PBS-T and incubated at 37°C for 30 minutes.
5. The milk was decanted without washing and 100 μl of enzyme labelled alkaline phosphatase (Ab-AP) diluted 1:1000 in coating buffer was pipetted into each well of micro titre plate and incubated at 37°C for 2hrs. Note that the concentration of antibody and conjugate were predetermined by titration at different concentrations of antigen, antibody, and conjugate.
6. This was washed with PBS-T thrice at intervals of 3 minutes each and tapped dry and 100 μl of 0.6mg / ml of 4-nitrophenyl phosphate in substrate buffer PH 9.8 were pippeted into each well of the microtitre plate and incubated at 37°C for 30 minutes. The colourless

substrate p-nitrophenyl phosphate gave rise to a yellow p-nitrophenol product which was observed visually and also measured with an ELISA reader (Spectrophotometer) at 405 nm. Absorbance values were accepted as positive when the reading was greater than twice the mean absorbance of the virus free control sample. The absorbance values for virus free sample were in most cases similar to those of extraction buffer controls indicating that the background was clear.

CHAPTER FOUR

4.0 RYMV INFECTIVITY, LONGEVITY, AND INDEXING IN RICE SEEDS.

4.1. INTRODUCTION.

Some seed transmitted viruses have been shown to be in the embryo (Murant, 1970; Uyemoto & Grogan, 1972; Gallo & Ciampar, 1977). Thus, bean common mosaic virus which is transmitted through seed was detected in blossoms, young pods, cotyledons, embryos, but not in seed coats of phaseolus (Ekpo and Saettler, 1974). It is also evident that transmission through the seed is largely due to contamination of the seedlings by mechanical means for which a small proportion may be infected in the embryo via pollen, gametophyte or both (Murant, 1970; Agrois, 1988; Hull, 1988; Matthews, 1991). If a virus was unable to enter the embryo, or if it entered but was later eliminated, seed transmission will not occur (Caroll, 1972; Matthews, 1991). This could be due to the fact that viruses that are confined to vascular tissues may be unable to enter the ovule which has no connection with the parent (Mathews, 1991), or due to the rate of inhibitors such as low molecular weight (MW) RNA involved in inhibition of the initiation of protein synthesis (Gunnery & Datta, 1987).

Although in recent years there have been many reports of virus transmission through seeds (Thresh, 1991; Thottappilly, 1992), only a small portion (1-30%) (Agoios, 1988), or about one fifth (Matthews, 1991) of the seeds derived from virus infected plants transmit the virus.

It is the usual practice to harvest seeds from virus infected plants and plant in pasteurized soil in a screen house that is free of insects and other virus vectors and observe for symptoms of virus infections (IITA, 1985; Davies & Mizuki, 1986; Providenti & Robinson, 1987; Gleanson and Providenti, 1990; Hu *et al.*, 1996). Thus, in a similar way Acuna and Ramos

(1958), Galvez and Jennings (1959), and Lamey *et al.* (1961) observed plants raised from seeds of hoja blanca virus (HBV) infected rice plants and concluded that there was no transmission of the virus through the rice seeds.

While Bakker (1974,1975), Fauquet & Thouvenel (1977), IITA (1985), and Anonymous (1988) reported that RYMV is apparently not seedborne, Awoderu (1987, 1991a), obtained evidence that points to seed borne nature of transmission of the virus and thus called for a re-examination of rice from infected plants to confirm or disprove the possibility of the virus being seed borne (Awoderu, 1991c).

Living infected plants may sometimes release large amounts of virus into surrounding soil (Hollings *et al.*, 1977). Several viruses such as TRSV, carnation ringspot virus, and SBMV are known to infect roots through virus contaminated soil without fungi or arthropods being involved (Kleinhempel and Kegler, 1982; Brown and Trudgill, 1984; Teakle, 1986). Viruses transmitted to the roots of a plant may subsequently infect the entire root system and may be translocated to the foliage where symptoms are produced and to stems and trunks (Forer *et al.*, 1984).

Virus transmission may take place between closely spaced plants after a strong wind that could cause the leaves of adjacent plants to rub together and if wounded to exchange some of their saps and thus, transmit any virus present in the sap e.g. Potato virus X (PVX) (Clinch *et al.*, 1938). Some viruses can be readily transmitted through contaminated hands, clothing, and from farm implements (Todd, 1958; Wetter, 1975; Jones and Fribourg, 1977; Tsuboi *et al.*, 1995).

In Mali 100% RYMV infection have been reported to occur only in transplanted fields

and not in direct seeded fields indicating a possible connection with infection from the nursery (Bakker, 1974; Sy, 1994) or infected stubble and leaf debris from the previous harvest.

Since transmission through leaf debris and other sources of contaminants may result in short or long distance dissemination of RYMV, this study is designed to investigate the role of rice seeds, empty rice spikelets, stubble, roots intertwinning, leaves and other sources from RYMV infected plants in the transmission of the virus. The study will further seek to determine the longevity of the virus in leaves and empty spikelets under different storage conditions.

4.2. MATERIALS AND METHODS.

4.2.1. The Transmission of RYMV Through Rice Seeds from Infected Plants.

Seeds were harvested from rice plants showing typical symptoms of RYMV in the field and screenhouse from Mali, Niger, and Cote d'Ivoire. These samples were rated as highly or moderately resistant, and symptomless by visual inspection (Thottappilly and Rossel, 1993).

For precise and easy assessments in ELISA, 100 seeds from each of the 27 samples collected were drawn from the pool and planted in plastic pots containing sterilized soil and maintained in an insect proof screenhouse in the lowland at M'be, WARDA headquarters, Cote d'Ivoire. The plants were watered with clean tap water twice weekly after germination till the experiment lasted. While samples 23-26 were harvested from artificially inoculated plants in the screenhouse, samples 1-22 were harvested from naturally infected plants in the field. Sample 1 was harvested from symptomless rice plants in the field, while sample 27 was harvested from healthy plants in the screenhouse. Seeds were harvested from rice plants inoculated mechanically at 2, 5, 8, and 10 WAT and raised in the insect proof screenhouse by direct seeding in sterilized

soil. Each seedling was monitored by visual assessment in combination with ELISA for foliar symptoms of RYMV infection up to 63 days (9 weeks) after planting as outlined in sections 3.7 and 3.7.1.

4.2.2. RYMV Indexing in Whole Rice Seeds, Seed Components, and Empty Rice Spikelets by ELISA.

Samples were drawn from seeds and empty spikelets harvested from RYMV infected rice plants in the field and from those artificially inoculated in the screen-house at different plant ages of 2, 5, 8, and 10 WAT. The seeds from each sample were mixed thoroughly and 4 lots were drawn from it. About 0.2g of samples were measured from each lot and ground with mortar and pestle in 2ml extraction buffer + 2% PVP. The homogenate was squeezed through cotton wool to get a clear solution. Similarly the spikelets were weighed and ground.

In another experiment the seed samples were thoroughly mixed once again and 100 seeds were drawn from it. These were sub-divided into 4 lots with each lot containing 25 seeds. The seeds were germinated for 7 days in petri dishes containing moistened filter paper (Whatman student grade) and on the seventh day the germinating seeds were excised with a scalpel blade into 3 parts (endosperm with hull, plumule, radicle) (Figure 5). About 0.2g of each part from each lot were ground with pestle and mortar in 2 ml extraction buffer + 2% PVP. The homogenate were squeezed through cotton wool and clear extracts were obtained. All the samples were collected in 2 ml eppendorf tubes for ELISA test. Each lot was replicated in duplicate wells of ELISA.

The seed samples that gave a consistent positive test in ELISA were selected and indexed for the location of the virus in the seeds. Here also, the samples were thoroughly mixed and

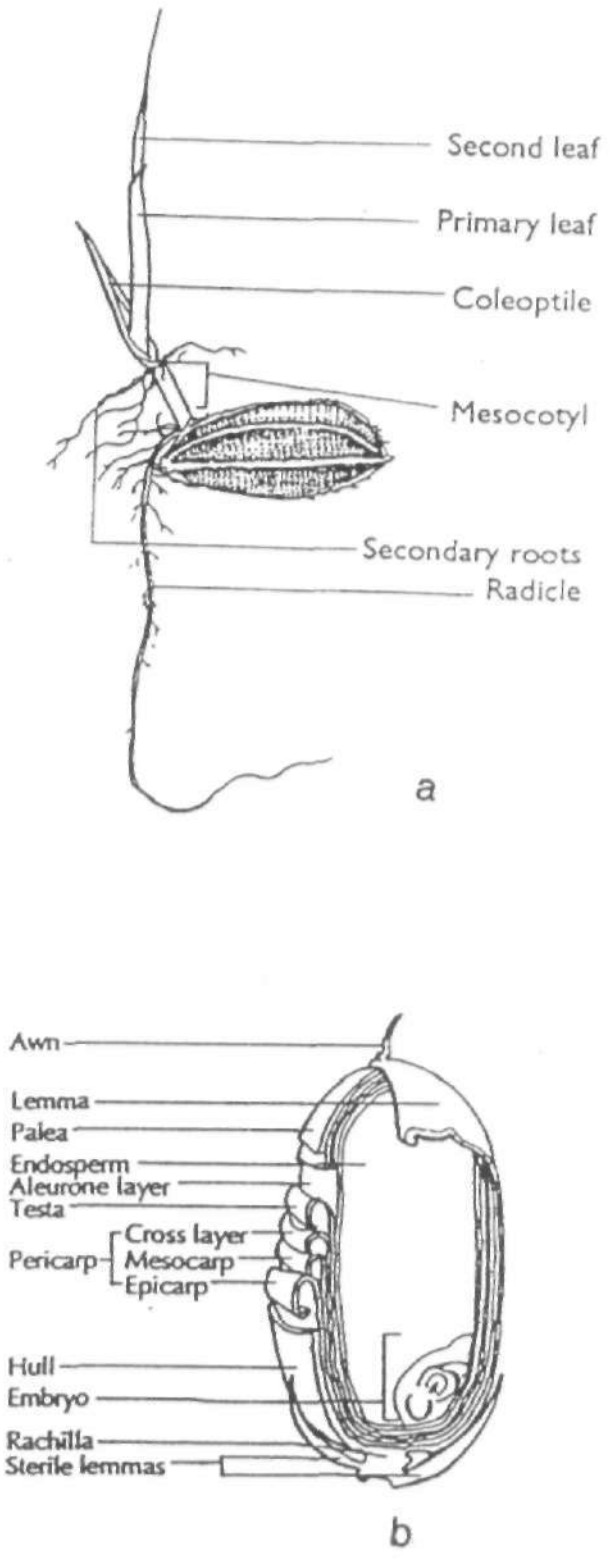


Fig. 5 Different organs and components of a rice seed used in RYMV indexing by ELISA. a = radicle and plumule from a germinating seed, b = embryo, endosperm and testa (husk/hull) from a rice grain. Source: Jones (1995) with slight modifications.

100g of each sample were drawn and separated into the husk/hull (testa), endosperm, and embryo (e.g Maury *et al.*, 1987). The husk/hull were separated with a dehusker, while the embryos were removed from the endosperm with a pin (needle). Also 0.2g of each component were weighed and ground with mortar and pestle in 2 ml of extraction buffer + 2% PVP for ELISA test. Each component was replicated in duplicate wells of ELISA.

For the purpose of developing an appropriate working dilution for the detection of RYMV in rice seeds and spikelets in ELISA, titration of the samples was carried out at different working dilutions for both the antibody and conjugates. Polyclonal antiserum produced in rabbits immunized with purified virus particles was used. The antiserum was donated by Dr G.Thottappilly of IITA, Ibadan, Nigeria. The working dilutions compared were 1:500, 1:1,000, 1:5,000, 1:10,000 for antibody and 1:1,000, 1:5,000, 1:10,000 for conjugates. The seeds and the Spikelets were diluted at 1:10 and 1:50 in extraction buffer + 2% PVP. Thus, the appropriate working dilution obtained that gave the best and economical result in ELISA test for detection of RYMV in both the seeds and spikelets was 1:1000 for both antibody and conjugates, while the antigen dilution was 1:10. ACP-ELISA was performed according to the protocol outlined in section 3.7.1.

4.2.3. The Influence of Time of Infection and Position of Inoculated Leaf on The Detection of RYMV in Rice Seeds.

Healthy seedlings of Bouaké 189, TOX 3440-171-1-1-1-1 (WITA 7), TOX 3440-175-1-2-1 (WITA 8), TOX 3233-31-6-2-1-2 and IR 47686-15-1-1 were transplanted in the screenhouse when 21 days old. They were inoculated at different plant ages of 2, 5, 8, and 10 WAT with RYMV crude sap expressed from leaves of Bouaké 189. The extracts were prepared in distilled water using 60g to 1000ml of distilled water (6% w/v) containing 2 ml carborundum. The seeds from these varieties were harvested at maturity, winnowed and separated into fully filled grains and empty spikelets. Four lots from each variety per sample were drawn and ground with mortar and pestle in PBS + 2% PVP. The extracts were expressed through cotton wool in eppendorf tubes for ELISA test to detect the presence of RYMV in the seeds. The working dilution of 1:1000 for both the antibody and conjugates as worked out in section 4.2.2 was utilised for both the seeds and spikelets. The antigen dilution used was 1:10.

The second experiment involved the inoculation of flag leaf, penultimate leaf, flag/penultimate leaves, and non-inoculated leaves (controls) of Bouaké 189, RYMV susceptible check and IR 47686-15-1-1 a resistant check at booting stage. The plants were raised in sterilized soil. Seeds were harvested from the varieties at full maturity, winnowed and separated into fully filled grains and empty spikelets. These were similarly analysed by ELISA as outlined in Section 3.7.1 to detect the presence of RYMV in the seeds and spikelets.

4.2.4. Virus Contamination, Plant Infection, and Infectivity of Virus Extract from Rice Husks.

The samples used in this study were drawn from the same pool with those used for direct seeding, virus indexing in seeds, seed components, and spikelets in sections 4.2.1 and 4.2.2. Also sterilized soil was used throughout this study.

The first experiment involved healthy rice seeds, seeds from RYMV infected rice plants, mixed with leaves and spikelets from RYMV infected rice plants and planted in the soil in the screenhouse and monitored for up to 63 days (9 weeks). Four rice varieties, Bouaké 189, BG 90-2, and Tox 902-5-10B-101-1-2, with high level of susceptibility and Moroberekan, resistant to RYMV were used with ten treatment combinations as indicated in Table 9. Twenty grammes of RYMV infected and non-infected leaf debris and empty spikelets samples were measured and thoroughly mixed with one kilogramme of sterilized soil in a 15 cm plastic buckets for each treatment. The seeds from the infected and healthy rice plants were seeded directly in these buckets and watered with clean tap water as required. At germination the seedlings were thinned to four in each bucket and monitored for symptom development of RYMV. Visual assessment of foliar symptoms (IRRI, 1988) and ELISA of leaf samples were carried out periodically at 3 weeks intervals up to 63 days after seeding.

In another experiment healthy seeds of Bouaké 189, BG 90-2, and Moroberekan were mixed with plant saps from RYMV infected rice leaves. The extracts were prepared in distilled water using 60g of infected leaves to 1000 ml of the distilled water (6% w/v) without abrasive. In the first treatment virus extracts were mixed with healthy seeds and planted immediately. While in the second treatment the virus extracts were mixed with healthy seeds and left at room temperature (28$\pm$ 2° c) for 12 hours before planting. In the third treatment virus extracts were mixed with healthy seeds and sundried for 12 hours before planting. The fourth treatment involved planting the seeds without the virus extracts. They were also watered with clean tap water regularly as required.

To test for the infectivity of RYMV particles detected in the husks, seeds of Bouaké 189, TOX 3233-31-2-1-2, and IR 47686-15-1-1 harvested from plants infected with RYMV were dehusked with a dehusker. Ten grammes of the husks from each variety were weighed and

ground to powder with an automatic grinder (ISO 901 Retsch). The powder was dissolved in 167 ml of distilled water and 0.33ml of carborundum was added to the inoculum. The plants were inoculated at seedling stage (21 day old) with inoculum from the husks by moistened fingers. Prior to the inoculation, inocula were tested and confirmed by ELISA that they contained RYMV particles.

ELISA and Visual assessment were conducted on leaf samples at 63 days after planting (DAP). The working dilution used for detecting the virus in rice leaves was 1:10000 for the detecting antibody and 1:1000 for the enzyme labelled conjugate. For each sample 0.2g was ground in 2 ml of extraction buffer + 2% PVP (1:10 w/v) in the ELISA tests. Each sample was replicated in two wells of ELISA.

4.2.5. The Role of Intertwining of Rice Roots in The Transmission of RYMV.

Healthy 21 day old seedlings of Bouaké 189 were transplanted into plastic buckets of 15cm in diameter containing sterile soil. Four seedlings were transplanted into each bucket. The foliage of the seedlings from each bucket were separated into pairs on both sides with cardboard papers. One pair of seedlings on one side was inoculated with RYMV 14 days after transplanting (DAT) while the pair on the opposite side was left un-inoculated (Plate I). The spacing between the pairs of the inoculated and non-inoculated was 5cm. The RYMV extract was prepared from infected leaves of Bouaké 189 in distilled water (6% w/v) containing 2 ml of carborundum. Altogether 30 pairs of rice seedlings were inoculated with the RYMV crude sap. Equal number of non-inoculated plants were maintained in the same insectproof screenhouse to serve as control. At grain filling stage the main crop was ratooned with sterile knives. The infection to non-inoculated and the ratooned plants was determined by visual assessment (IRRI, 1988) and ELISA test of leaf samples.



Plate I. The inoculated rice plant (left) and the uninoculated healthy plants (right) with roots intertwined together

The detection of RYMV in roots of the test plants was carried out only in the ratoon stage by unearthing and washing the roots of the entire plants with clean tap water. The roots were excised close to the stalk with a sharp and sterile blade and 0.2g from each of the samples was weighed and ground with mortar and pestle in PBS+2%PVP for ELISA test. ELISA was carried out as previously outlined in section 3.7.1.

4.2.6. The Role of Rice Stubble in The Transmission of RYMV.

Rice stubble from rice plants heavily infected mechanically with RYMV (Plate II) were dug and incorporated in the soil on the same spot in the screenhouse after harvest. The stubbles were flooded and completely submerged in water for 21 days. Thereafter, the water was drained from the plots and 21 day old seedlings of Bouaké 189 and BG 90-2 (RYMV susceptible varieties) were transplanted on the stubble in 4 plots (2 plots per variety) measuring 20 x 5 m each. In each plot 1140 seedlings of a variety were transplanted on the RYMV infected stubbles previously incorporated in the soil. Three rows of each variety were planted on both sides where previously non-inoculated plants were raised but were not assessed for infection because of the lack of barrier from the RYMV infected and non-infected stubbles. They were used simply as barrier crops. The experiment was monitored for up to 63 days (9 weeks) by visual assessment (IRRI, 1988). The test rice plants and *Echinocloa* spp in the plots (Plate III) were analysed by ELISA Infection was expressed as the number of infected plants over number of non-infected plants.



Plate II. The artificially inoculated rice plants exhibiting typical and classical symptoms of RYMV in the screenhouse. Infected plants (arrows) are showing yellowing symptoms.



Plate III. The RYMV infected rice plants (yellow symptoms) in the circled patch at the foreground are a consequence of the RYMV infected stubble incorporated-into the soil.

4.2.7. *The Role of Leaf and Body Contacts in The Transmission of RYMV.*

In the first experiment twenty five healthy seedlings of Bouaké 189 were raised in sterile soil side by side with RYMV infected plants of the same variety in the same plastic bucket measuring 15 cm in diameter. At the time of inoculation of the plants a cardboard paper was placed between the inoculated and non-inoculated plants to act as a barrier and then removed 2 weeks later when the first noticeable symptoms of RYMV were obvious. Thus, leaf contact was established between the infected and non-inoculated plants. Root contact was avoided by placing a plywood permanently between the RYMV infected and non-inoculated test plants. In addition root diffusion was avoided by planting the seedlings in polythene bags before placing them in the buckets. In the second experiment twenty five healthy seedlings of Bouaké 189 were rubbed vigorously (Jones and Fribourg, 1977) against RYMV infected plants for 3 minutes. While in the third experiment RYMV infected plants were stroked with palms of the hands and then back-stroked to healthy seedlings of Bouaké 189. The infected plants were stroked 10 times and equally back-stroked 10 times to the healthy plants in a sequential manner of one stroke on infected plant to one backstroke to healthy plant and so on. In both tests direct seeding was employed and then the seedlings were thinned to 4 at two weeks after germination. Equal number of non-treated healthy plants of Bouaké 189 were maintained in the screenhouse as control. The experiment was maintained in an insect proof screenhouse in the lowland at Mbe, Cote d'Ivoire and monitored for RYMV symptoms for up to 63 days (9 weeks) by visual assessment (IRRI, 1988).

4.2.8. The Role of Direct Seeding and Transplanting of Rice in The Transmission of RYMV.

One kilogramme of heat sterilized soil was thoroughly mixed with 20 grammes of RYMV infected leaf debris from rice (Bouaké 189) which have been cut into pieces with a knife. The mixture was dispensed in plastic buckets (15cm diameter) and maintained in an insect proof screenhouse in the lowland environment at M'be. In the first treatment healthy seeds of Bouaké 189 were seeded directly in 30 plastic buckets. At germination the seedlings were thinned to 4 per pot when 21 days old. In the second treatment 21 day old seedlings of Bouaké 189 with intact roots (not trimmed) were transplanted in another 30 plastic buckets containing the soil and infected leaf debris. In the third treatment also 21 day old seedlings of Bouaké 189 had their roots trimmed with a sterile knife and transplanted in 30 plastic buckets containing the soil and infected leaf debris. One seedling was maintained in each bucket for both treatments. The experiment was monitored by visual assessment (IRRI, 1988) for up to 63 days (9 weeks) for RYMV transmission.

4.2.9. The Longevity of RYMV Infected Plant Materials in Storage.

Young seedlings of Bouaké 189, RYMV susceptible variety were sap inoculated with RYMV in the screenhouse. At maturity, seeds, empty spikelets, and the infected leaves were harvested from the mother plants. Clean seeds were separated from the empty spikelets by a winnowing machine. Some clean seeds were then dehusked with a dehusker to obtain the husks/hull. These infected leaves as well as empty spikelets and the husks were divided into 5 portions each. Each portion of a particular sample was preserved at room temperature ($28\pm2^{\circ}c$), sterile dry soil, non-sterile dry soil, sterile waterlogged soil and non-sterile waterlogged soil. Thus, the longevity in these plant materials was tested by ELISA at intervals of 3 months up

to 9 months. The dilution used for the antibody and conjugates coating was 1:1000 while the dilution for the antigen extraction was 1:10.

4.3. RESULTS.

None of the seedlings that were germinated from seeds of the infected plants and monitored in the insectproof screenhouse elicited any symptom of RYMV infection by visual assessment and ELISA at 63 days after germination (Tables 1 and 2).

The results of RYMV indexing in dry seeds by ELISA are presented in Table 3. Although RYMV was detected in seeds of the rice plants by ELISA the frequency of detection of the virus varied with the seeds harvested from the field and screenhouse. At least 1 out of 4 lots, tested positive for RYMV in susceptible varieties harvested from the field and the screenhouse. However, the frequency of detecting RYMV in more than two lots per variety was higher for the artificially inoculated than the naturally infected plants. The result from the RYMV indexing of the empty rice spikelets presented in Table 4 shows that at least 2 out of 4 lots drawn from the infected plants tested positive for RYMV. RYMV particles were not detected in the immune variety IR 47686-15-1-1 which obviously was not infected by the virus.

The virus was not detected in the plumule and the radicle but was detected in the extracts of husks and endosperm ground together (Table 5). Thus, when the seeds were dehusked and the embryo removed the virus was detected only in the husk. No virus particle was detected by ELISA in the endosperm or embryo (Table 6). RYMV was detected in all but the immune varieties inoculated with it at different growth stages of 2,5,8,10 WAT and leaf positions (flag leaf, penultimate leaf, flag leaf/penultimate leaf) (Tables 7 and 8).

Table 1: Determination of the infection by ELISA in leaf samples of screenhouse reared plants raised from seeds of RYMV infected rice plants.

S/No	Location & Site	Type of Variety & Status of Plants	ELISA Absorbance Values of leaves (A405nm)[b]		
			21 DAS	42 DAS	63 DAS
1	N9,Niono, Mali Plot 6	BG90-2,Healthy,No Symptoms	0.02[c](-)	0.04(-)	0.05(-)
2	N9,Niono, Male, Plot 3	BG90-2,Infected, Moderate	0.03(-)	0.02(-)	0.05(-)
3	N9,Niono, Mali, Plot 2	BG90-2,Infected, High	0.02(-)	0.02(-)	0.05(-)
4	N9,Niono, Mali Plot 4	BG90-2,Infected, High	0.03(-)	0.03(-)	0.05(-)
5	N9,Niono, Mali, Plot 1	BG90-2,Infected High	0.03(-)	0.05(-)	0.07(-)
6	N9,Niono, Mali, Plot 5	BG90-2,Infected,Moderate	0.03(-)	0.03(-)	0.06(-)
7	KM36,Niono,Mali,Plot 1	Seberang MR,Infectd, Moderate	0.03(-)	0.03(-)	0.05(-)
8	KM36,Niono, Mali,Plot5	BG90-2,Infected, Moderate	0.04(-)	0.03(-)	0.06(-)
9	KM36, Niono,Mali,Plot3	BG90-2,Infected, High	0.04(-)	0.04(-)	0.07(-)
10	KM36, Niono,Mali,Plot1	BG90-2,Infected,Moderate	0.04(-)	0.04(-)	0.90(-)
11	KM36, Niono, Mali,Plot2	BG90-2,Infected, High	0.04(-)	0.05(-)	0.07(-)
12	KM36,Niono, Mali,Plot3	BG90-2,Infected,High	0.04(-)	0.04(-)	0.06(-)
13	KM36,Niono, Mali	BG90-2,Infected,Moderate	0.03(-)	0.04(-)	0.06(-)
14	N6,Niono, Mali	IET2911,Infected, High	0.03(-)	0.04(-)	0.06(-)
15	N6, Niono, Mali	BG90-2,Infected, Moderate	0.03(-)	0.03(-)	0.06(-)
16	N10,Niono, Mali	BG90-2,Infected, High	0.03(-)	0.03(-)	0.05(-)
17	N4,Niono, Mali	BG90-2,Infected, High	0.03(-)	0.03(-)	0.06(-)
18	Selingue, Mali	BG90-2, Infected, Moderate	0.03(-)	0.03(-)	0.05(-)
19	SAY1, Niger	IR 1529-680-3,Infected,Moderate	0.04(-)	0.04(-)	0.05(-)
20	SAY1, Niger	IR54,Infected, Moderate	0.04(-)	0.50(-)	0.05(-)
21	SAY1, Niger	IR54/IR1529-680-3,Infected, Moderate	0.04(-)	0.03(-)	0.06(-)
22	Sakassou,Cote d'Ivoire	Bouake 189, Infected, High	0.03(-)	0.03(-)	0.05(-)
23	Mahiboull,Gagnoa,Cote d'Ivoire	Bouake 189, Infected, High	0.04(-)	0.03(-)	0.05(-)
24	WARDA, MBe,Cote d'Ivoire	Bouake 189, Infected, High	0.04(-)	0.03(-)	0.05(-)
25	WARDA, Mbe, Cote d'Ivoire	TOX 902-10B-101-2, Infected,High	0.06(-)	0.05(-)	0.05(-)
26	WARDA, Mbe, Cote d'Ivoire	TOX 3519, Infected, High	0.06(-)	0.07(-)	0.09(-)
27	WARDA, Mbe, Cote d'Ivoire	Bouake 189, Healthy, Non-infected	0.03(-)	0.04(-)	0.09(-)
	CONTROLS	Positive (RYMV infected rice leaves)	2.60(+)	2.60(+)	2.60(+)
		Negative (Non-infected rice leaves)	0.10(-)	0.10(-)	0.10(-)

[b] ELISA cut off point(minimum level) for positive values = 0.200

[c] Values were considered as positive when the reading was greater or equal to twice the mean absorbance of negative controls

(-) Negative values

(+) Positive values

DAS Days after seeding.

Table 2. Determination of infection by ELISA in leaf samples of screenhouse reared rice plants raised from seeds of plants inoculated with RYMV at different plant ages[a]

Time of Inoculation After Transplanting (Weeks)	Varieties	ELISA Absorbance Values of leaves (A405nm)[b]		
		21 DAS	42 DAS	63 DAS
2	Bouaké 189	0.03[c] (-)	0.03 (-)	0.04 (-)
	TOX 3440-171-1-1-1-1 (WITA 7)	0.04 (-)	0.04 (-)	0.04 (-)
	TOX 3440-176-1-2-1 (WITA 8)	0.03 (-)	0.04 (-)	0.05 (-)
	TOX 3233-6-2-1-2	0.03 (-)	0.03 (-)	0.05 (-)
	IR 47686-15-1-1	0.04 (-)	0.04 (-)	0.06 (-)
5	Bouaké 189	0.04 (-)	0.03 (-)	0.05 (-)
	TOX 3440-171-1-1-1-1 (WITA 7)	0.04 (-)	0.05 (-)	0.04 (-)
	TOX 3440-176-1-2-1 (WITA 8)	0.05 (-)	0.06 (-)	0.06 (-)
	TOX 3233-6-2-1-2	0.04 (-)	0.04 (-)	0.04 (-)
	IR 47686-15-1-1	0.05 (-)	0.05 (-)	0.05(-)
8	Bouaké 189	0.04 (-)	0.05 (-)	0.06 (-)
	TOX 3440-171-1-1-1-1 (WITA 7)	0.04 (-)	0.06 (-)	0.05 (-)
	TOX 3440-176-1-2-1 (WITA 8)	0.05 (-)	0.05 (-)	0.05 (-)
	TOX 3233-6-2-1-2	0.04 (-)	0.05 (-)	0.05 (-)
	IR 47686-15-1-1	0.05 (-)	0.05 (-)	0.06 (-)
10	Bouaké 189	0.05 (-)	0.04 (-)	0.05 (-)
	TOX 3440-171-1-1-1-1 (WITA 7)	0.05 (-)	0.05 (-)	0.06 (-)
	TOX 3440-176-1-2-1 (WITA 8)	0.04 (-)	0.04 (-)	0.05 (-)
	TOX 3233-6-2-1-2	0.05 (-)	0.05 (-)	0.04 (-)
	IR 47686-15-1-1	0.04 (-)	0.05 (-)	0.06 (-)
CONTROLS	Positive (RYMV infeeted rice leaves)	2.60(+)	2.60 (+)	2.60 (+)
	Negative (Non-infected rice leaves)	0.10 (-)	0.10 (-)	0.10 (-)

[b] ELISA cut off point (minimum level) for positive values = 0.200

[c] Values were considered as positive when the reading was greater or equal to twice the mean absorbance of negative controls

(-) Negative values

(+) Positive values, DAS = Days after seeding

Table 3: Determination of the presence of RYMV in whole rice seeds of naturally RYMV infected plants collected from the field.

		ELISA absorbance values (A405nm)			
S/No.	Types of Variety & Status of Plants	Number of lots drawn			
		1	2	3	4
1	BG 90-2 Healthy, no symptoms	0.06	0.11	0.08	0.07
2	BG 90-2, Infected, moderate	0.13	0.24	0.14	0.17
3	BG90-2,Infected, High	0.22	0.21	0.18	0.22
4	BG90-2,Infected, High	0.14	0.13	0.23	0.18
5	BG90-2,Infected High	0.13	0.22	0.11	0.15
6	BG90-2,Infected,Moderate	0.06	0.10	0.20	0.06
7	Seberang MR,Infectd, Moderate	0.13	0.22	0.12	0.23
8	BG90-2,Infected, Moderate	0.22	0.14	0.09	0.09
9	BG90-2,Infected, High	0.23	0.24	0.22	0.20
10	BG90-2,Infected,Moderate	0.12	0.21	0.21	0.09
11	BG90-2,Infected, High	0.22	0.23	0.11	0.13
12	BG90-2,Infected,High	0.22	0.09	0.11	0.06
13	BG90-2,Infected,Moderate	0.22	0.09	0.11	0.12
14	IET2911,Infected, High	0.24	0.34	0.15	0.33
15	BG90-2,Infected, Moderate	0.09	0.06	0.16	0.07
16	BG90-2,Infected, High	0.16	0.22	0.21	0.23
17	BG90-2,Infected, High	0.22	0.21	0.15	0.13
18	BG90-2, Infected, Moderate	0.19	0.27	0.15	0.22
19	IR 1529-680-3,Infected,Moderate	0.10	0.25	0.12	0.12
20	IR54,Infected, Moderate	0.13	0.10	0.21	0.07
21	IR54/IR1529-680-3, Infected, Moderate	0.18	0.09	0.22	0.10
22	Bouake 189, Infected, High	0.21	0.22	0.18	0.21
23	Bouake 189, Infected, High	0.31	0.24	0.20	0.20
24	Bouake 189, Infected, High	0.36	0.35	0.17	0.26
25	TOX 902-10B-101-2, Infected,High	0.21	0.26	0.17	0.24
26	TOX 3519, Infected, High	0.35	0.27	0.33	0.23
27	Bouake 189, Healthy, Non-infected	0.07	0.06	0.07	0.06
CONTROLS	Positive (RYMV infected rice leaves)	2.60	2.60	2.60	2.60
	Negative (Non-infected rice leaves)	0.10	0.10	0.10	0.10

ELISA cut off point for positive values = 0.200

Values were considered as positive when the reading was greater or equal to twice the mean absorbance of the negative controls.

Table 4: Determination of the presence of RYMV in empty rice spikelets of naturally RYMV infected plants collected from the field.

S/No.	Types of Variety & Status of Plants	ELISA absorbance values (A405nm) Number of lots drawn			
		1	2	3	4
1	BG 90-2 Healthy, no symptoms	0.10	**0.19**	**0.15**	0.19
2	BG 90-2, Infected, moderate	0.27	**0.17**	**0.22**	0.21
3	BG90-2,Infected, High	0.24	**0.20**	**0.22**	0.21
4	BG90-2,Infected, High	0.18	**0.22**	**0.21**	0.23
5	BG90-2,Infected High	0.35	**0.19**	**0.21**	0.16
6	BG90-2,Infected,Moderate	0.13	**0.12**	**0.21**	0.20
7	Seberang MR,Infectd, Moderate	0.21	**0.21**	**0.21**	0.24
8	BG90-2,Infected, Moderate	0.23	**0.24**	**0.17**	0.14
9	BG90-2,Infected, High	0.27	0.13	**0.24**	0.20
10	BG90-2,Infected,Moderate	0.12	**0.28**	**0.21**	0.16
11	BG90-2,Infected, High	0.20	**0.36**	**0.14**	0.16
12	BG90-2,Infected,High	0.27	0.17	**0.19**	0.25
13	BG90-2,Infected,Moderate	0.21	0.15	**0.23**	0.14
14	IET2911,Infected, High	0.41	0.33	**0.40**	0.42
15	BG90-2,Infected, Moderate	0.18	0.13	**0.25**	0.18
16	BG90-2,Infected, High	0.28	**0.28**	**0.25**	0.36
17	BG90-2,Infected, High	0.18	**0.26**	**0.17**	0.23
18	BG90-2, Infected, Moderate	0.17	**0.19**	**0.25**	0.28
19	IR 1529-680-3,Infected,Moderate	0.27	**0.18**	**0.19**	0.18
20	IR54,Infected, Moderate	0.16	**0.12**	**0.21**	0.18
21	IR54/IR1529-680-3, Infected, Moderate	0.27	**0.17**	**0.27**	0.18
22	Bouake 189, Infected, High	0.22	**0.25**	**0.24**	0.22
23	Bouake 189, Infected, High	0.36	**0.30**	**0.32**	0.31
24	Bouake 189, Infected, High	0.35	**0.35**	**0.35**	0.36
25	TOX 902-10B-101-2, Infected,High	0.31	**0.31**	**0.33**	0.34
26	TOX 3519, Infected, High	0.36	**0.33**	**0.31**	0.34
27	Bouake 189, Healthy, Non-infected	0.12	**0.11**	**0.10**	0.17
CONTROLS	Positive (RYMV infected rice leaves)	2.60	**2.60**	**2.60**	2.60
	Negative (Non-infected rice leaves)	0.10	**0.10**	**0.10**	0.10

ELISA cut off point for positive values = 0.200.

Values were considered as positive when the reading was greater or equal to twice the mean aborbance of the negative controls.

Table 5: Determination of the presence of RYMV in the organs of germinating rice seeds of naturally RYMV infected plants.

		ELISA absorbance values (A405nm)		
S/No.	Types of Variety & Status of Plants	Endosperm/ Husks	Plumule	Radicle
3	BG 90-2 Infected, High	0.31	0.11	0.14
7	Seberang MR, Infected, Moderate	0.30	0.06	0.14
9	BG 90-2, Infected, High	0.31	0.08	0.06
10	BG 90-2, Infected, moderate	0.25	0.06	0.13
11	BG 90-2, Infected, High	0.25	0.07	0.11
14	BG 90-2, Infected, High	0.41	0.06	0.14
16	BG 90-2, Infected, High	0.34	0.06	0.15
17	BG 90-2, Infected, High	0.24	0.07	0.16
18	BG 90-2, Infected, High	0.26	0.06	0.11
22	Bouake 189, Infected, High	0.39	0.06	0.12
23	Bouake 189, Infected, High	0.22	0.06	0.15
24	Bouake 189, Infected, High	0.35	0.08	0.15
25	TOX 902-10B-101-2, infected, High	0.32	0.10	0.16
26	TOX 3519, Infected, High	0.33	0.08	0.15
CONTROLS	Positive (RYMV infected rice leaves)	2.60	2.60	2.60
	Negative (Non-infected rice leaves)	0.10	0.10	0.10

ELISA cut off point for positive values = 0.200.

Values were considered as positive when the reading was greater or equal to twice the mean aborbance of the negative controls.

Table 6: Determination of the presence of RYMV in the rice components of naturally RYMV infected plants.

		ELISA absorbance values (A405nm)		
S/No.	Types of Variety & Status of Plants	Husks/Hull	Endosperm	Embryo
3	BG 90-2 Infected, High	0.82	0.16	0.13
7	Seberang MR, Infected, Moderate	1.19	0.17	0.11
9	BG 90-2, Infected, High	1.13	0.18	0.12
10	BG 90-2, Infected, moderate	1.00	0.19	0.15
11	BG 90-2, Infected, High	0.59	0.17	0.14
14	BG 90-2, Infected, High	1.15	0.19	0.15
16	BG 90-2, Infected, High	0.50	0.16	0.13
17	BG 90-2, Infected, High	0.97	0.16	0.13
18	BG 90-2, Infected, High	0.87	0.15	0.14
22	Bouake 189, Infected, High	0.82	0.15	0.14
23	Bouake 189, Infected, High	0.86	0.17	0.15
24	Bouake 189, Infected, High	1.32	0.15	0.13
25	TOX 902-10B-101-2, infected, High	1.21	0.15	0.13
26	TOX 3519, Infected, High	1.00	0.16	0.12
CONTROLS	Positive (RYMV infected rice leaves)	2.60	2.60	2.60
	Negative (NM-infected rice leaves)	0.10	0.10	0.10

ELISA cut off point for positive values = 0.200.

Values were considered as positive when the reading was greater or equal to twice the mean aborbance of the negative controls.

Table 7. The effect of time of RYMV infection on the translocation of the virus particles in the seeds of rice.

Varieties	Time of Inoculations After Transplanting (Weeks) / Ratio of positive to negative tests							
	2		5		8		10	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Bouaké 189	7	3	6	4	8	3	3	7
TOX 3440-171-1-1-1-1 (WITA 7)	6	4	7	3	7	3	4	6
TOX 3440-176-2-1-2 (WITA 8)	6	4	4	6	6	4	4	6
TOX 3233-15-1-1	5	5	5	5	6	4	4	6
IR 47686-15-1-1	0	0	0	0	0	0	0	0
SEM	1.24		1.21		1.40		0.78	

Table 8. The effect of leaf inoculation positions with RYMV on the translocation of the virus particles in seeds of rice

Varieties	Inoculation Positions / Ratio of Positive to Negative Tests					
	Flag Leaf		Penultimate Leaf		Flag & Penultimate Leaves	
	Positive	Negative	Positive	Negative	Positive	Negative
Bouaké 189	3	7	4	6	5	5
IR 47686-15-1-1	0	0	0	0	0	0
SEM	1.50		2.00		2.50	

Results on the investigation on the role of plant debris (virus infected leaves and spikelets), and contamination of rice seeds with virus extracts from rice leaves are shown in Tables 9 and 10. The highest RYMV transmission was obtained through treatment combinations involving infected rice leaves and empty spikelets. No infection was obtained through rice seeds planted alone or with non infected rice stubbles. No RYMV transmission was obtained on Bouaké 189 with the extracts expressed from the rice husks of the seeds from Bouake 189, TOX 3233-31-2-1-2 and IR 47686-15-1-1.

Evidence of RYMV transmission through root fusion in the main and the ratooned crops of Bouaké 189 is indicated in Table 11. A total of 11 out of 90 plants were infected in the main crop while 26 out of 90 plants became infected in the ratoon crop. The ELISA test detected RYMV particles only in the roots of the plants that had visual symptoms of RYMV in the main crop. No virus particle was detected in the roots of plants that appeared symptomless.

The RYMV infected rice stubble that were dug and incorporated into the soil transmitted the virus to Bouaké 189 and BG 90-2. About 45 out of 2280 (1.97%) plants became infected for Bouaké 189 while 34 out of 2280 (1.50%) plants were infected for BG90-2 after 63 days of transplanting.

RYMV transmission was demonstrated by leaf contact, brushing the leaves of the infected plants against the healthy ones, backstroking healthy plants with hands previously stroked against infected plants. The actual proportion of infected plants in each test are presented in Table 12. No infection was obtained on the control plants.

The result of the role of direct seeding and transplanting of rice in the transmission of RYMV is presented in Table 13. RYMV Infection was obtained with both treatments. However, the highest infection levels were obtained when roots were trimmed before transplanting (19/90) as against 8/90 and 2/90 for untrimmed roots and direct seeding respectively.

Table 9. The infectivity of rice seeds, leaf debris, and empty rice spikelets from non-infected and RYMV infected rice plants[a]

Status of Seeds/Treatment Levels	Number of Infected Plants out of 25[d]/Varieties			
	Bouaké 189[b]	Tox902-10B-101-2[b]	BG90-2[b]	Morobereka
Seeds from non-infected rice plants	0[c] (0)	0 (0)	0 (0)	0 (0)
Seeds from non-infected rice plants mixed with leaf debris of RYMV infected rice plants	3 (12)	2 (8)	2 (8)	1 (4)
Seeds from RYMV infected rice plants	0 (0)	0 (0)	0 (0)	0 (0)
Seeds from non-infected rice plants mixed with empty spikelets from RYMV infected rice plants	0 (0)	0 (0)	0 (0)	0 (0)
Seeds from non-infected rice plants mixed with leaf debris and empty spikelets from RYMV infected rice plants	4 (16)	3 (12)	2 (8)	0 (0)
Seeds from RYMV infected rice plants mixed with leaf debris from RYMV infected rice plants	2 (8)	2 (8)	3 (12)	0 (0)
Seeds from RYMV infected rice plants mixed with empty spikelets from RYMV infected rice plants	1 (4)	1 (4)	0 (0)	0 (0)
Seeds from RYMV infected rice plants mixed with leaf debris and empty spikelets from RYMV infected rice plants	3 (12)	4 (16)	3 (12)	1 (4)
Seeds from non-infected rice plants mixed with empty spikelets from non-infected rice plants	0 (0)	0 (0)	0 (0)	0 (0)
Seeds from RYMV infected rice plants mixed with empty spikelets from healthy rice plants	0 (0)	0 (0)	0 (0)	0 (0)

Values in brackets are percentages

[a] Infectivity was determined both by visual rating (IRRI, 1988) and ELISA

[b] RYMV susceptible varieties

[c] RYMV resistant variety

[d] 25 plants tested per variety per treatment.

[e] Values are means of three repeatitions.

Table 10. Determination of infectivity of RYMV particles adhering to the surface of rice seed[a]

Treatment Levels	Number of Infected Plants out of 25[d]/Varieties			
	Bouaké 189[b]	Tox 902-10B-101-2[b]	BG90-2[b]	Moroberekan[c]
Immediate direct planting of healthy seeds mixed with virus extracts	3[d] (12)	2 (8)	1 (4)	0 (0)
Healthy seeds mixed with virus extracts and stored at room temperature (c.30°c) for 12 hours before planting	1 (4)	1 (4)	0 (0)	0 (0)
Healthy seeds mixed with virus extracts and sundried for 12 hours before planting	0 (0)	0 (0)	0 (0)	0 (0)
Healthy seeds without virus extracts	0 (0)	0 (0)	0 (0)	0 (0)

Values in brackets are percentes

[a]Infectivity was determined both by visual rating (IRRI, 1988) and ELISA

[b]RYMV susceptible varieties

[c]RYMV resistant varieties

[d]25 plants tested per test variety per treatment.

[e]Values are means of three repetitions.

Table 11. The effect of roots fusion in the main an ratoon crop stages on the transmission of RYMV[b].

Treatments[a]	Test Number	No of Infected Plants/No of Plants tested	ELISA Mean Values	% Infection
Main Crop				
	1	3/30	0.27	10
	2	3/30	0.26	10
	3	5/30	0.36	17
SEM = 0.032				
Ratoon Crop				
	1	9/30	0.31	30
	2	7/30	0.29	23
	3	10/30	0.31	33
SEM = 0.007				

[a] Seeds and seedlings of Bouaké 189 were used as test plants

[b] Visual assessment based on SES (IRRI, 1988) was used to determine infection or no infection

[c] Percentage infection was calculated as number of infected plants divided by the total number of plants tested

[d] Roots infection was assayed by ELISA

Ratooning was carried out with sterile knives at grain filling stage

Table 12. The effects of leaf and body contacts on the transmission of rice yellow mottle virus

Treatments[a]	Test Number[b]	No of Infected Plants/No of plants tested	ELISA Mean Values	% Infection
Leaf Contact				
	1	2/15	0.28	13
	2	3/15	0.29	20
SEM = 0.009	3	3/15	0.31	20
Brushing RYMV Infected Plants Against Healthy Plants[d]				
	1	3/15	0.27	20
	2	1/15	0.26	7
SEM = 0.012	3	2/15	0.30	13
Backstroking of Healthy Plants with Hands Previously Stroked Against RYMV Infected Plants[e]				
	1	2/15	0.29	13
	2	1/15	0.28	7
	3	2/15	0.29	13
SEM = 0.003				

[a] Seedlings of Bouaké 189 were used as test plants

[b] Equal number of non treated seedlings were included in each test number to serve as healthy controls

[c] Visual assessment based on SES (IRRI, 1988) was used to score for infection or no infection

[d] Infected and healthy plants were brushed against each other for 30 seconds

[e] Each plant was backstroked 10 times

Table 13. The incidence of RYMV due to direct seeding and transplanting of rice on the virus infected stubble incoporated in the soil[b]

Treatments[a]	Test Number	No of Infected Plants/No of tested Plants	ELISA Mean Values	% Infection[c]
Direct Seeding				
	1	1/30	0.25	3
	2	1/30	0.26	3
	3	0/30	0.00	0
SEM = 0.085				
Transplanting of seedlings with roots untrimmed (unwounded)				
	1	3/30	0.26	10
	2	3/30	0.27	10
	3	2/30	0.27	7
SEM = 0.003				
Transplanting of seedlings with roots trimmed (wounded)				
	1	5/30	0.25	17
	2	6/30	0.27	20
	3	8/30	0.29	27
SEM = 0.012				

[a] Seeds and seedlings of Bouaké 189 were used as test plants

[b] Visual assessment of leaves based on SES (IRRI, 1988) and ELISA were used to determine infection or no infection

[c] Percentage infection was calculated as number of infected plants divided by the total number of plants tested

The results of the study on the longevity of rice plant materials in storage are presented in Tab 14 to 18. RYMV was detected by ELISA in leaves, empty rice spikelets and husks/hull preserved at room temperature (28± 2°c) up to 9 months. The detection of RYMV was only possible up to the 6th month for both the spikelets and husks preserved in the sterile dry soil whereas the virus was detec up to the 3rd month only when preserved in non-sterile dry soil. For the spikelets and husks preserv in sterile and non-sterile waterlogged soils the detection of RYMV particle was only possible up to the 3rd month for the spikelets and less than 3 months for the husks. It was possible to detect RYM particle up to the 9th and 6th months for leaves preserved in sterile dry soil and non-sterile dry s respectively. Furthermore, RYMV was detected up to the 3rd month only in leaves preserved in waterlogged soils.

Table 14. The longevity of RYMV in plant materials of Bouaké 189 preserved at room temperature[b]

Time of Test (Months)	Plant Materials / Ratio of Positives to Negative Values in ELISA Test					
	Leaves		Empty Spikelets		Husks/Hull (Testa)	
	Positive	Negative	Positive	Negative	Positive	Negative
0	10	0	9	1	6	4
3	10	0	7	3	4	6
6	6	4	4	6	5	5
9	5	5	4	6	3	7
SEM	1.32		1.13		0.65	

[b] Bouaké 189 is RYMV susceptible check

((0))

Table 15. The longevity of RYMV in plant materials of Bouaké 189 incorporated in sterile dry soil[b]

Time of Test (Months)	Plant Materials / Ratio of Positive to Negative Values in ELISA Test					
	Leaves		Empty Spikelets		Husks/Hull (Testa)	
	Positive	Negative	Positive	Negative	Positive	Negative
0	10	0	9	1	7	3
3	6	4	4	6	2	8
6	3	7	2	8	1	9
9	2	8	0	10	0	10
SEM	1.80		1.93		1.94	

[b] Bouaké 189 is RYMV sceptible check variety

((0))

Table 16. The longevity of RYMV in plant materials of Bouaké 189[b] incorporated in non-sterile dry soil

Time of Test (Months)	Plant Materials / Ratio of positive to Negative Values in ELISA Test					
	Leaves		Empty Spikelets		Husks/Hull	
	Positive	Negative	Positive	Negative	Positive	Negative
0	10	0	7	3	8	2
3	4	4	3	7	5	8
6	2	8	1	10	1	9
9	0	10	1	10	0	10
SEM	2.16		1.41		1.85	

[b] RYMV susceptible check

((0))

Table 17. The longevity of RYMV in plant materials of Bouaké 189[b] incorporated in sterile waterlogged soil

Time of Test (Months)	Plant Materials / Ratio of Positive to Negative Values in ELISA Test					
	Leaves		Empty Spikelets		Husks/Hull	
	Positive	Negative	Positive	Negative	Positive	Negative
0	10	1	8	2	6	4
3	2	8	1	9	0	10
6	0	10	0	10	0	10
9	0	10	0	10	0	10
SEM	2.38		1.93		1.50	

[b] RYMV susceptible check

((0))

Table 18. The longevity of RYMV in plant materials of Bouaké 189[b] incorporated in non-sterile waterlogged soil[b]

Time of Tests (Months)	Plant Materials / Ratio of Positive to Negative Values in ELISA Test					
	Leaves		Empty Spikelets		Husks/Hull	
	Positive	Negative	Positive	Negative	Positive	Negative
0	10	0	6	4	5	5
3	2	8	1	9	0	10
6	0	10	0	10	0	10
9	0	10	0	10	0	10
SEM	2.38		1.44		1.25	

[b] RYMV susceptible check

4.4. DISCUSSION.

The non detection of RYMV in all lots drawn from seeds and spikelets of virus infected plants could be due to environmental factors and the virus-host interactions which could lead to uneven distribution of the virus in plant organs and tissues. According to Maule (1991) host genes, viral genes, and environmental factors affect the rate and extent of virus movement through plants. However, irrespective of time of inoculation RYMV was translocated in rice seeds and detected by ELISA. As with most other non-seed transmitted viruses (Ekpo and Saetler, 1974; Gallo and Ciampor, 1977) RYMV is located in the husk of the rice seed. This result indicates that RYMV is unlikely to be seed transmissible.

Although, it was established from the foregoing section that RYMV was present in the lots drawn from the pool of seed samples used in this study thus, confirming that the seeds planted in this test had the virus, no evidence of seed transmission of RYMV was obtained. Seeds harvested from rice plants inoculated with RYMV at 2, 5, 8, and 10 WAT did not produce evidence that the virus is transmitted through seeds. The result is convincing for the fact that for a virus to be transmitted through seeds it must enter and survive in the embryo (Owusu *et al.*, 1968; Caroll, 1972; Gunnery & Datta, 1987). Laviolette and Athow (1971) found that seed transmission of tobacco ringspot virus (TRSV) was associated with the presence of the virus in embryonic tissue of the seed but not the seed coat. In this work RYMV has been found to be located in the husk/hull and therefore, apparently not transmitted through rice seeds. Earlier reports elsewhere involving planting of rice seeds from RYMV infected plants also indicated that RYMV

is not transmitted through rice seeds (Bakker, 1974,1975; Fauquet & Thouvenel, 1977; IITA, 1985; Anonymous, 1988). No evidence of RYMV transmission was also obtained when rice seed samples of IR 1529-680-3 from infected plants which were grown and analysed at the Danish Government Institute of Seed Pathology for Developing Countries, Denmark (SY, A.A., personal communication-See Appendix 3).

Irrespective of time of rice infection and leaf inoculation positions, RYMV is translocated in the seeds of the susceptible varieties. The frequency of detection of the virus by ELISA also varied with the varieties (Bouaké 189, WITA 7, WITA 8, TOX 3233-15-1-1). There was no marked difference in the frequency of detection of RYMV from seeds harvested from plants inoculated at 2, 5, and 8 WAT however, the frequency of detection was lower for the seeds harvested from plants inoculated at 10 WAT (flowering stage). Thus, it is likely that the final distribution of RYMV in rice seeds is uneven.

No infection was detected in seeds of infected rice plants germinated in the soil without the RYMV infected debris. However, infection was obtained when seeds from both the virus free and virus infected rice plants were planted with the infected leaf debris. Higher levels of infection was obtained with infected leaf debris alone or in combination with the infected empty rice spikelets. RYMV infection was only possible when infected rice seeds were planted in combination with empty rices pikelets from the infected plants. Very low level of infection was obtained with virus adhering outside the seed coat of the susceptible rice plants. Although it is evident that some infection could be caused by the infected spikelets in the soil the real source of infection is from the

infected leaf debris. There is evidence that this virus which is known for its high infectivity and stability (Bakker, 1974; Fauquet and Thouvenel, 1987), and rapid rate of multiplication (Bakker, 1974; Fauquet *et al.*, 1995) is transmitted through surface contamination of seeds of susceptible rice varieties abiotically though at very low level. This evidence is convincing because in other viruses such as Tobacco mosaic virus (TMV) in Tomato, transmission is largely due to contamination of the seedling by mechanical means (Broadbent, 1965).

The RYMV extract from rice seeds was not infectious to rice seedlings in artificial inoculations and when RYMV infected seeds were germinated in nature, thus suggesting that a mechanism exists in the seeds that is inhibiting the expression of the virus. Bakker (1974) and Campbell *et al.*, (1996) also failed to recover virus in expressed sap of seedcoats of rice and melon respectively.

RYMV transmission did occur when roots of rice were intertwinned together especially in young and ratooned plants that were closely spaced. The infection in the ratooned non-inoculated plants increased by 50 percent on Bouaké 189. The plants that were rated as not infected by visual inspection of leaves also contained no virus particle in their roots when tested by ELISA. The release of RYMV particles through the root system is therefore, a source of virus spread without a soil vector being involved.

Another good source of RYMV infection was demonstrated through the infected stubble that was dug into the soil after the harvesting of rice. Although it may seem to the ordinary eye that the incidence in this study was not high yet the initial inoculum is surely the source through which the spread of the virus could take place if a mobile

vector is involved.

The brushing of leaves against each other through leaf contact in nature and through contaminated hands transmitted the virus. Although in the process of this trial care was taken not to wound the healthy plants unnecessarily while brushing and stroking, it was observed that some leaves from both the infected and healthy plants were slightly bruised during the processes. It was some of these healthy plants that became bruised in the process that developed symptoms of RYMV. No abrasive was used to aid this transmission other than the slight pressure that was created during the backstroking and brushing of plants against each other. Thus, it is interesting to note that no abrasive is necessary in the transmission of RYMV and a slight pressure on the rice leaves with fingers is sufficient to cause infection. This observation was also made by Raymundo and Konteh (1980).

RYMV was transmitted through direct seeding and transplanting where RYMV infected stubble were incorporated into the soil. Also wounding by trimming of rice seedlings during transplanting increased the incidence of RYMV. However, this result does not give any indication that this mode of transmission is responsible for the fast spread of RYMV in the field. However, this mode of transmission may serve as a source of inoculum for the mobile vectors such as insects that are suspected to be responsible for the fast and distant spread of RYMV disease.

Strong evidence was obtained which indicates that RYMV persists longer than 9 months in the dry leaves, empty rice spikelets and husks/hull preserved at room temperature. Other workers also found a long persistence of RYMV in dry leaves readily

preserved (Bakker, 1974; Fauquet and Thouvennel, 1987). However, the virus is lost after 6 months in leaves incorporated in the dry soils. The virus appeared to be lost after 3 months in leaves preserved in waterlogged soils. These evidences indicate that decomposition and micro-organisms might have played a role in eliminating the virus from the leaves. Also the non-detection of RYMV in the husks and to a lesser extent the spikelets seems likely that there is an internal elimination mechanism for the virus in the spikelets and the husks apart from the decomposition factor and the action of micro-organisms in the soil. However, the fact that the virus is not transmitted through seeds is an added evidence that there exists such a mechanism of inhibition or elimination.

These results provide evidence that RYMV is not seed-borne but could persist in rice leaves and empty rice spikelets or probably in the soil and later cause infection to new plants when suitable conditions are created.

CHAPTER FIVE

5. 0 SCREENING OF SOME RICE INSECTS FOR THEIR ABILITY TO TRANSMIT RICE YELLOW MOTTLE VIRUS.

5. 1. INTRODUCTION.

In Africa more than 80 species of insects have been reported to attack rice, but only 15 species are considered important (Alam *et al.*, 1984). However, because of diverse conditions under which rice is grown, pest problems also vary from one area to another (Alam and Akibo-Betts, 1984; Alam *et al.*, 1984).

Thus, in East Africa the major insect vectors of RYMV have been identified as *Chaetocnema* spp., *Trichispa sericea* and other beetles belonging to the family Chrysomelidae (Bakker, 1971, 1974, 1975). In Madagascar, the major insect vector of the virus has been identified as *Dicladispa gestroi* while *Trichispa sericea* was found in the Central High Plateau but evidence of RYMV disease was not established there (Reckhaus and Andriamasintseheno, 1995). In West Africa *T. sericea* Guerin has been reported to cause considerable damage to young rice crops in Niger, Mali (Sy, 1994) and the damage is widespread in Cote d'Ivoire. Grist and Lever (1969) reported some *Chaetocnema spp* which damaged rice in Africa. High populations of other insects such as *Locris spp.*, *Diopsis spp.*, grasshoppers, leafhoppers, aphids, plant hoppers, and coccinelids have been observed on rice fields across the West African sub-region (Akibo-Betts and Raymundo, 1980; Breniere, 1983; Alam *et al.*, 1984) where RYMV incidence is increasing (Sy, 1994). However, their population peaks were not correlated with RYMV incidence in the rice field (Heinrichs *et al.*, 1995b).

Thus, considering the differences in species of insect pests, disease patho-systems

in Africa and elsewhere and in view of the above facts, it has become necessary to determine if and which rice insects occurring in rice fields in Cote d'Ivoire, West Africa, transmit RYMV and to establish how long the *T. Sericea*, *C. pulla* and *Chnootriba similis* (*Epilachna similis*) encountered in this study could retain and transmit RYMV.

5.2. MATERIALS AND METHODS.

5.2.1. Screening of some rice insects for their ability to transmit rice yellow mottle virus.

Most of the insects were collected from rice fields with a sweep net and maintained in various entomological cages appropriate for each insect. *Trichispa sericea* was picked by hand using a camel hair paint brush and with a sweep net. The life cycle of the beetle indicated in Appendix 4, was used as a guide in rearing the insects. The eggs, larvae and nymphs were collected by cutting the entire leaves on which they were found and transported in aerated bottles to the laboratory for rearing and transmission tests. The larvae hatched within 5 days, bored into the leaves and lasted for about 8-10 days before turning into nymphs. The nymphs hatched to adult beetles in another 5 days. The newly hatched adult beetles were first placed on RYMV-infected and later on healthy rice plants for virus acquisition and transmission tests respectively. Adult beetles collected from the field at Sakassou irrigation scheme, Cote d'Ivoire where there was an outbreak of the beetles were also placed on healthy rice seedlings for virus transmission test to establish if they carried the virus from the field. Sakassou is an endemic area for RYMV in Cote d'Ivoire.

For the ability of adult *Trichispa sericea* to acquire and transmit the virus in

screening tests, the young adults reared in the entomology cages on Bouaké 189 were picked and placed on RYMV infected rice plants for 3 days for virus acquisition and thereafter 10 insects were picked with a camel hair brush and placed on each of the 20 healthy 21-day old seedlings of Bouaké 189, a susceptible check, for another 3 days for feeding transmission test. The insects were then removed, killed with insecticide and the plants were transferred to separate insect proof cages and monitored for up to 63 days (9 weeks) for development of RYMV disease symptom. These plants were also constantly checked for newly hatched beetles which could have been hatched from eggs laid by the adult beetles during the virus transmission test period. They were always removed from the test plants and killed by crushing between fingers when found.

The transmission test with *Chaetocnema pulla* was carried out with adults that were collected from the rice field in the upland environment at M'be, Cote d'Ivoire. This is because information on the biology of the insect was not available (Nacro and Heinrichs, 1996). Thus, the adult beetles were collected from the field with sweep net and aspirators and similarly treated like the adults of *T. sericea*. Longer virus acquisition period was used because the efficiency of some insects such as the beetles in acquiring and retaining the virus increases with longer feeding period (Bakker, 1974; Fulton *et al.*, 1987).

The leafhoppers were also collected from the field and reared in cages in the screenhouse. Both the adults collected from the field and the newly hatched nymphs were used in the screening tests. For the leafhoppers, 5 viruliferous insects were transferred onto healthy seedlings of Bouaké 189 for 3 days after it had acquired the

virus for 3 days. At the end of the former the plants were removed and kept in separate insect proof cages and observed for up to 63 days (9 weeks) for RYMV symptoms by visual assessment (IRRI, 1988), and ELISA of leaf samples as outlined in section 3.6.2.

Adults from other group of insects were also given 3 days of virus acquisition and 3 days of transmission access periods. Thereafter, the test plants were monitored for RYMV symptoms in insect proof cages for up to 63 days (9 weeks) as mentioned above.

In the screening test each group of the insects were subjected to 3 hours starvation (non-feeding) before being placed on virus infected plants for the virus acquisition feeding. Similarly the insects were starved for 3 hours after acquiring the virus before they were transferred on healthy plants for virus transmission tests. However, Walters (1969) reported that fasting the beetles had no effect on their ability to acquire and to transmit the viruses.

For leafhoppers and other insects tested their method of transfer from RYMV infected to healthy test plants was carried out easily by removing the infected plants and replacing them with the healthy test plants in the cages containing the viruliferous insects for virus transmission feeding tests.

The proportion of insects transferred on healthy rice plants for virus transmission tests depended on the size of each group of insect that was recovered from the infected plants after the virus-acquisition period.

The method for assessing successful transmission by each group of the insects was based on visual assessment (IRRI, 1988) and ELISA of leaf samples as outlined in section 3.7.1, although visual assessment on indicator plants like Bouaké 189 is adequate

to prove that the insect was able to transmit RYMV (Thottappilly and Rossel, 1993).

5.2.2. Detection and infectivity test with faecal droplets of grasshoppers.

The faecal droplets of the short and long-horned grasshoppers feeding on RYMV infected plants were collected and carefully sorted out from contaminants such as pieces of leaves of the plants from which the insects were feeding on. These were divided into two portions. One portion of the faecal droplets was measured and 0.2g of each lot was ground in 2ml *PBS + 2% PVP* for *ELISA* test. Antigen dilution of 1:10 and the antibody and conjugate dilution of 1:1000 were used for the ELISA test. The other portion was used to test for the infectivity of faeces of the short and long-horned grasshoppers. For the inoculation test 4g of the faeces were measured and ground in 67ml distilled water and 0.13ml of carborundum was added to the inoculum. Young seedling of Bouaké 189 were inoculated with the inoculum and maintained in insect proof screen house and monitored visually and by ELISA for symptoms of RYMV.

5.2.3. Investigation on The Mode of Transmission and Retention Period of RYMV by *Trichispa sericea* Guerin, *Chaetocnema pulla* Chapius and *Chnootriba similis* Thunberg.

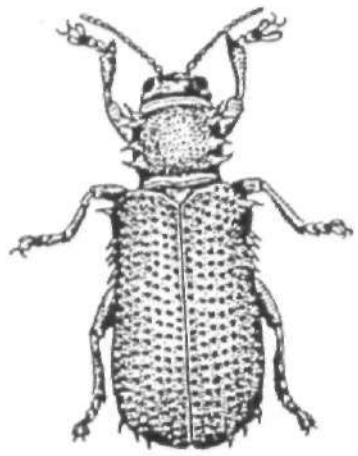
The adults of *T. sericea* used in this study were reared in the laboratory. The adults of *C. pulla* were collected directly from the rice field because their life cycle and method of rearing in the laboratory is yet to be established (Nacro and Heinrichs, 1996). The adults of *C. similis* Thunberg were also collected from the rice field. For the mode of transmission 14 day old seedlings of Bouaké 189 were placed in the cages and 10

insects that had acquired RYMV for 3 days were picked and placed on the healthy plants. Each insect was placed on a separate plant. Six separate testing time regime of 15, 30, 45, 60 minutes and 1, 2 days were employed. At the end of each testing time regime the insects were removed and killed by spraying with insecticide. Thereafter, the test plants were placed in the screenhouse and monitored for RYMV symptoms by visual assessment of leaves up to 63 days (9 weeks) after test. Symptomatic and non symptomatic plants were also tested by ELISA as outlined in section 3.7.1.

Retention period of RYMV in *T. sericea*, *C. pulla* and *C. similis* was similarly carried out with the adults as previously explained above. However, single insect that had acquired RYMV for 3 days was picked and placed on a single 14 day old healthy seedling of Bouaké 189 on the first day. At the end of day 1 and subsequent days the insect was picked using an aspirator or camel hair brush and transferred on to the next healthy test plant for the day. The daily transfer of the insects was carried out up to 10 days. The test plants were maintained and tested for RYMV infection as outlined above.

5.3. RESULTS.

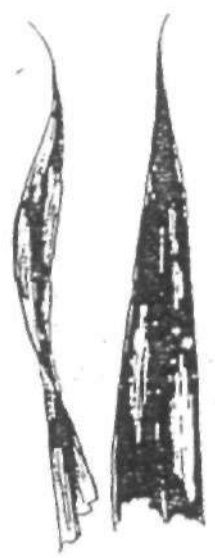
The results of the transmission test on the insects show that *Trichispa sericea* Guerin, *Chaetocnema pulla* and *Chnootriba similis* (Figure 6) transmitted RYMV (Table 19). The adults of *T. sericea* caused whitish feeding streaks on leaves of young rice plants (Plate IV) during rearing and transmission tests. Its larvae also fed on and mined into leaves and metamorphosed into nymphs and caused wilting or drying up of leaves (Plate V). The adult beetles of *C. pulla* caused similar foliar damage on rice leaves as



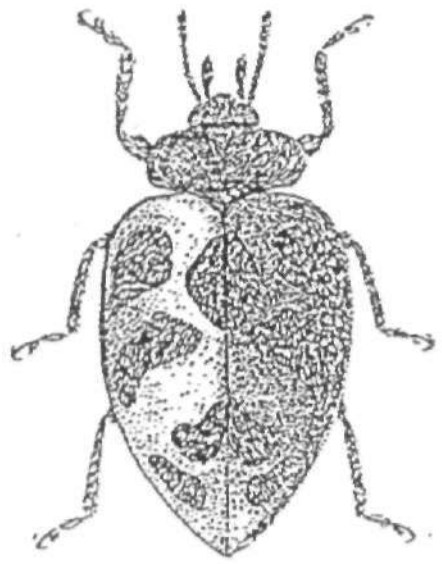
A



B



D



C

Fig. 6 . Insect vectors of Rice yellow mottle virus in Cote d'Ivoire, West Africa. **A**. *Trichispa sericea* Guerin (Hispinae), **B**. *Chaetocnema pulla* Chapius (Halticinae), **C**. *Chnootriba similis* Thunberg (*Epilachna similis* Mulsant) (Coccinelidae), **D**. Common typical leaf damage associated with the insects. Source of Diagrams: Bakker, 1974; Breniere, 1983; Booth *et al*., 1990.

Table 19. The transmission and non transmission of rice yellow mottle virus by some rice insects in screening tests

Common Name		Scientific Name & Family	Number of Insects per Plant	Number of Test Plants[c]	Source of Insects	Number of Infected Plants/ Number of Plants Tested
Hispid beetle	(i)	*Trichispa sericea*, Chrysomelidae (Hispinae)	10	15	a	5/15
			10	15	b	7/15
Flea beetles	(i)	*Chaetocnema pulla*, Chrysomelidae (Halticinae)	10	15	b	8/15
	(ii)	*Altica spp*, Chrysomelidae (Halticinae)	10	10	b	0/10
Ladybird bettle	(i)	*Chnootriba similis*, Coccinellidae	10	10	b	7/15
Leafhoppers	(i)	*Cofana unimaculata*, Cicadellidae	10	20	a	0/20
			10	20	b	0/20
	(ii)	*Cofana spectra*, Cicadellidae	5	10	a	0/10
			5	10	b	0/10
	(iii)	*Nephotettix afer*, Cicadellidae	10	20	a	0/20
			10	20	b	0/20
Spittlebugs	(i)	*Locris maculata*, Cercopidae	5	10	b	0/10
	(ii)	*Locris rubra*, Cercopidae	5	5	b	0/10
Stalked-eyed flies	(i)	*Diopsis macrophthalma*, Diopsidae	10	10	b	0/10
	(ii)	*Diopsis apicalis*, Diopsidae	10	10	b	0/10
Long-horned grasshoppers	(i)	*Conocephalus longipennis*, Tettigonidae	5	10	b	2/10*
Short-horned grasshoppers	(i)	*Oxya hyla*, Acrididae	5	10	b	0/10
Aphids	(i)	*Hysteroneura setariae*, Aphididae	10	15	b	0/15

a = Field collected insects, b = Laboratory reared insects,
c = Bouake 189, RYMV susceptible check, * = This seems as a consequence of virus contamination through the mouthparts.



Plate IV. Typical whitish feeding streaks of the adults of *Trichispa sericea* Guerin on all the rice leaves



Plate V. The typical devastating feeding damage of the adults and larvae of *Trichispa sericea* Guerin on the rice foliage in the screenhouse

do the adults of *T. sericea* and *C. similis* (Plates VI and VII). They also transmitted RYMV during this feeding process.

The adults of *T. sericea* collected from the field from Sakassou, Cote d'Ivoire on RYMV infected ratooned/volunteer rice successfully transmitted the virus to healthy rice seedlings of Bouaké 189 in the screen house.

A low level of RYMV transmission was obtained with the long-horned grasshoppers (*Conocephalus longipennis)*. No transmission was obtained with the short-horned grasshopper (*Oxya hyla* Serville). The long and short-horned grasshoppers caused considerable damage by defoliating the rice leaves in a very short period when confined in the cage. The leafhoppers, spittle bugs (*Diopsis spp*), stalk-eyed flies (*Locris spp*), and aphids (*Hysteroneura setariae*) did not transmit the virus. *Locris* spp caused yellowing of rice leaves after prolonged feeding and such plants became wilted and died. Also wilting and dying off of RYMV infected plants for which the *Locris* spp were used for virus acquisition feeding was observed. The leaves of the wilted healthy plants contained no RYMV particle when tested by ELISA. RYMV transmission was obtained with the adults of the lady-bird beetle (*C. similis*). Adults of the leaf beetle *(C. similis)* caused whitish feeding streaks on the rice leaves (Plates VI and VII) similar to those caused by the hispa (*T. sericea*) and flea (*C. pulla*) beetles.

The faeces of the long-horned grasshoppers did not infect rice seedlings of Bouaké 189. They contained no RYMV particle when tested by ELISA. RYMV particles were detected in all lots tested from the faeces of the short-horned grasshopper (*Oxya hyla*). However, when the virus was extracted in distilled water and inoculated to young

seedlings of Bouaké 189, there was no transmission with the extracts. This suggests that a mechanism is involved in inhibiting the expression of the virus.

The results on the mode of transmission and retention period of RYMV by the insects are presented in Tables 20 and 21. *T. sericea* transmitted RYMV for up to 1 day while *C. pulla* transmitted the virus consistently up to 2 days. *T. sericea* retained and transmitted RYMV for 1 day and once for 2 days. On the other hand *C. pulla* retained and transmitted RYMV for 5-6 days while *C. similis* transmitted it consecutively up to 3 days.



Plate VI *Chnootriba similis* Thunberg and its typical feeding damage (whitish streaks/chlorosis) on rice leaves



Plate VII *Chnootriba similis* Thunberg and whitish streaks/chlorosis with drying of rice leaves occasioned by its feeding habit

Table 20. Mode of transmission of RYMV by *Trichispa sericea* Guerin, *Chaetocnema pulla* Chapius and *Chnootriba similis* Thunberg (*Epilachna similis* Mulsant).

Insect Group	Test Number	Number of insects per plant[a]	Time regime/Infectivity					
			Minutes				Days	
			15'	30'	45'	60'	1	2
Trichispa sericea Guerin, Chrysomelidae (Hispinae)								
	1	1	-	+	+	+	+	-
	2	1	+	+	+	+	+	-
	3	1	+	+	+	+	+	-
Chaetocnema pulla Chapuis, Chrysomelidae (Halticinae)								
	1	1	+	+	+	+	+	+
	2	1	+	+	+	+	+	+
	3	1	+	+	+	+	+	+
Chnootriba similis Thunberg, Coccinellidae								
	1	1	+	+	+	+	+	+
	2	1	+	+	+	+	+	+
	3	1	+	+	+	+	+	+

[a] Bouaké 189, RYMV susceptible check.

Table 21. Retention period of RYMV by *Trichispa sericea* Guerin, *Chaetocnema pulla* Chapius and *Chnootriba similis* Thunberg in daily transfer test on healthy seedlings of Bouaké 189.

Insect Group	Test Number	Number of insects per plant plant[a]	Daily transfer of viruliferous insects/Infectivity[b]									
			1	2	3	4	5	6	7	8	9	1
Trichispa sericea Guerin												
	1	1	+	-	-	-	-	-	-	-	-	-
	2	1	+	+	-	-	-	-	-	-	-	-
	3	1	+	-	-	-	-	-	-	-	-	
Chaetocnema pulla Chapius												
	1	1	+	+	+	+	+	-	-	-	-	-
	2	1	+	+	+	+	-	-	-	-	-	-
	3	1	-	+	+	+	+	+	-	-	-	-
Chnootriba similis Thunberg												
	1	1	+	+	+	-	-	-	-	-	-	-
	2	1	+	+	+	-	-	-	-	-	-	-
	3	1	+	+	-	-	-	-	-	-	-	-

[a] Number of insects decreased as the days increased due to mortality. Ten plants were maintained for each daily transfer test and each insect was serialy transferred on the next plant seperately.

[b] Infectivity was determined by visual observation of typical RYMV symptoms and ELISA

\+ Positive transmission of RYMV

\- No transmision of RYMV

5.4. DISCUSSION.

The adults of *T. sericea*, *C. pulla* and *C. similis* have been proven in this study to be vectors of RYMV in Cote d'Ivoire, West Africa. The symptoms of RYMV were noticed on test plants at about 21 days after the insect feeding transmission test. These symptoms appeared first on the youngest emerging leaves. The adults of *T. sericea*, *C. pulla* and *C. similis* transmitted the virus and at the same time caused serious foliar damage to rice seedlings leading to wilting and dying off of the plants. *T. sericea* and *C. pulla* have been reported in Kenya, East Africa as vectors of RYMV (Bakker, 1971,1974 & 1975). Also *Chaetocnema spp* was suspected by Raymundo and Buddenhagen (1976) at IITA, Ibadan, Nigeria, West Africa as a potential vector of RYMV. Raymundo (1980) also suspected the transmission of RYMV by *C. pulla* in Sierra Leone. The transmission of RYMV by *C. similis* has not been reported elsewhere and it is the first time this insect has being implicated in the transmission of the virus. *C. similis* is phytophagous, but is particularly attracted by graminaceous plants especially rice, sugarcane, maize and sorghum (Breniere, 1983). These three vectors of RYMV are prevalent throughout West Africa (Breniere, 1983) other African countries (Grist and Lever, 1969) and its island of Madagascar (Bouriquet, 1946).

Although no RYMV transmission was obtained with *Locris* spp their feeding habit leading to the yellowing of leaves and eventual death of plants is of economic significance to rice production. Harris (1937) and Bakker (1974) reported similar yellowing discolouration and death of rice shoots after feeding by *Locris* spp. The long-horned grasshopper (*Conocephalus longipennis)* was demonstrated to transmit the virus in the third attempt. The consequence of RYMV transmission from the faecal droplets of *C. longipennis* is ruled out because no virus

particle was detected in them by ELISA and the extracts were not infectious to rice in mechanical transmission test. The detection of RYMV particles in the faeces of the short-horned grasshopper (*Oxya hyla*) is a strong indication that the rice seedling on which it feeds could be contaminated with the virus. However, no RYMV transmission was obtained with this insect in screening test.

The leafhoppers and other insect groups screened are also apparently non-vectors of RYMV. Bakker (1974) and Raymundo *et al.* 1979 failed to obtain RYMV transmission with these groups of insects. Infact the leafhoppers transmit their viruses in a persistent manner and none of the viruses have been reported to be transmitted by mechanical means (Nault and Amar, 1989; Matthews, 1991) and thus, they could not possibly be a vector of RYMV which is transmitted mechanically by the beetles (Bakker, 1974, 1975). Furthermore the rest of the insects screened except aphids (Harris, 1983; Alegbejo, 1986) have not been so far associated with the transmission of any virus (Matthews, 1991). The inability of other insects to transmit RYMV could be explained by the fact that viruses transmitted by beetles belong to the tymovirus, comovirus, bromovirus, and sobemovirus groups and are not transmitted by members of other arthropod groups (Walters, 1969; Matthews, 1991) except few insects with biting mouthparts (Fulton *et al.*, 1987).

T. sericea retained and transmitted RYMV consecutively for 1-2 days, and *C. similis* up to 3 days and they could be described as transmitting RYMV in a semi-persistent manner. For *C. pulla* Chapius which retained and transmitted RYMV for 6 days it could be described as transmitting the virus in a persistent manner according to classification scheme of Papkova (1989). This finding is in line with earlier report in Kenya by Bakker (1974) that *T. sericea* retained and transmitted RYMV for 1 day only while *C. pulla* retained and transmitted the virus for 8 days.

CHAPTER SIX

6.0 THE REACTION OF SOME WEEDS AND CULTIVATED CEREALS TO RICE YELLOW MOTTLE VIRUS IN SCREENING TEST AND IN NATURE.

6.1. INTRODUCTION.

Outside the genus *Oryza* some Graminaceous species particularly in the tribe Eragrostideae were found to be systemic hosts of RYMV (Bakker, 1974, 1975; Ou, 1985). Bakker (1974) wrote that the virus was probably present around lake Victoria on these grass hosts before rice cultivation was introduced and that it then spread to rice. Inoculation tests in Kenya with RYMV did not infect the forest rice *(O. eichingeri* Peter), *Eleusine spp* (L.), *Leersia hexandra*, other grass species but rice was the only host found naturally infected (Bakker, 1974). On the other hand Raymundo *et al.* (1979) reported artificial infection of the virus in *Eleusine indica* Gaertiner and *Echinochloa crus-galli* L.P. Beak at Rokupr, Sierra Leone. Bakker (1974) and Fauquet and Thouvenel (1977) found that the cotyledons could not be infected mechanically with the virus. While Bakker (1974) obtained infection of a localised nature with *E. coracana* in Kenya. Fauquet and Thouvenel (1977) did not obtain any mechanical infection with *E. coracana* and *E. indica* in Cote d'Ivoire. On the other hand, Coulibaly and N'guessan (1994) indicated infection of *E. indica* in host range studies in Cote d'Ivoire using back inoculation to a susceptible rice host. According to Okioma *et al.* (1983) in East Africa and Awoderu (1991a) in West Africa, the grasses (*Dinebra retroflexa, Eleusine indica, Eragrostis tenifolia, Phleum arenarium)* and wild *Oryza* species which occur abundantly around rice fields are likely important reservoirs of the virus during the off-season. RYMV was also found in *Oryza longistaminata*, a wild species of rice growing in marshy areas and in rice fields in Niger, Mali (John *et al.*, 1984), Senegal (Mbodj *et al.*, 1984), and Burkina Faso (Konate, 1995). Sy (1994) reported that RYMV-like symptoms were observed visually on *Oryza longistaminata, O.*

glaberrima and *Imperata spp* in the lowland in Mali. However, in spite of intensive searching in Kenya and Nigeria for natural weed hosts of the virus no evidence for the natural occurrence of RYMV neither in indigenous *Oryza* species nor in other wild grasses has been obtained not even in the vicinity of heavily infected rice crops on farmers' fields (Bakker, 1974; Rossel, 1986). RYMV is transmitted mechanically from rice to rice (Bakker, 1974; Fauquet and Thouvenel, 1977; Thottappilly and Rossel, 1993) but there is no concrete information on cross-infectivity tests from rice to weeds and vice versa.

Dale (1994) reported that both larvae and adults of *T. sericea* Guerin and other hispinae fed on rice leaves and those of *Chloris virgata, Echinochloa holubii, Eragrostis aethiopica, E. heteromera, Digitaria zeyleri* and *Diplache fusca*. Bakker (1974) caught *T. sericea* Guerin on rice only, however, *Sesselia pussila* was caught on ratooned rice, *Cyperus* spp., *Leersia hexandra, Panicum repens,* other sedges and grasses. He caught *Chaetocnema* spp on ratooned rice, *E. Colona, Scirpus confuses, Dinebra retroflexa* in Kenya. Hargreaves (1937) listed *Arachis* spp., maize, millet, rice, and ginger as hosts of *C. pulla (zeae). T. sericea* was found to feed on rice and *Eragrostis aethiopica*, the systemic hosts of RYMV (Bakker, 1974) in Swaziland (MOD, 1970).

Thus, the objectives of this study are to establish infection link between some weeds and rice through sensitive serological tests (e.g ELISA) and back-inoculation of expressed virus sap to a susceptible check, Bouaké 189. And to demonstrate if RYMV could be transmitted to *L. hexandra* by direct feeding of viruliferous *T. sericea*. The study will also seek to identify plant species that are infected in nature by RYMV and obtain preliminary evidence as to how the virus subsists in nature.

6.2. MATERIALS AND METHODS.

6.2.1. Screening of some weeds and cultivated cereals for susceptibility to RYMV.

Seeds of some weed species were obtained from the Weed Science unit while rice varieties and accessions were collected from the Plant Breeding and Plant Pathology units of WARDA. Seeds of some cultivated cereals were purchased directly from the local market. Some seedlings were raised from splits and rhizomes. For both the seedlings raised by seeds, splits or rhizome inoculation with RYMV crude sap was carried out at 14 days after germination or establishment. The virus sap was expressed from leaves of Bouaké 189 artificially infected with RYMV. About 60g of the infected leaves were ground in one litre of distilled water (6%w/v). Prior to inoculation of the test materials, carborundum was added to the crude sap to aid penetration of the virus into the leaf tissues. For each test sample, 20 seedlings were inoculated with the virus crude sap using fingers moistened with the inoculum. For each plant species, 5 seedlings were left uninoculated to serve as a check in the assessment of true symptom manifestation of RYMV on the inoculated plants. The test plants were maintained in an insect proof screenhouse for 45 days and visual assessment of RYMV symptom was carried out using SES (IRRI, 1988) procedures and ELISA test as outlined in section 3.7.1. For ELISA test of weed samples the best working dilution of 1:10 for antigen extraction and 1:1000 for the antibody and conjugate coatings was used. Each sample was replicated in two wells of ELISA. As for the ELISA test proper, systemic sampling of leaves were carried out by harvesting the newly formed topmost leaves and the lowermost leaves on the plants separately. These were tested separately in ELISA as outlined above. As for back-inoculation of the expressed saps from the test plants to Bouaké 189, the whole leaves on the plant were pooled and ground in distilled water (6%w/v) and 2ml of carborundum were added to the sap before the back-inoculation using fingers moistened with the inoculum. When the expressed saps from the pooled leaves of the

plant species (e.g *O. longistaminata*, *E. crus-pavonis*, *E. pyramidalis*, *Eragrostis ciliaris*) did not infect all the seedlings of the test plants in back-inoculation the subsequent samples from the topmost and the lowermost leaves were inoculated separately. The symptoms of RYMV were also monitored at 45 days after inoculation by using the SES procedure as indicated above. ELISA test was performed on both the symptomatic and non-symptomatic indicator plants.

6.2.2. Investigation on the Transmission of RYMV to *Leersia hexandra* Sw by *Trichispa sericea* Guerin in Direct Feeding Test.

Some young seedlings of *L. hexandra* Sw were collected from the lowland area and transplanted in plastic buckets. At 14 days after transplanting viruliferous adults of *T. sericea* Guerin that were allowed 3 days virus acquisition feeding on RYMV infected leaves of Bouaké 189 were picked and placed on the test plants. They were allowed to feed on the leaves of the *L. hexandra* for another 3 days after which they were removed and killed using insecticidal spray. Twenty seedlings of the test plants were maintained and 5 insects were transferred on to each seedling for each test period and the test was repeated 3 times. At 45 days after virus inoculation by direct feeding of the insects in each test period the leaves from each plant were sampled and analysed by ELISA as outlined in section 3.7.1. The leaves were pooled from each plant and ground in distilled water (6%w/v) for back-inoculation to Bouaké 189. The same inoculum was used to inoculate a susceptible host, BG 90-2, and Bouaké 189 to confirm the infectivity of the inoculum. Prior to inoculation 2ml of carborundum were added to the expressed saps to facilitate the penetration of RYMV particles into the leaves.

6.2.3. Field Surveys.

Monthly surveys were carried out on WARDA rice farms at M'be and at the Irrigation rice scheme, Sakkasou, Cote d'Ivoire from July, 1996 to July, 1997. At M'be the surveys and sampling for the insects and weed species were carried out between July, 1996 to April, 1997.

When the outbreaks of *C. pulla* was noticed in the third week of February, 1997 in the upland rice fields at Mbe belonging to the breeding unit, young seedling bait plants of Bouaké 189 were placed round the fields at intervals of 14 days for a period of 2 months (March to April) to establish if the insects were viruliferous. At the end of the 14 days the plants were removed to the screen house and observed for RYMV infection. At the same time at M'be leaves of *Eleusine indica, Echinochloa colona, E. Crus-pavonis, E. pyramidalis, Pennisetum purpereum, Imperata cylindrica* and rice with or without suspected symptoms of RYMV were sampled once a month on the bunds, edges of rice fields and within the rice fields and tested by ELISA as outlined in section 3.7.1 for RYMV infection.

At Sakkassou, the surveys were carried out from January to July, 1997 using random sampling. Here, various weed species and rice with or without symptoms of RYMV were sampled once a month on the bunds, edges of rice fields, within the fields and in the vicinity of RYMV infected rice plants. The plant samples were similarly tested by ELISA as outlined in section 3.7.1 to detect RYMV infection.

6.3. RESULTS.

The results on the reaction of some weeds and cultivated cereals to RYMV are presented in Tables 22 and 23. RYMV infection and particles were observed and detected in both the topmost and lowermost leaves of *Oryza sativa* (Bouaké 189, BG 90-2, Moroberekan), *O.*

barthii, and *O. glaberrima* (CG 14) both visually and by ELISA. The expressed saps from these plants were highly infectious to all the seedlings inoculated in back-inoculation to the indicator host. On the other hand the RYMV was detected only in the lowermost and the inoculated leaves of *O. longistaminata*, *Echinochloa crus-pavonis*, *E. pyramidalis* and *Eragrostis ciliaris* by ELISA. It was also found that only the oldest and close to dry leaves from the lowermost part of the plants were infectious to the indicator plants. The typical symptoms of RYMV were not observed on these plants. No transmission test was possible with *Eleusine indica*, *Echinocloa colona*, *Leersia hexandra*, *Imperata cylindrica*, TOG 5681 (*O. glaberrima*), maize (*Zea mays*), millet (*Pennisetum* spp), wheat (*Triticum aestivum*), Sorghum (*Sorghum bicolor*), the dicotyledons as well as a host of other weed species screened.

Echinocloa colona and *E. cruspavonis* exhibited no obvious symptoms when inoculated with the virus or when grown within the vicinity of RYMV infected plants. No virus particle was detected in the leaves and roots that have come in contact with the infected plants as demonstrated in Plates VIII and IX. Also no transmission test was successful with *Chenopodium amaranticolor*, *Nicotiana benthamiana*, *N. tabaccum* widely used as indicator plants for many other plant viruses.

The results presented in Table 24 indicate that *T. sericea* did not transmit RYMV to *L. hexandra* Sw by direct feeding. No seedling became infected for the 3 test periods. The expressed saps from the mechanically inoculated leaves of *L. hexandra* were not infectious to Bouaké 189 in back-inoculation indicating that they harboured no RYMV particle. On the other hand the seedlings of BG 90-2 inoculated with the same sap that was used to inoculate *L. hexandra* became infected and the leaves were also infectious to Bouaké 189 in back-inoculation.

No RYMV infection was detected in the weeds except rice samples collected at M'be during the study period. Outbreaks of *C. pulla* were recorded in July, 1996 and February, 1997 in the upland rice fields. The *C. pulla* was noticed to cause whitish feeding streaks on the attacked rice seedlings and those leaves wilted. Similarly an outbreak of *T. sericea* Guerin was observed on rice (mostly *O. glaberrima* varieties) and *L. hexandra* in the lowland environment (Continuum field) in June, 1997.

The result of ELISA test on rice and weed samples collected from Sakassou showed that RYMV infection was detected in almost all the rice samples collected and throughout the period of sampling (Table 25). The virus was detected by ELISA in *E. crus-pavonis* in June and July only during the sampling period and only from the field previously attacked by *T. sericea*. An outbreak of *T. sericea* was noticed on *L. hexandra* (Plate X) and volunteer/ratooned rice (Plate XI) at Sakassou, Cote d'Ivoire. This caused wilting, drying up and eventually, death of some plants attacked (Plates X and XI).

Table 22. Sensitivity status of some weeds and wild rice species to rice yellow mottle virus in mechanical inoculation test.

Plant Species	Family / Habitat	Mode of Raising Test Plants[b]	Typical RYMV Symptom on Leaves of Test Plants	Mode of Test/Reaction[a]	
				ACP-ELISA	Back-Inoculation[a]
Grasses:					
Oryza sativa L. (Bouaké 189)	Graminae, Lowland	Seeds	Stunting, yellow, mottle	+ + +	+ + +
Oryza barthii A. Chev et Roehr (Guinea accession)	Graminae, Lowland	Seeds	Yellow, mottle	+ + +	+ + +
Oryza barthii A. Chev et Roehr (Senegal accession)	Graminae, Lowland	Seeds	Yellow, mottle	+ + +	+ + +
Oryza longistaminata A. Chev et Roehr (Senegal accession)	Graminae, Lowland, Swamps, Hyrdromorphic, Drains	Seeds/ Rhizome	None	±	±
Oryza longistaminata A. Chev et Roehr (Foro Foro-Cote d'Ivoire accession)	Graminae, Lowland Swamps, Hyrdromorphic, Drains	Rhizome	None	±	±
Leersia hexandra Sw.	Graminae, Lowland, Hyrdromorphic, Drains	Seedlings	None	-	-
Echinochloa colona Link	Graminae, Moist Upland, Hydromorphic	Seeds / Seedlings	None, but yellowing of leaves is common	-	-
Echinochloa crus-pavonis Schultes	Graminae, Lowland	Seeds / Seedlings	None	+	+
Echinochloa pyramidalis (Lam.) Hitch. & Chase	Graminae, Lowland	Seeds / Seedlings	None	+	+
Rottboellia cochinchinensis (L.) Clayton	Poaceae, Upland, Hydromorphic	Seeds / Seedlings	None	-	-
Acroceras zizanoides (Kunth) Danddy	Graminae, Drainage, Ditches	Seedlings	None	-	-
Eragrostis ciliaris	Graminae, Upland	Seeds	None	±	±

Digitaria horizontalis Willd.	Graminae, Upland	Seeds	None	-	-
Pennisetum purpureum Schum.	Graminae, Upland, Hydromorphic,	Seedlings	None	-	-
Eleusine indica (L.) Gaertner	Graminae, Upland, Hdromorphic	Seedlings	None	-	-
Imperata cylindrica (L.)	Graminae, Upland,	Seedlings/ Rhizome	None	-	-
Leptochloa caerulescens Steud.	Graminae, Lowland, Hydromorphic	Seedlings	None	-	-
Sedges:					
Cyperus difformis L.	Cyperaceae, Lowland	Seeds / Seedlings	None	-	-
Fimbrystilis littoralis Gaud.	Cyperaceae, Lowland	Seeds	None	-	-
Broad Leaves:					
Ludwigia abyssinica A. Rich.	Onagraceae, Lowland	Seedlings	None	-	-
Sphenoclea zeylanica Gaert.	Sphenochleaceae, Lowland	Seeds / Seedlings	None	-	-
Euphorbia heterophyilla L.	Euphorbiaceae, Upland	Seeds	None	-	-
Ageratum conyzoides Linn.	Asteraceae, Upland	Seedlings	None	-	-
Eclipta prostrata L.	Compositae, Lowland, Hdromorphic, Drains, Widespread	Seedlings	None	-	-
Nicotiana tabaccum L.	Solanaceae, Upland	Seeds	None	-	-
Nicotiana benthamania L.	Solanaceae, Upland	seeds	None	-	-
Chenopodium amaranticolor Coste et Rayn	Chenopdiaceae, Upland	Seeds	None	-	-

[a] Back-inoculation was carried out on healthy young sedlings of Bouaké 189, RYMV susceptible check

[b] Visual assessment for symptoms was based on SES (IRRI, 1988), - Negative (No virus particle detected by ELISA).

+++ Very high virus content in ELISA. All the inoculated plants in the back-inoculation became infected from the expresssed sap.

\+ Low virus content. Not all the inoculated plants in the back-inoculation became infected from the expressed sap

Very low virus content. Irregular recovery of the virus even from the older leaves previously inoculated with RYMV

Table 23. Sensitivity status of some cultivated cereals to rice yellow mottle virus in mechanical inoculation test.

Scientific Name/Cultivar & Family	Typical RYMV Symptoms on Leaves of Test Plants[b]	Mode of Test/Reaction	
		ACP-ELISA	Back-Inoculation[a]
Graminae:			
Oryza sativa L:			
(i) Bouaké 189	Stunting, yellow mottle of leaves	+ + +	+ + +
(ii) BG90-2	Stunting, yellow mottle of leaves	+ + +	+ + +
(iii) Moroberekan	Green & yellow with orangre patches/ Sparse dots on leaves	+ +	+ + +
Oryza glaberrima Steud:			
(i) CG 14	Yellow, mottle	+ + +	+ + +
(ii) TOG 5681	None	-	-
Sorghum bicolor (L.) Moench [(Red) Bouake local)]	None	-	-
Sorghum bicolor (L.) Moench [(White) Bouake local]	None	-	-
Zea mays L:			
(i) EV 8728SR	None	-	-
(ii) DRT.COMP.EW	None	-	-
Triticum aestivum L. (Zaria local)	None	-	-
Pennisetum typhii L. (Amakama local)	None	-	-

[a] Back-inoculation was carried out on healthy young sedlings of Bouaké 189, RYMV susceptible check

[b] Visual assessment for symptoms was based on SES (IRRI, 1988)

+ + + Very high virus content in ELISA. All the inoculated plants in the back-inoculation became infected from th expresssed sap.

+ + High virus content in ELISA

- Negative

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Table 24. Non-susceptibility reaction of *Leersia hexandra* Sw. to RYMV by direct feeding of viruliferous *Trichispa sericea* Guerin and mechanical inoculation.

Treatments	Test Number	Mode of Test / Reaction	
		ACP-ELISA	Back-inoculation
Direct feeding by viruliferous *Trichispa sericea* Guerin on *Leersia hexandra* Sw.			
	1	-	-
	2	-	-
	3	-	-
Mechaical inoculation of *Leersia hexandra* Sw. with expressed sap from RYMV infected rice leaves			
	1	-	-
	2	-	-
	3	-	-
Indicators (Checks)	Bouaké 189	+	+
	BG 90-2	+	+

- The same inoculum used to inoculate *L. hexandra* Sw was used for Bouaké 189 and BG 90-2.

\+ Positive transmission

\- Negative transmission



Plate VIII. *Echinochloa colona* Link grown in the same spot with RYMV infected rice plant for infectivity test



Plate IX. *Echinochloa crus-pavonis* schultes growing in nature in the same vicinity and in close contact with RYMV infected rice plants (yellow symptoms)

Table 25. Result of Enzyme-linked immunosorbent assay (ELISA) test on weeds and rice samples collected from irrigated rice field endemic to RYMV at Sakkassou, Cote d'Ivoire.

Plant Species and Family	Symptoms Observed on the Plants at Sampling*	Monthly ELISA Test Results of Leaf Samples						
		Jan.	Feb.	March	April	May	June	July
Graminae:								
Oryza sativa L.*	Yellow, mottle	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Echinochloa colona Link	Yellowing of some leaves	-	-	-	-	-	-	-
Echinochloa crus-pavonis Schultes	Yellowing of some leaves	-	-	-	-	-	+	+
Echinochloa pyramidalis Hitche & Chase	Mild mosaic of some leaves	-	-	-	-	-	-	-
Eleusine indica (L.) Gaertner	Mostly green interpersed with yellow leaves	-	-	-	-	-	-	-
Leersia hexandra Sw.	Green and spotted leaves	-	-	±	-	-	-	-
Imperarta cylindrica (L.) Raeuschel	Green and yellow mosaic leaves with streaks	-	-	-	-	-	-	-
Leptochloa caerulescens Steudel	Green	-	-	-	-	-	-	-
Panicum laxum Sw.	Green	-	-	-	-	-	-	-
Pennisetum purpureum Schm.	Green and yellow leaves	-	-	-	-	-	-	-
Rottboellia cochinchinensis (Lour.) Clayton	Green and light yellow mosaic leaves	-	-	-	-	-	-	-
Sorghum arundinaceum (Desv.) Stapf.	Light green leaves	-	-	-	-	-	-	-
Onagraceae:								
Ludwigia abyssinica A. Rich.	Green	-	-	-	-	-	-	-

Malvaceae:								
Sida urens L.	Green	-	-	-	-	-	-	-
Sphenocleaceae:								
Sphenoclea zeylanica Gaertner	Green	-	-	-	-	-	-	-
Cyperaceae:								
Cyperus difformis L.	Light green	-	-	-	-	-	-	-
Fimbrisytilis littoralis Gaud.	Green	-	-	-	-	-	-	-
Compositae:								
Ageratum conyzoides L.	Green	-	-	-	-	-	-	-
Eclipta prostrata L.	Green	-	-	-	-	-	-	-
Solanaceae:								
Physalis angulata Linn.	Green	-	-	-	-	-	-	-

[a] Assessment was based on leaf colour

[b] Lowland indica varieties widely cultivated at the irrigated rice field at Sakassou, Cote d'Ivoire (e.g Bouaké 189)

+ + Very high virus content detected by ELISA

+ Low and irregular virus content detected by ELISA

± Low virus particles were detected from the wilted leaves harvested from plants attacked by the adults and larvae of *T. Sericea* Guerin

- Negative (No virus particle detected)



Plate X. Fallow rice field populated with *Leersia hexandra* Sw (*Oryza* Tribe) attacked by the adults and larvae of *Trichispa sericea* Guerin at Sakassou, Cote d'Ivoire



Plate XI. Volunteer/regrowth rice field attacked by *Trichispa sericea* Guerin at Sakassou, Cote d'Ivoire

6.4. DISCUSSION.

In this work, systemic infection of *Oryza sativa, O. barthii,* and *O. glaberrima* by RYMV has been established. Infection of a localised nature was obtained with *O. longistaminata, E. crus-pavonis, E. pyramidalis,* and *Eragrostis ciliaris*. No RYMV infection was possible with *Eleusine indica, E. colona, Imperata cylindrica* contrary to other reports that they are susceptible to the virus (Okioma *et al.*, 1983; Awoderu, 1991a). However, since rice varieties also exhibited different levels of susceptibility or tolerance to the virus it is likely that these weed species reacted differently to the virus and that some plant species could be tolerant while the other could be completely immune (Bakker, 1974; Rossel, 1986; Thottappilly and Rossel, 1993). This is probably because it is known that in some plant hosts capsid protein of a virus may be synthesised but nucleic acid replication is inhibited or nucleic acid is replicated but no or little capsid protein is synthesised (Hunger and Sherwood, 1985). For example in the *O. glaberrima* varieties, CG 14 is highly susceptible to the isolate while TOG 5681 is not. It is also possible that quite closely related strains of a virus may differ in the host range of plants they will infect (Matthews, 1991; Fauquet *et al.*, 1995).

Closer inspection and ELISA test with non artificially inoculated leaf samples from *Eleusine indica, E. Colona, E. Crus-pavonis*, and *E. pyrimadilis* that appeared yellow in pot experiment or in nature in the field did not prove that such yellowness is associated with RYMV infection. More so none of these weeds was found as a systemic host in this study. Furthermore, ELISA test on leaves and roots of *E. Crus-pavonis* that have germinated on RYMV infected rice stubble and within the vicinity of the virus infected plants did not detect any virus particle or infection. Bakker (1974) and Rossel (1986) could not find any RYMV infection in nature on

weeds except rice even with those samples collected within the vicinity of infected rice plants. The result confirms the earlier screening result in Kenya (Bakker, 1974) that *L. hexandra* Sw is apparently a non host of RYMV. There were no infections either through the direct feeding of *T. sericea* or by artificial inoculation with the virus sap.

The consistent detection of RYMV in rice samples is an indication that the virus subsists most on rice (Bakker, 1974; John *et al.*, 1986; Rossel, 1986). Bakker (1971) observed fast spread of RYMV at the Ahero pilot scheme in Kenya where high populations of *C. pulla* were found. A similar incidence was also observed by the author at the WARDA upland research farms between August and October, 1996 cropping season in which a lowland RYMV susceptible check variety, Bouaké 189, grown under irrigation was severely attacked by RYMV. A survey of the rice and maize fields within the vicinity revealed large numbers of *C. pulla* in the fields. Also some *C. similis* were seen in the rice fields. *T. sericea* the other vector of RYMV was not found in the upland environment where the RYMV attack took place. When there was an outbreak of *C. pulla* in 1997 accompanied by foliar damage to rice in the WARDA's irrigated upland fields the RYMV virus was not detected in the samples collected from the varieties attacked. The reason for this was in part due to the fact that all the varieties in the field were of the upland japonica type which have high level of resistance and immunity to RYMV. On the other hand some of the bait plants placed round the fields were infected and developed RYMV symptoms in the screen house. This indicates that *C. pulla* and/or *C. similis* which were encountered in the field might have been responsible for the transmission of the virus. However, other chrysomelidae that share the same ecology with *C. pulla* but were unnoticeable could not be ruled out as been responsible for the transmission of RYMV.

CHAPTER SEVEN

7.0 SCREENING OF SOIL NEMATODES FOR THEIR ABILITY TO TRANSMIT RICE YELLOW MOTTLE SOBEMOVIRUS.

7.1. INTRODUCTION.

Out of 2600 nominal species of plant parasitic nematodes only 30 species are known to transmit viruses (Weischer, 1993). These belong to the families Longidoridae and Trichodoridae in the order Dorylaimida. Longidoridae belongs to the sub-order Dorylaimida while Trichodoridae belongs to the sub-order Diptherophorina. Although viruses can be transmitted experimentally by nematodes, a biologically unequivocal role of nematodes as virus vectors has been ascertained only for nepoviruses by longidorids and tobraviruses by trichodorids (Weischer, 1993). It has been speculated however, that migratory endoparasite nematodes may be able to pass virus particles to feeding sites through contaminated mouthparts (Matthews, 1991; Weischer, 1993).

It is a common procedure to plant virus susceptible indicator plants into soils containing nematodes suspected as vectors of the virus. Should the indicator plant develop disease symptoms and the virus isolated and identified, an indication of transmission by nematodes is obtained (Hussey & McGuire, 1987).

Trudgill *et al.* (1983) developed a test system that satisfies all requirements for proof of nepovirus transmission by longidorid nematodes using well defined virus free nematodes placed around source plant roots infected with a virus. For trichodorid nematodes and tobraviruses, Brown *et al.* (1989) developed a special testing system. The technique involves in testing for the ability of soil nematodes to transmit plant viruses has been used by various authors (Raski and Hewitt, 1963).

The objective of this study is to establish if common nematodes in lowland rice environment are involved in the transmission of RYMV.

7.2. MATERIALS AND METHODS.

RYMV infected rice plants and the soil around them were collected from 5 geographic locations in West Africa: 3 in Cote d'Ivoire (Mbe, Sakassou, Gagnoa), and 2 in Mali (Niono, Selingue). The rhizospheric soil and plant samples were either scooped by hands or dug and collected with a hand trowel or hoe. These were placed in plastic polythene bags and transported to the screen house and laboratory in moist conditions for nematode extraction from soil and roots, identification, and virus transmission tests. The criterion for sampling was based on the identification of classical cases of RYMV in rice plants in the fields. In each field samples were taken from at least 5 spots where infected plants were sited. Sampling was carried out twice (1995 and 1996) in sites from Cote d'Ivoire and once (1996) in sites from Mali. All sites were sampled at flowering stage in August of each year. Nematodes were recovered from 5g fresh weight roots using a modified Baerman filter technique (Hooper, 1986). For the wet soil, nematodes were extracted using a modified flotation and sieving method (Hooper, 1990). Soil samples were dispersed in approximately 12L of water in a bucket. After a settling time of one minute the water was decanted through nested 90 μm and 53 μm mesh sieves. The process was repeated and nematodes washed from the sieves were extracted using a modified Baerman filter technique (Hooper, 1986). All samples were examined after extraction for a period of 48 hours and counts and identification of nematode genera were made using a Leica Wild M3C stereo microscope.

The rhizospheric soils collected were pooled for each site and dispensed in pots. Five healthy young seedling of Bouaké 189 known to be highly susceptible to RYMV were transplanted in each pot and maintained in an insect proof screen house in the lowland environment at Mbe, Cote d'Ivoire. A total of 50 plants were tested for each site.

Further experiments were conducted to verify the status of two nematode *Hirschmaniella oryzae* (endoparasitic and encountered in all rice ecologies where RYMV is

prevalent), *Xiphinema* spp and *Paratrichodorus* spp (ectoparsitic and confirmed as virus vectors). These nematodes were collected from the fields within and around WARDA research farms. The nematodes were extracted and identified as previously described above. The nematodes were individually picked using a fine eyelash hair pin and placed directly near the roots of RYMV infected plants for virus acquisition for 7 days. Thereafter the nematodes were re-extracted, counted and divided into groups. For *Hirschmaniella oryzae*, 40 viruliferous nematodes were inoculated on each of the 21 day old seedlings of Bouaké 189, raised in 100ml plastic pots. For *Xiphinema* spp, 10 viruliferous nematodes were placed on to a single seedling in the similar 100ml plastic pots. In order to compensate for the low recovery rate of *Xiphinema spp* in pure cultures (Raski and Hewitt, 1963) more soil samples were collected around infected plants from a field (B2N, Plot 17-18) at WARDA Research farms at Mbe, Cote d'Ivoire where *Xiphinema* spp was found. *Xiphinema* spp that were extracted from the root region were incorporated in sterile soil dispensed in the small plastic pots (100ml) and seedlings of Bouaké 189 were planted in them and monitored for RYMV infection.

In each case equal number of healthy plants transplanted in sterilised soil were maintained as controls. The test plants were monitored for foliar symptoms of RYMV by visual assessment based on standard evaluation system on rice (IRRI, 1988), and ELISA (Clark & Adams, 1977) at 63 DAT. The ELISA protocol used was the same as in section 3.7.1. except that antibody coating was 1:10000 and conjugate was 1:1000 for leaf samples.

7.3. RESULTS.

The results are presented in Tables 26 to 28. The visual rating and ELISA results indicated that only 1 plant out of 200 seedlings planted and screened in the soils collected from different locations in Cote d'Ivoire and Mali became infected with the virus. No seedling became infected in the soil containing the virus transmitting nematodes, and in the control plants transplanted in the sterilised soil.

Table 26. Reaction of Bouaké 189[a] seedlings transplanted in soil containing nematodes collected around RYMV infected Rice Plants

Location	Ecology	Species of Nematodes Within each Soil Sample	No. of Infected Plants out of 25[c]	
			1995	1996
Rice garden I (B2 plot No. 24), WARDA research farm, Mbe, Cote d'Ivoire	Lowland, irrigated	i	None	None
P6, WARDA research farm, Mbe, Cote d'Ivoire	Upland, irrigated	ii, x, xi	None	None
B2N, plot No. 17-18, WARDA research farm, Mbe, Cote d'Ivoire	Lowland, rainfed/irrigated	ii, iii[b], iv, v, vi[b], vii, viii, ix, x, xi	None	None
Farmers' field, Sakassou, Cote, d'Ivoire	Lowland, irrigated	i, ii, v, ix	None	None
Farmers' field, Bariyo, Gagnoa, Cote d'Ivoire	Lowland, rainfed	i, v	1*	None
Farmers' field, Mahibou II, Gagnoa, Cote d'Ivoire	Lowland, rainfed	i, v	None	None
Farmers' field, Garayo, Gagnoa, Cote d'Ivoire	Lowland, rainfed	i, v	None	None
Km10, Niono, Mali	Sahel, irrigated	i#	None	-
Km36, Niono, Mali	Sahel, irrigated	i#	None	-
Farmers' field, Selingué, Mali	Sahel, irrigated	i#	None	

i	*Hirschmaniella* oryzae	ii	*Helicotylenchus* spp.,	iii	*Xiphinema* spp+
iv	*Macroposthonia* spp.	v	*Tylenchorynchus* spp.	vi	*Paratrichodorus* s
vii	*Meloidogyne* spp.	viii	*Scutellonema brachyurus*	ix	*Heterodera oryza*
x	*Pratylenchus* spp.	xi	*Criconemoides* spp.		

* RYMV susceptible check variety and Visual rating (IRRI ,1988) and ELISA were used to assess for the virus infection

[b] Virus transmitting nematodes

[c] Equal number of healthy plants were transplanted in sterilized soil and maintained as controls

\- No Data

* Infection was suspected as a consequence of virus contamination from infected debris that could h remained in the soil

\+ The species contains a mixture of *Xiphinema* cf. *neobasiri*, *X. fatikae*, and *X. savanicola* (Ref. Sawadogo, A., Nematologist. In: Memo to Dr A.A. Sy, WARDA/ADRAO).

\# The species also contained a mixture of *H. spinicaudata* in this test

Table 27. Reaction of Bouaké 189[a] to viruliferous nematodes previously raised in pure culture on RYMV infected rice plants.

Nematode Groups	Inoculation Population Per Plant	No. Of Test Plants[a]	Total Populations Of Nematodes	No. Of Infe Plants/No. o Plants Teste
Hirschmaniella oryzae	40	30	1200	0/30
Xiphinema spp[b#]	2	5	10	0/5

[a] RYMV susceptible check

[b] Recovery of this nematode species on infected plants was very low

[#] Likely species: *Xiphinema* cf. *neobasiri* (Ref. Sowadogo, A., Nematologist, In: Memo to Dr A.A. Sy, WARDA/ ADRAO)

Table 28. Reaction of Bouaké 189[a] to nematodes extracted from the root zones of RYMV infected rice plants[a].

Nematode Groups	Inoculation Population per plant	Test Number	Total Populations of Nematodes	Number of Test Plants[a]	Number of Infected Plants/Total Number of Plants Tested
Hirschmaniella oryzae	40	1	640	16	0/17
	40	2	680	17	0/16
	40	3	600	15	0/15
Xiphinema spp[#]	5	1	20	4	0/4
	4	2	20	5	0/5
Paratrichodorus spp	6	1	18	3	0/3
	4	2	8	2	0/2

[a]RYMV susceptible check

[#]Likely species: *Xiphinema cf. neobasiri* (Ref. Sawadogo, A., Nematologist, In: Memo to Dr A.A. Sy WARDA/ ADRAO)

7.4. DISCUSSION.

RYMV was not transmitted by the nematodes encountered within the soil samples used in this study. The fact that no single infection occurred in the soil containing nematode transmitting viruses (*Xiphinema* spp; and *Paratrichodorous* spp.) indicates that possible contamination of the seedling might have taken place. Further more none of the control plants in sterilised soil were contaminated with the virus. Also Bakker (1974) and Raymundo *et al.* (1979) could not obtain any substantive evidence for the transmission of RYMV by nematodes from soils collected from infested rice fields in previous studies. The non-transmissibility of RYMV by these nematodes may be explained by the fact that this virus does not belong to the group of nematode transmitting viruses, nepo and tobra viruses (Harrison *et al.*, 1971; Trudgill *et al.*, 1983; Ploeg *et al.*, 1989). Although *Xiphinema* and *Longidorous* spp transmit nepoviruses, while trichodorous and paratrichodorous spp transmit tobraviruses (Lamberti *et al.*, 1975), the other nematode listed in this study are not virus vectors (Harrison et., 1971; Trudgill *et al.*, 1983; Hussey & McGuire, 1987; Ploeg *et al.*, 1979; Weischer, 1993).

Further screening of *Hirschmaniella oryzae*, *Xiphinema* spp., and *Paratrichodorus* spp collected from the RYMV infested fields from the roots/root zones of rice and raised in pure culture on RYMV infected plants also failed to demonstrate that they are capable of transmitting RYMV. Thus, none of these nematode transmitting viruses listed in Tables 27 and 28 transmitted RYMV in this study. It is most likely that the recognition mechanism needed for the release of this virus by the nematodes is inhibited (McGuire and Douthit, 1978). For excample the sobemoviruses such as RYMV have sperical monopartite genome whereas the tobraviruses have rod-shaped and nepoviruses have sperical bipartite genomes respectively although all are positive sense RNA viruses (Bakker, 1974; Robinson, 1991).

CHAPTER EIGHT

8.0 SUMMARY AND CONCLUSIONS.

The seed analysis by ELISA detected RYMV in the husk / hull (testa) of rice seeds. No transmission of RYMV was obtained with the virus infected rice seeds as do seeds of other plants for which viruses were detected in their husks (Owusu, 1968; Laviolette and Athow, 1971; Gunnery and Datta, 1987). Although RYMV was not transmitted through seeds in this study and other studies (Bakker, 1974; Fauquet and Thouvenel, 1977; IITA, 1985), this provides evidences of infections through virus containing leaf debris, empty rice spikelets and surface contaminants of the virus on rice seeds. The virus could be transported in rice sacs containing contaminated seeds and partially winnowed rice seeds to new areas far from its origin and it could be spread there. RYMV has been demonstrated in this study to survive for many months in the leaf debris, empty rice spikelets, and in the husks of rice seeds thus, making these plant organs potential sources of virus contamination when favourable conditions are available. Although no RYMV transmission was demonstrated with the extract from the husks it is not sufficient to conclude that some contamination could not take place in other circumstances.

RYMV was also proved in this study to be transmitted when rice roots were intertwined, leaf contact in closely spaced plants, contaminated hands and leaf stubbles incorporated into the soil. This result indicates that RYMV is stable and highly infectious (Bakker, 1974; Fauquet and Thouvenel, 1977). It has also being demonstrated that trimming of rice roots before transplanting and ratooning have a high risk of increasing infection of RYMV thereby serving as sources for further spread of the disease in the field.

This work has established the ability of some rice insects to transmit RYMV. Out of the three Chrysomelid beetles (*T. sericea*, *C. pulla*, *Altica* spp) screened only *T. sericea* (40%), and *C. pulla* (53%) successfully transmitted the virus. In captivity, it was observed that *C. pulla* did not stay long on one plant while *T. sericea* prefers to feed for longer times on the same plant. By this behaviour it is suspected that *C. pulla* which is more agile than *T. sericea* might be responsible for the fast spread of RYMV in the field. However, other beetles that could be responsible for fast spread of RYMV in the field (Bakker, 1974, 1975) were not encountered in this study. For example *Sessilia pussila* (50%), and insects of a genus near *Apophylia* (Bakker, 1975). *C. pulla* Chapius retained the virus for up to 6 days and transmitted it in a persistent manner whereas *T. sericea* retained the virus for about 1-2 days only and transmitted it in a semi-persistent manner. *Altica* spp preferred *Ludwigia repens* than rice on the field and the latter was greatly attacked when outbreaks occurred. A Coccinelidae (*Chnootriba similis*) was demonstrated for the first time to transmit RYMV (47%) as efficiently as *T. sericea* and *C. pulla* and could also be responsible for the spread of RYMV in the field. Coccinelids are generally predators but *C. similis* is phytophagous and is attracted by graminaceous plants (Breniere, 1983). The long-horned grasshopper (*Conocephalus longipennis*) with biting mouthparts also transmitted RYMV though at very low level. Although it has been established that beetle transmitted viruses fall into four groups; the comovirus, tymovirus, bromovirus, and sobemovirus and are usually not transmitted by other arthropod groups (Fulton *et al.*, 1987). The detection of RYMV particles in the faecal droplets of the short-horned grasshopper (*Oxya hyla*) which could not transmit the virus and none in those of the *C. longipennis* that transmit the virus probably

infer that the virus moved into the hemolymph and was expelled by the former and thus, did not become available for transmission by the insect through its mouthparts. On the other hand the non detection of RYMV particles in the faeces of *C. longipennis* shows that the virus was retained in the hemolymph and then deposited on the leaf surface in regurgitant during feeding. No insects screened in the families Cicadellidae, Cercopidae, Diopsidae, Acrididae, and Aphididae could transmit RYMV.

The earlier reports that most of the *Oryza* species are systemic hosts and the cotyledons and other cultivated cereals are non-hosts (Bakker, 1974; Fauquet and Thouvenel, 1977) were confirmed in this study to be valid and consistent. On the other hand no RYMV infection was obtained with the following plant species that have been reported as potential hosts of RYMV: *Eleusine indica* and *Echinochloa colona* (Raymundo *et al.*, 1979; Awoderu, 1991a). Similarly, Bakker (1974), Fauquet and Thouvenel (1977) could not infect *Eleusine indica* and *Echinocloa colona* mechanically with RYMV. Nevertheless, the works of Bakker (1974, 1975), Fauquet and Thouvenel (1977) and this current work have proven beyond reasonable doubt that the host range of RYMV is narrow and restricted to the monocotyledons (Seghal *et al.*, 1993). The fact that *T. sericea* overwinters on *L. hexandra* which is not susceptible to RYMV shows that the virus inoculum subsists most on rice. The most susceptible and systemic hosts of RYMV in this study have been identified as the *Oryza sativa* indica varieties such as Bouaké 189 and BG 90-2. They are also suitable as indicator plants. The non-detection of systemic hosts in weeds in this study does not imply that they do not exist. Bakker (1974, 1975) has reported systemic hosts other than rice which were not encountered in the location where this study took place.

The nematodes extracted, identified and screened in this study did not transmit

RYMV. These include: *Hirschmaniella oryzae*, *Macroposthonia* spp., *Meloidogyne* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Tylenchorynchus* spp., *Scutellonema brachyurus*, Criconemoides spp., *Xiphinema* spp., *Paratrichodorus* spp., and *Heterodera oryzae*. Bakker (1974) also failed to obtain RYMV transmission with some of these nematodes in Kenya, East Africa.

Evidences from field surveys showed that ratooned or volunteer rice is mostly affected by RYMV and serve as sources of inoculum from which insect vectors such as *T. sericea*, *C. pulla*, *C. similis* and *C. longipennis* could acquire the virus and transmit it to newly transplanted rice. It was also found that *T. sericea* overwinters on *Leersia hexandra* and ratooned / volunteer rice and later move to newly transplanted / planted rice in the field. The outbreaks of *T. sericea* and *C. pulla* cause foliar damage to rice crops. *L. hexandra* turned out not to be susceptible to RYMV thus, proving a point that a plant will be a host to a vector that transmits a particular virus for feeding purposes only but may not necessarily be susceptible to the virus and vice versa (Bakker, 1974; Daniel *et al.*, 1996).

The means by which RYMV is transmitted in this study will serve as sources of inoculum in the field for the insect and further vectors yet to be identified for quick spread of the virus. Although RYMV has only been found in Africa and its neighbouring islands, other continents also stand the risk of having the disease in future if phytosanitory situations are relaxed. For example Bakker (1974,1975) reported a European grass, *Phleum arenarium* as a systemic host of RYMV.

This study therefore, provides vital information on the inoculum sources and vectors of RYMV from which management strategies (Abo and Alegbejo, 1997; Abo *et al.*, 1998; Abo and Sy, 1998) will be focused or modified.

REFERENCES

Abo, M.E., & Alegbejo, M.D. (1997). Strategies for Sustainable Control of Virus Diseases of Some Crops in Nigeria. *Journal of Sustainable Agriculture 10(2/3)*:57-80.

Abo, M.E., & Sy, A.A. (1998). Rice Virus Diseases: Epidemiology and Management Strategies. *Journal of Sustainable Agriculture 11(2/3)*:113-134.

Abo, M.E., Sy, A.A., & Alegbejo, M.D. (1998). Rice Yellow Mottle Virus (RYMV) in Africa: Evolution, Distributions, and Economic Significance on Sustainable Rice Production and Management Strategies. *Journal of Sustainable Agriculture 11(2/3):85-111.*

A'Brook, J., & Benigno, D.A. (1972). The transmission of cocksfoot and Phleum mottle viruses by *Oulema melanopa* and *O. lichens. Annals of Applied Biology 72:169.*

Acuna, J., & Ramos, L. (1958). Informes de interes general en relacion anel arroz. *Admin. Estab. Anoz, Cuba Biol-10.*

Adams, O.B., & Kuhn, C.W. (1977). Seed transmission of plant mottle virus in peanuts. *Phytopathology 7:1126-1129.*

Adesina, A.A., Johnson, D.E., & Heinrichs, E.A. (1994). Rice pests in the Ivory Coast, West Africa: Farmers' perceptions and management strategies. *International Journal of Pest Management 40(4):293-299.*

Afun, J.V.K., Heinrichs, E.A., Johnson, D.E., & Russel-Smith, A. (1995). The influence of weeds in the natural control of upland rice insect pests in Cote d'Ivoire. *Brighton Crop Protection Conference (Weeds). 3C-7:181-186.*

Agyen-Sampong, M. (1982a). The major pest problems of irrigated, upland and mangrove swamp rice ecosystems in the humid tropical, Guinea Savanna, and Sahel climatic zones. Pages 318-328. In: Proc. of a course on Integrated Pest Management in Rice in Africa. 10-28 Jan. 1982. WARDA, Monrovia, Liberia.

Agyen-Sampong, M. (1982b). Status and prospects of rice Integrated Pest Management in the humid tropical zones. Pages 329-338. In: Proc. of a course on Integrated Pest Management on rice in West Africa 10-28 January, 1982, Monrovia, Liberia.

Agrios, N.G. (1988). "Plant Pathology". Academic Press, Inc. New York. 3rd Edition, 803pp.

Akibo-Betts, D.T., & Raymundo, S.A. (1980). Aphids as rice pests in Sierra Leone. *International Rice Research Newsletter 3(6):15-16.*

Akobundu, I.O., & Fagade, S.O. (1978). Weed problems of African ricelands. In: Rice in Africa (Buddenhagen I.W., & Persley G.J., eds), London Academic Press, PP.181-192.

Alam, M.S. (1988). Evaluation of rice cultivars for resistance to *Diopsis longicornis* (Diptera:Diopsidae). *Journal of Economic Entomology. 81(3)*:934-936.

Alam, M.S., & Akibbo-Betts, D.T. (1984). Field and Storage insect pests of rice and their control. In: Rice Production Manual, IITA, Ibadan, Nigeria.

Alam, M.S., John, V.T., & Zan, K. (1984). Insect Pests and Diseases of rice in Africa. In: Rice in Africa. IITA Rice Production Manual, IITA, Ibadan, Nigeria.

Alegbejo, M.D. (1986). Aphid transmission of pepper veinal mottle virus. *Samaru Journal of Agricultural Research 4(1 & 2):71-77.*

Alegbejo, M.D. (1987). Identification of a weed host of pepper veinal mottle virus in northern Nigeria. *Samaru Journal of Agricultural Research* **5**:65-70.

Alegbejo, M.D. (1996). A viral leaf curl disease of Sesame at Samaru,northern guinea savanna zone of Nigeria. *The Nigerian Journal of Agricultural Extension* **II (I)**: In Press.

Allen, W.R. (1981). Dissemination of tobacco mosaic virus from soil to plant leaves under glasshouse conditions. *Canadian Journal of Plant Pathology 3:163-168.*

Alfaro, A., & Goheen, A.C. (1974). Transmission of strains of Grapevine fanleaf virus by *Xiphinema index. Plant Disease Reporter 58:549-552.*

Ali, F.H., & Abubakar, Z.M. (1995). Incidence of RYMV in Zanzibar. Paper presented at the 1st International Symposium on RYMV, 18-22 September, 1995, Mbe, WARDA/ADRAO,Bouake, Cote d'Ivoire.

Andriese, W. (1986). Area and distribution of rice. In: The wetlands and Rice in Sub-Saharan Africa (Juo, A.R., & Lowe, J.A., eds), IITA, Ibadan, Nigeria.

Anonymous, (1988). Resume des travaux sur la Mosaic Jaune du riz. Rapport annuel du Ministere de l'Agriculture et de l'Enviroment. Direction de la Protection et des Vegetaux, Service Diagnostic et de Surveillance, Niamey, Niger, pp.1-9.

Arrandeau, M., & Harahap, Z. (1986). Relevant upland rice breeding objectives. Pages 189-197. In: Proceedings of the 1985 Jakarta Conference, progress in upland rice research. International Rice Research Institute, Los Banos, The Philippines.

Athow, K.L., & Bancroft, J.B. (1959). Development and transmission of tobacco ringspot virus in soybean. *Phytopathology 49:697-701.*

Athow, K.L., & Lavoilette, F.A. (1962). Relation of seed position and pod location to tobacco ringspot virus seed transmission in soybean. *Phytopathology 52:714-715.*

Atiri, G.I. (1984). The occurence and importance of Okra mosaic virus in Nigerian weeds. *Annals of Applied Biology 104:261-265.*

Attere, A.F., & Fatokun, C.A. (1983). Reaction of *Oryza glaberrima* accessions to rice yellow mottle virus. *Plant Disease 67:420-421.*

Awoderu, V.A. (1991a). Rice yellow mottle virus in West Africa. *Tropical Pest Management 37:356-362.*

Awoderu, V.A. (1991b). The rice yellow mottle situations in West Africa. *Journal of Basic Microbiology 31(2):91-99.*

Awoderu, V.A. (1991c). Varietal reaction to rice yellow mottle virus in upland rice ecology n Cote d'Ivoire. *Nigerian Journal of Botany 4:181-187.*

Awoderu, V.A., Alam, M.S., Thottappilly, G., & Alluri, K. (1987). Occurrence of rice yellow mottle virus in upland rice in Cote d'Ivoire. *FAO-Plant Protection Bulletin 35(1):32-33.*

Ayassi, M.N., Ritzenthaler, C., Brugidou, C., Fauquet, C., & Beachy, R.N. (1994). Nucleotide sequence and genome characterisation of rice yellow mottle virus RNA. *Journal of General Virology 15*:249-257.

Babalola, J.O. 1983. Pathogenicity of *Heterodere sacchari* on rice. *Nematologica Mediterranea* 11: 21-25.

Babalola, J.O. 1984. Rice nematology in Nigeria. The occurence, distribution and pathogenesis. *Tropical Pest Management* 30: 256-266.

Bakker, W. (1970). Rice yellow mottle, a mechanically transmissible virus disease of rice in Kenya. *Netherlands Journal of Plant Pathology 76:53-63.*

Bakker, W. (1971). Three new beetle vectors of rice yellow mottle virus in Kenya. *Netherlands Journal of Plant Pathology 77:201-206.*

Bakker, W. (1974). Characterization and ecological aspects of rice yellow mottle virus in kenya. Agricultural Research, Doctoral Thesis, University of Wageningen, The Netherlands, 152p.

Bakker, W. (1975). Rice yellow mottle virus. CMI/AAB Descriptions of Plant Viruses, No. 149.

Barr. B.A., Koechler, C.S., & Smith, R.F. (1975). Crop losses to insects, diseases, weeds and other pests. USAID Pest Management and Related Environmental Protection Project, University of California, Berkeley USA.

Beagle, E.C. 1978. Rice-Husk conservation to energy. Agricultural Science Bulletin, 31 Food and Agricultural Organisation of the United Nations, Rome,175pp.

Bennett, C.W., & Costa, A.S. (1961). Sowbane mosaic virus caused by a seed transmitted virus. *Phytopathology 51:546.*

Bigger, M. (1981). The relative abundance of the mealy bugs vectors (Hemiptera, Coccidae and Pseudococcidae) of Cocoa Swollen shoot disease in Ghana. *Bulletin of Entomological Research 71:435-448.*

Bouriquet, G. (1946). Maladies du Riz. In: Lechevalier (Ed.): Les maladies des plantes cultivées à Madagascar. *Encyclopaedia Mycological, Paris, 12:374-382.*

Breniere, J. (1983). The principal insect pests of rice in West Africa and their control. West African Development Association, Monrovia, Liberia, 87p.

Bridge, J.M., Luc, M., & Plowright, R.A. 1990. Nematode of rice. In: Plant parasitic nematodes in sub-tropical and tropical agriculture, pp 69-108 (eds Luc, M., Sokra, A., and Bridge, J.). Wellington, UK. CAB International

Broadbent, L. (1965). The epidemiology of tomato mosaic virus: Infection through tomato roots. *Annals of Applied Biology* 55:57-66.

Brown, D.J.F., Ploeg, A., & Robinson, D.J. (1989). A review of reported associations between *Trichodorus* and *Paratrichodorus* species and tobraviruses with a description of laboratory methods for examining virus transmission by Trichodorids. *Revue de Nematologie 12:235-341.*

Brown, D.J.F., & Trudgill, D.L. (1984). The spread of carnation ringspot virus in soil with or without nematodes. *Nematologica 30:102-104.*

Brugidou, C., Bonneau, C., Ngon, A., Ayassi, M., Holt, C., Beachy, R.N., & Fauquet, C.M. (1995). An infectious full length cDNA clone of RYMV. A molecular tool for Engineering virus Resistance and Developing a viral Expression vector. Paper presented at the 1st Interl. Symp. on RYMV, 18-22 Sept.,1995, Mbe, WARDA, Bouake, Cote d'Ivoire.

Buser, A. (1990). Untersuchungen Uber die Pfeffingerkrankheif der Susskrrsche Und deren vektor *Longidorus macrosoma*, Eidgernossiche Technische Hochschulē Zurich,

dissertation No. 9194.

Campbell, R.N., Wipf-Scheibel, C., & Lecoq, H. (1996). Vector-assisted seed transmission of melon necrotic spot virus in melon. *Phytopathology 86*: 1294-1298.

Caroll, T.W. (1972). Seed transmissibility of two strains of barley stripe mosaic virus. *Virology 48:328-336.*

Caroll, T.W., & Chapman, S.R. (1970). Variation in embryo infection and seed transmission of barley stripe mosaic virus within and between two cultivars of barley. *Phytopathology 60:1079-1081.*

Carpenter, A. (1978). The history of rice in Africa. In: Rice in Africa (I.W. Buddenhagen & G.B. Persley, eds). Academic Press, London, U.D.

Chang, T.T. (1976). The origin, evolution, cultivation, dissemination and diversification of Asian and African rices. *Euphytica 25*:425-441.

Clark, M.F., & Adams, A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology 34:475-483.*

Clinch, P., Louqhmans, J.B., & Murphy, P.A. (1938). A study of the infiltration of viruses into seed potato stocks in the field. *Sci. Proc. R. Dublin Soc. 22:18-31.*

Converse, R.H., & Lister, R.M.(1969). The occurrence and some properties of black raspberry latent virus. *Phytopathology 59:325-333.*

Converse, R.H., & Martin, R.R. (1982). Use of the Babara Clark $F(ab')_2$ indirect ELISA test for detection of viruses in small fruits. *Acta Horticulture 129:21.*

Cooper, J.J., & Harrison, B. (1973). The role of weed hosts and the distribution and activity of vector nematodes in the ecology of tobacco rattle virus. *Annals of Applied Biology 73:53-66.*

Coulibaly, F., & N'guessan, K.P. (1994). Lutte Integrée Contre la maladie de la Bigarrure Jaune du Riz: Recherche de plantes Reservoirs. Rapport Presenté à Troisieme Reunion du group de Recherche de "Systeme de protection Integrée du riz" pour l'Afrique d'Louest, 13-15 Mars, 1994 au Siege de l'ADRAO, Mbe/Bouake, Cote d'Ivoire.

Couch, H.B. (1955). Studies on the seed transmission of lettuce mosaic virus. Virology 48:323-336.

Coyne, D.L., Plowright, R.A, & Fofana, I. 1996. Preliminary investigations of nematodes associated with rice in Guinea, Benin and Togo. *Afro-Asian Journal of Nematology* 6(1):

70-73

Cung, H.A. (1981). Weeds in some crops and procedures to control them. Ph.D Thesis, Agriculture and Science Technology Institute, Hanoi, Vietnam.

Dale, D. (1994). Insect pests of rice plants - Their Biology and Ecology. Pp 363-485, In: Heinrichs, E.A., Ed.), Biology and Management of Rice Insects. Wiley eastern, New Delhi, 779p.

Daniel, L., Ochoa, M., Zavaleta-Mejia, E.,Tohansen, R.M., Herrera, G., and Cardenas, S.E. (1996). Toposviruses, weeds and Thrips associated with Chrysanthemum (*Dendrathema grandiflora* Tzvelvev cv. *Polaris*). *Interenational Journal of Pest Management 42(3):157-159.*

Davies, R.F., & Mizuki, M.K. (1986). Lack of evidence for virus transmission in seeds of Squash. *Cucurbit Genetic Co-operatin 10:81.*

Day, M.F., & Venables, D.G. (1961). Transmission of Cauliflower mosaic virus by aphids. *Australian Journal of Biological Science 14:163-193.*

Delouche, J.C. (1988). Weeds as rice seed contaminants. In: Rice seed Health (Manila, Philippines International Rice Research Institute), pp.179-187.

Desjardins, P.R., Drake, R.J., & French, J.V. (1969). Transmission of Citrus ringspot virus to Citrus and non-citrus hosts by dodder (*Campestris subinclusa*). *Plant Disease Reporter 53:947-948.*

Dias, H.F. (1970). The relationship between cucumber necrosis virus and its vector, *Olpidium cucurbitacearum. Virology 42:204-211.*

Dias, H.F., & Watermoth, H.E. (1967). The identity of a seedborne mosaic virus of *Chenopodium amaranticolor* and *C. quinosa. Canadian Journal of Botany 45: 1285.*

Dimick, A.W., Geissinger, C.M. & Herst, R.K. (1971). A new adaptation of tissue for the study of virus and mycoplasma diseases. *Phytopathology 61*:429-430.

Diomande, M. 1982. Response of upland rice cultivars to *Meloidogyne* spp. *Revue de Nematologie* 70(1): 57-63.

Doval, S.L., Mathur, B.P., & Kaul, C.K. (1975). Studies on the Biology and Control of *Chaetocnema basalis* Baly (Chrysomelidae: Coleoptera). *Annals of Arid Zone 4(1)*:57-62.

Duffus, J.E. (1971). Role of weeds in the incidence of virus diseases. *Annual Review of Phytopathology 9:319-394.*

Ekpo, E.J.A., & Saettler, A.W. (1974). Distribution pattern of bean common mosaic virus in developing bean seed. *Phytopathology 64:269-270.*

Elias, R., Granados, G., & Ortega, A. (1962). El estado actual de la hoja blancha en mexico. *Agricultural Technico Mexico 24:2-7.*

Eslick R.F., & Afanasiev, M.M. (1955). Influence of time of infection with barley stripe mosaic on symptoms, plant yield and seed infection of barley. *Plant Disease Reporter 39:722-724.*

Estessami, P., Callis, R., Ellwood, S., & Stanley, J. (1988). Delimitation of essential genes of Cassava latent virus DNA 2. *Nucleic Acids Research 16:4811-4829.*

Fademi, O.A. 1987. Nature and importance of nematodes on rice in Nigeria. *Pakistan Journal of Nematology* 5(1): 5-13.

Fademi, O.A.,& Fawole, B. 1995. Effect of rice resistance on infection by *Meloidogyne incognita*, *Nematropica* 25(2): 177-180.

Fagade, S.O., Pillai, P.G., & Kehinde, J.K. (1988). Improved rice varieties released in Nigeria. *International Rice Research Newsletter 3:1.*

Fauquet, C.M., Brugidou, C., Kouassi, N., Bonneaus, C., Ngon, A., Yassi, M., Opalka, N., Yeager, M., & Beachy, R.N. (1995). Rice Yellow Mottle Virus: A Threat to African Farmers and a Model for Plant Virologists. Paper presented at the 1st International Sympossium on RYMV, 18-22 September, 1995, Mbe, WARDA / ADRAO, Bouake, Cote d'Ivoire.

Fauquet, C.M., & Thouvenel, J.C. (1977). Isolation of the rice yellow mottle virus in Ivory Coast. *Plant Disease Reporter 61:443-446.*

Fauquet, C.M., & Thouvenel, J.C. (1987). Plant viral diseases in the Ivory Coast. Initiations-Documentation Techniques No. 46, ORSTOM-PARIS, 243pp.

Fomba, S.N. (1984). Rice disease situation in mangrove and associated swamps in Sierra Leone. *Tropical Pest Management 30:73-81.*

Fomba, S.N. (1986). Crop losses and other effects of attack caused by the rice yellow mottle virus on the rice varieties, ROK 5 and Angthata at Rokupr, Sierra Leone. *West AFrica Rice Development Association Technical Newsletter 6:24-25.*

Fomba, S.N. (1988). Screening for seedling assistance to rice yellow mottle virus in some rice cultivars in Sierra Leone. *Plant Disease 72:641-642.*

Fomba, S.N. (1990). Rice yellow mottle virus on swamp rice in Guinea. *IRRN 15:6.*

Forer, L.B., Powell, C.A., & Stouffer, R.F. (1984). Transmission of tomato ringspot virus to apple rootstock cuttings and to Cherry and peach seedlings by *Xiphinema rivesi*. *Plant Disease 68:1052-1054.*

Francki, R.I.B., & Miles, R. (1985). Mechanised transmission of Sowbane mosaic virus carried on pollen from infected plants. *Plant Pathology 34:11-19.*

Frosheiser, F.I. (1974). Alfalfa mosaic virus transmission to seed through alfalfa gametes and longevity in alfalfa seed. *Phytopathology 64:102-105.*

Fujisaka, S., Guino, R.A., Lubigan, R.I., and Moody, K. (1993). Farmers' rice seed management practices and resulting weed seed contamination in the Philippines. *Seed Science and Technology 21:149-157.*

Fulton, J.P., Gergerich, R.C., & Scott, H.A. (1987). Beetle transmission of plant viruses. *Annual Review of Phytopathology 25:111-123.*

Gallo, J., & Ciampor, F. (1977). Transmission of alfalfa mosaic virus through *Nicandra physaloides* seeds and its localization in embryo and cotyledons. *Acta Virologica 21: 344-346.*

Galvez, E.G.E., & Jennings, P.R. (1959). Transmission de la hoja blanca del arroz en Colombia. *Agriculture Tropicale 15:507-515.*

Galvez, E., Thurston, H.D., & Jennings, P.R. (1961a). Host range and insect transmission of the hoja blanca disease of rice. *Plant Disease Reporter 45:949-953.*

Galvez, E., Thurston, H.D., & Jenings, P.R. (1961b). Transmission of the hoja blanca disease of rice. *Proceeding on Rice Technologists Working group. 1960: 20 (Abstr.).*

Gergerich, R.C., Scott, H.A., and Fulton, J.P. (1983). Regurgitant as a determinant of specificity in the transmission of plant viruses. *Phytopathology 73:936-938.*

Gibbs, A.J. 1968. Cryptograms. In: Martyns (Ed.): Plant Virus Names. Annontated list of names and synonyms of plant viruses and diseases. *Phytopathological Papers 9*:135-149.

Gibbs, K.S., & Randles, J.W. (1988). Studies in the transmission of velvet tobacco mottle virus by the miriad *Crytopeltis nicotianae*. *Annals of Applied Biology 45:427-437.*

Givord, L. (1981). Southern bean mosaic virus isolated from cowpea in Ivory Coast. *Plant Disease 65(9):755-756.*

Gleason, M.L., & Providenti, R. (1990). Absence of transmission of Zucchini yellow mosaic virus from seeds of pumpkin. *Plant Disease 74(10):828.*

Gordon, D.T., Bradfute, O.E., Gingery, R.E., Nault, L.R., & Uyemoto, J.K. (1984). Maize Chlorotic mottle virus, CMI/AAB Descriptions of plant viruses No. 234.

Grimsley, N., Hohn, T., Davies, J.W., & Hohn, B. (1987). Agrobacterium mediated delivery of infectious maize streak virus into maize plants. *Nature (London) 325:177-179.*

Grist, D.H., and Lever, R.A.J.W. (1969). Pests of Rice. Longmans, London, pp.3-9.

Gunnery, S., & Datta, A. (1987). An inhibitor RNA of translation from barley embryo. *Biochemical and Biophysical Research Commission 142:383-388.*

Harder, D.E., & Bakker, W. (1973). African cereal streak, a new disease of cereals in east Africa. *Phytopathology 63:1407-1411.*

Hargreaves, E. (1937). Some insects and their food plants in Sierra Leone. *Bulletin of Entomololgical Research 28*: 505-520.

Harlan, J.R., & Stemler, A. (1976). The races of Sorghum in Africa. In: Origins of African Plant Domestication. (J.R. Harlan, Dewit J.M., & Stemler, A.D., eds), Mouton Publishers, Hague, The Netherlands.

Harris K.F. (1983). Stenorrhynchus vectors of plant viruses: Virus- vector interactions and transmission mechanisms. *Advances in Virus Research 28:113-140.*

Harris, W.V. (1937). Annual report of the entomologist. In:Rep. Dep. Agric. Tanganyika for 1936. Government Printer, Dares Salam, p.88-94.

Harris, K.F., & Maramorosch, K. (1980). Vectors of plant Pathogens. Academic Press, New York, 467pp.

Harrison, B.D. (1977). Ecology and control of viruses with soil inhabiting vectors. *Annual Review of Phytopathology 15:331-360.*

Harrison, B.D. (1985). Advances in geminivirus research. *Annual Review of Phytopathol. 23:55-82.*

Harrison, B. D., Finch,J.T., Gibbs,A. J., Hollings, M., Shephard, R. J.,Valenta,V., Wetter, C. (1971). Sixteen group of plant viruses. *Virology 45:*233-247.

Harrison, A.N., & Gudauskas, R.T. (1968). Effects of some viruses on growth and seed production of two cowpea cultivars. *Plant Disease Reporter 52:509-511.*

Harrison, B.D., Winslow, R.D. (1961). Laboratory and field studies on the relation of arabis mosaic virus to its nematode vector, *Xiphinema diversicaudatum Micoletzky). Annals of Applied Biology 49:621-633.*

Heard, A.J., & Chapman, P.F. (1986). A field study of the pattern of local spread of ryegrass mosaic virus in Mown grassland. *Annals of Applied Biology 108:341-345.*

Heinrichs, E.A. (1994). Development of Multiple Pest Resistant crop Cultivars. *Journal of Agricultural Entomology 11(3):225-253.*

Heinrichs, E.A., Johnson, D.E., Afun, J.K., & Robinson, D.J. (1995a). Rice Pests of Shifting Cultivation in Cote d'Ivoire, West Africa. In: Fragile lives in Fragile ecosystems (K.L. Koenig, ed.). Proc. International Rice Research Conference, 13-17 Feb., 1995, Manila, The Philippines 976pp.

Heinrichs, E.A., & Miller, A.T. (1991). Preface: Pages vii-viii In: Rice insects: Management Strategies. (Heinrichs, E.A., & Miller, T.A., eds), Springe - Verlag New York. Inc.

Heinrichs, E.A., Sy, A.A., Akator, S.K., and Oyediran, I. (1995b). Seasonal occurrence of rice yellow mottle virus on the WARDA research farm. Paper presented at the 1st International Symosium on RYMV, at WARDA, Mbe, Bouake, Cote d'Ivoire, 18-22 September, 1995.

Herdt, R.W. & Palacpac, A.C. 1983. World rice facts and trends. Department of Agricultural Economics, International Rice Research Institute, Los Banos, Laguna, Philippines, 41pp.

Hewitt, W.B., Raski, D.T., & Goheem, A.C. (1958). Nematode vector of soil borne fanleaf virus of grapevines. *Phytopathology 48:568-595.*

Hiruki, C. (1987). Recovery and identification of tobacco stunt virus from air-dried nesting spores of *Olpidium brassicae*. *Plant Pathology 36:224-228.*

Hollings, M., Stone, O.M., & Barton, R.J. (1977). Pathology, soil transmission and characterisation of Cymbidium ringspot, a virus from Cymbidium orchids and white clover (*Triticum repens*). *Annals of Applied Biology 85*:233-248.

Hooper, D.J. (1986). Extraction of free living stages from soil. In: Laboratory methods for work with plant and soil nematodes. pp.5-30 (ed.J.F.Southey). Reference Book 402, Ministry of Agriculture, Fisheries and Food. London: Her Majesty's Stationery Office.

Hooper, D.J. (1990). Extraction and processing of plant and soil nematodes. In: Luc, M., Sikora, R.A., & bridge, J. (Eds). Plant parasitic nematodes in sub-tropical and tropical Agriculture. CAB International, Wallington, UK, P45-68.

Hsieh, S.P.Y. (1969). Multiplication of the rice transistory yellowing virus in its vector. *Nephotettix apicalis* Motsch. *Plant Protection Bulletin (Taiwan) 11:159-190.*

Hu, Q.W., Pu, Z.Q., XU, G.Z., and Fuang, D.Z. (1996). A viral disease of broad bean caused by a non aphid transmissible strain of Turnip mosaic virus. *Plant Pathology*

45:843-847.

Huelma, K., Moody, K., and Mew, T.W. (1996). Weed seeds in rice seed shipments: A case study. *International Journal of Pest Management 42(3): 147-150.*

Hull, R. (1988). The Sobemovirus group. In: The plant viruses Vol. 3. Polyhedral virus with monopatite RNA genomes (I. R. Koenig, ed), Plenum Press, New York.

Hunger, R.M., & Sherwood, J.L. (1985). Use of Symptomatology and Virus Concentrations for Evaluating Resistance to Wheat soilborne mosaic virus. *Plant Disease 69*:848-850.

Hussey, R.S., & McGuire, J.M. (1987). Interaction of nematodes with other organisms. In: Principles and Practice of Nematode Control in Crops. R.H. Brown & B.A. Kerry (eds). Academic Press, 1987.

International Institute of Tropical Agriculture (IITA), (1976). Annual report, Research highlights, IITA ,Ibadan, Nigeria, pp. 154, 181-183.

IITA, (1978). Annual Report, Research highlights, IITA, Ibadan, Nigeria, pp. 33-34.

IITA, (1980). Annual Report, Rice Research Programme, IITA, Ibadan, Nigeria, pp.137, 109-116.

IITA, (1981. Annual Report, Rice Improvement Proghramme IITA, Ibadan, Nigeria, pp.121-122.

IITA, 1982). Annual Report. Research highlights, IITA, Ibadan, Nigeria, pp. 66.

IITA, (1984). Annual report, Rice Improvement Programme, IITA, Ibadan, Nigeria, pp.19-22.

IITA, (1985). Annual Report, Rice Improvement Programme, IITA, Ibadan, Nigeria, pp.17-21, 113.

IITA, (1986). Annual Report, Research Highlights, IITA, Ibadan, Nigeria, PP.130-132.

Imeokparia, O.P. (1996). Effects of land preparation and planting and water depth on weeds. Progress rerport presented at the fifth Annual meeting of The integrated Management Task Force (IPM-TF) for West Africa at WARDA, Bouake, Cote d'Ivoire, 20-22 March, 1996 (Abstract).

IRRI (International Rice Research Institute), (1988). The International Rice Testing Program-Standard Evaluation System (IRTP-SES) for rice. Los Banos, Laguna, The Philippines. 3rd Edition, pp. 23.

IRTP (International Rice Testing Program), (1989). Disease and Insect Pests. In: Summary report of the Monitoring Tour and Workshop, IRRI, Los Banos, The Philippines, PP.12-13,

Ishihara, T. (1964). Revision of the genus *Nephotettix* (Hemiptera: Deltocephalidae). *Shikoku entomological Society 8(2):39-44.*

Ishikawa, R. (1928). The merit of Hatzuzo Hashimoto, the earliest investigator of dwarf disease of rice (In Japanese). *Journal of Plant protection 15:218-222.*

John, V.T., Mosajo, T.M., and Thottappilly, G. (1986). Breeding rice for resistance to rice yellow mottle virus. *IITA Research Briefs 7:4-5.*

John, V.T., Thottappilly, G., & Awoderu, V.A. (1984). Occurrence of rice yellow virus in some Sahelian countries in West Africa. *FAO-Plant Protection Bulletin 32:86-87.*

John, V.T., Thottappilly, G., Masajo, T.M., & Reckhaus, P.M. (1988). Rice Yellow Mottle Virus Disease in Africa. Unpublished contribution, International congress of plant pathology, Japan, 20-27, August, 1988.

Jones, M.P. (1995). The Rice Plant and its environment. WARDA Training Guide 2. WARDA/ADRAO publication, Bouake, Cote d'Ivoire. 32pp.

Jones, R.A.C., & Fribourg, C.E. (1977). Beetle, Contact and Potato true seed transmission of Andean Potato latent virus. *Annals of Applied Biology 86:133-128.*

Jones, R.A.C., & Harrison, B.D. (1969). The behaviour of Potato mop top virus in soil, and evidence for its transmission by *Spongospora subterranea. Annals of Applied Biology 63:1-17.*

Juliano, B.O. 1979. The chmical basis of rice grain quality. Pages 69-90. In: Proceedings of workshop on chemical aspects of rice grain quqlity. International Rice Research Institute, Los Banos, Laguna, Philippines.

Juliano, B.O. 1985a. Production and utilisation of rice. In: Rice Biochemistry and Technology (Juliano, B.O. ed), 2nd edition. The American Association of Cereals Chemists Incorporated, 1985, 773pp.

Juliano, B.O. 1985b. Rice Bran. In: Rice Biochemistry and Technology (Juliano, B.O. ed), 2nd edition. The American Association of Cereals Chemists Incorporated, 1985, 773pp.

Juliano, B.O. 1985c. Miscellaneous rice products. In: Rice Biochemistry and Technology (Juliano, B.O. ed), 2nd edition. The American Association of Cereals Chemists Incorporated, 1985, 773pp.

Juliano, B.O. 1985d. Rice hull and rice straw. In: Rice Biochemistry and Technology (Juliano, B.O. ed), 2nd edition. The American Association of Cereals Chemists Incorporated, 1985, 773pp.

Kaiser, W.J., Wyatt S.D., & Pesho G.R. (1982). Natural hosts and vectors of tobacco streak virus in eastern Washington. *Phytopathology 72:1508-1512.*

Kennedy, J.S., Day, M.F.I., & Eastop V.P. (1962). A conspectus of aphids as vectors of plant viruses. Commonwealth Institute of Entomology, London. 144p.

Kimble, K.A., Grogan, R.G., Greathead, A.S., Paulus, A.D., & House, I.K. (1975). Development, application and comparison of methods for indexing lettuce seed for mosaic virus in California. *Plant Disease Reporter 59:461-464.*

Kiyoshi, Y., & Sohtaro, K.1985. Rice in brewing. In: Rice Biochemistry and Technology (Juliano, B.O. ed), 2nd edition. The American Association of Cereals Chemists Incorporated, 1985, 773pp.

Kleinhempel, H., & Kegler, G. (1982). Transmission of tomato bushy stunt virus without vectors. *Acta Phytopathology Academy of Science,* Hungary *17:17-21.*

Konate, G. (1995). Les Hotes Relais du RYMV au Burkina Faso. Paper presented at the 1st International Sympossium on RYMV,18-22 September 1995, Mbe, WARDA, Bouake, Cote d'Ivoire.

Kongseree, N. 1979. Quality tests for waxy (glutinous) rice. Pages 303-311. In: Proceedings of workshop on chemical aspects of rice grain quality. International Rice Research Institute, Los Banos, Lagua, Philippines.

Lamberti, F., Taylor, C.E., & Seinhorst, S.W. (1975). Nematode vectors of plant viruses. Plenum press, London, 460pp.

Lamey, H.A., McMillian, W.W., & Hendrick, R.D. (1964). Host ranges of the hoja blanca virus and its insect vector. *Phytopathology 54:536-541.*

Lamey, A.A., McMillian, & McGuire, J.U. (1961). Transmission and host range studies on hoja blanca. *Proceedingon Rice Technologists Working group, 1960. P.20-21 (Abstract).*

Lampley, P.N.L., & Hamilton, R. (1974). A new Cowpea strain of southern bean mosaic virus from Ghana. *Phytopathology 64:1100.*

Laviolette F.A., & Athow K.L. (1971). Longevity of Tobacco ringspot virus in soybean seed. *Phytopathology 61:755.*

Lim, W.L., & Hogedorn, D.J. (1977). Bimodal transmission of plant viruses. In: Aphids as virus vectors (K.F. Harris & K. Maramorosch, eds), pp 237-251. Academic press, New York.

Ling, K.C. (1975). Rice virus diseases. 3rd Printing, IRRI. Los Banos, The Philippines, 142pp.

Lister, R.M. (1960). Transmission of soil borne virus through seed. *Virology* ***10:547-549.***

Litsinger, J.A. (1984). Cultural, mechanical and physical control of rice insects. In: Biology and management of Rice insects (E.A. Heinrichs, ed.) Wiley Eastern, New Delhi, India. pp 547-582.

Loevinsohn, M.E. (1994). Rice pests and agricultural environments. Pages 487-573. In:Biology and Management of rice insects (E.A.Heinrichs, ed), Wiley Eastern, New Delhi and IRRI, Los Banos, The Philippines.

Maramorosch, K. (1969). Effects of Pathogenic Viruses in Their Insect Vectors. In: The Virus Diseases of the Rice Plant. *Proc. of a Symposium at The International Rice Research Institute (IRRI), The Philippines, April, 1967.*

Matthews, R.E.F. (1991). "Plant Virology" 3rd edition Academic Press, London. 835pp.

Maule, A.J. (1991). Spread of viruses within plants. *Proc. Phytochem. Soc. Eur., Inter. Symp. Biochem. Mol. Plant Pathol. Interact.* In: Mathews, 1991, pp. 358,764.

Maury, Y., Bossenec, J.M., Boudazin, G., Hampton, R., Pieterson, G., & Macquine, J. (1987). Factors Influencing ELISA evaluation of bovine mosaic virus in infected pea seed. *Agronomic* 7:225-230.

Mbodj, Y., Sidibe, B.D., & Sere, Y.(1984). Rice Phytosanitary problems in the Sahel and attempts for solution. Paper presented at an International Seminar Organised by CILSSM on the Integrated Management of Pests and Diseases in the Sahel, Niamey, Niger, 6-13 Dec., 1984.

McElroy F.D. (1977). Nematode as vectors of plant viruses-acurrent review. *Proc. of The American Phytopathological Society 4:1-10.*

McGuire, J.M., & Douthit, L.B. (1978). Host effect on acquisition and transmission of tobacco ringspot virus by *Xiphinema americanum. Phytopathology 68:457-459.*

McGuire, J.M., & Schneider, I.R. (1973). Transmission of satellite of Tobacco ringspot virus by *Xiphinema americanum. Phytopathology 63:1429-1430.*

McGuire, J.M., & Wickizer, S.L. (1981). Association of *Xiphinema americanum* with spread of necrotic ringspot of blueberry. *Journal of Nematology 13:465-466 (Abstract).*

McLean, D.L., & Kinsey, M.G. (1984). The precibarial value and its role in the feeding behaviour of the pea aphid, *Cyrthosiphon pisum. Bulletin of Entomological Society of America. 30:26*-31.

Ministry of Overseas Development (MOD) (1970). Pest Control in rice. PANS Manual 3. Ministry of Overseas Development, London, p.173-174.

Mohammed, N.A., & Mossop, D.W. (1981). Cynosurus and cocksfoot mottle viruses: A comparison. *Journal of General Virology 55:63.*

Monis, J., Scott, H.A., & Gergerich, R.C. (1986). Effect of beetle regurgitant on plant virus transmission using the gross wounding technique. *Phytopathology 76:808-811.*

Mountain, W.L., Powell, C.A., Forer, L.B., & Stouffer, R.F. (1983). Transmission of tomato ringsport virus from dandelion via seed and dagger nematode. *Plant Disease 67:867-868.*

Murant, A.F., Chambers, J., & Jones, A.T. (1974). Spread of Raspberry bushy dwarf virus by pollination, its association with crumby fruit, and problems of control. *Annals of Applied Biology 77:271-281.*

Mustapha, S. (1994). The effect of training in rice sciences on job performance of Trainers. Paper presented at the WARDA/NARS Training Seminar held at Mbe/Bouaké, Cote d'Ivoire, 9-12 may, 1994.

Nacro, S., & Heinrichs, E.A. (1996). Flea beetles, *Chaetocnema* spp (Coleoptera: Chrysomelidae), In: Insects Pests of Rice in West Africa. IPM-TF Document, WARDA, 01 BP 2551, Bouaké 01, Cote d'Ivoire, 53p.

Nault, L.R., & Ammar, E.D. (1989). Leafhopper and plant hopper transmission of plant viruses. *Annual Review of Entomology 34:503-529.*

Nault, L.R., & Madden, L.V. (1988). Phytogenetic relatedness of maize chlorotic dwarf virus leafhopper vectors. *Phytopathology 78:1683-1687.*

Nayar, N.M. (1973). Origin and Cytogenetics of rice. *Advances in Genetics 17:153-192.*

Ng, N.Q. (1979). Plant exploration in Central Nigeria. In: Rice in Africa (I.W. Buddenhagen & G.B. Persley, eds). Academic Press, London, U.K.

Ng, N.Q. (1988). Use of African Rice germplasm. In: proceedings of the International Workshop on Rice Testing program for Africa, October, 1987, Saint Louis, Senegal.

Ng, N.Q., Buddenhagen I.W., & Sarkarung S. (1980). Screening rice germplasm for rice yellow mottle virus resistance. In: proc. of the 8th Ann. Conf. of Genetic Society of Nigeria, Univ. of Benin, Benin City, Nigeria, December, 1980.

Ng, N.Q., Chang, T.T., Vaughan, D.A., & Zuno Alto, V.C. (1988). Africa Rice Diversity: Conservation and Prospects for Crop Improvement. In: Crop Genetic Resources of Africa Vol. II (N.Q. Ng, P. Pernno, F. Attere, & H. Zedan, eds), IITA, Ibadan, Nigeria, 322pp.

Niblett, C.L., & Toler, R.W. (1977). Panicum mosaic virus, CMI/AAB Descriptions of plant viruses, No. 177.

Oka, H.I., Morishima, H., Sano, Y., & Koizuni, T. (1977). Observations of rice species and accompanying Savanna Plants on the Southern fringes of Sahara desert. Report of study tour in West Africa. National Institute of Genetics, Japan.

Okioma, S.N.M., Muchoki, R.N., & Gathuru, E.M. (1983). Alternate hosts of rice yellow mottle virus in the lake Victioria basin in Kenya. *Tropical Pest Management 29(3):295-296.*

Orlob, G.B. (1966). Feeding and transmission characlteristics of *Aceria tulipae* Keifer as a vector of wheat streak mosaic virus. *Phytopathologische Zeitschrift 55:218-238.*

Ou, S.H. (1985). Rice Diseases, 2nd edition. Kew, Commonwealth Mycological Institute, England, 368pp.

Owusu, G.K., Crowley, N.C., and Francki, R.I.B. (1968). Studies on the seed transmission of Tobacco ringspot virus. *Annals of Applied Biology 61:195-202.*

Papkova, K.V. (1989). General Phytopathology (In Russian). Agropromizhat, Moscow, 398p.

Paul, C.P. (1991). Diversity of *Oryza sativa* L., and *O. glaberrima* Steud. in Africa. M. Phil. Thesis Univ. of Ibadan, Nigeria, 185pp.

Piron, P.G.M. (1986). New aphid vectors of Potato virus Y^N. *Netherlands Journal of Plant Pathology 92:223-229.*

Ploeg, A., Brown, D.J.F., & Robertson, D. (1989). Transmission of tobraviruses by Trichodorid nematodes. *Eppo Bulletin 19:605-610.*

Plowright, R.A., Matius, D., Aung, T., & Mew, T. 1990. The effect of *Pratylenchus zeae* on the growth and yield of upland rice. *Revue de Nematologie* 13: 283-291.

Ponnamperuma, F.N. 1984. Straw as a source of energy of nutrients for wetland rice. Pages 117-136. In: Proceedings of the International conference on Organic Matter and Rice, 1982. International Rice Research Institute, Los Banos, Laguna, Philippnes.

Poteres, R. (1950). Vieilles agricultures de l'Afrique Intertropicale. *L'Agronomic Tropicale 5:489-507.*

Poteres, R. (1956). Taxonomy agrobotanique de riz cultives, *Oryza sativa* L. et *O. glaberrima* Steud. *Journal Agricultural & Tropical Botanique Applied 3:342-356.*

Poteres, R. (1976). African Cereals, In: Origins of African Plant Domestication (J.R. Harlan, J.M. De Wet & A.B. Stemler, eds), Mouton publishers, the Haque, Netherlands.

Powell, N.T. (1979). Internal Synergism among organisms inducing disease. In: *Plant Disease*. Vol. IV (J.G. Horsfall & E.B. Cowling, eds). pp 113-133. Academic Press, New York.

Providenti, R., & Robinson, R.W. (1987). Lack of seed transmission in squash and melon plants infected with Zucchins yellow mosaic virus. *Cucurbitis Genetic Co-operation 10:81-82.*

Purseglove, J.W. (1972). *Oryza* L Monocotyledons 1. Longman, London, P.161-198.

Raccah, B., Loebenstein, G., & Bar-Joseph, M. (1976). Transmission of Citrus tristeza virus by the melon aphid. *Phytopathology 37:809-816.*

Rader, W.E., Fitzpatrick, H.F., & Hilderbrand, E.M. (1947). A seed borne virus of muskmelon. *Phytopathology 37:809-816.*

Ranjhan, S.K. & Beagle, E.C. 1978. Utilisation of rice hulls for animal feeding. Egypt Technical Co-operation Programme consultant Report. 6/EGY/03/I. Food and Agricultural Organisation of the United Nations.

Rao, A.N., and Moody, K. (1990). weed seed contamination in rice seed. Seed Science and Technology *18:139-146.*

Raski, D.T., & Hewitt, W.B. (1963). Plant -Parasitic Nematodes as Vectors of Plant Viruses. *Phytopathology 53*:39-47.

Ratna, A.S., Rao, A.S., Reddy, A.S., Nott, B.L., Reddy, D.V.R., Vijayalakshmi, M., & McDonald, D. (1991). Studies on transmission f Indian Peanut Clump virus disease by *Polymyxa graminis. Annals of Applied Biology 118:71-78.*

Raymundo, S.A. (1980).Diseases of Rice in Sierra Leone. *Technical Bulletin No. 1.* Rokupr, Sierra Leone. Rice Research Station.

Raymundo, S.A., & Buddenhagen, I.W. (1976). A rice disease in West Africa. *International Rice Commission Newsletter 29:51-53.*

Raymundo, S.A., Buddenhagen, I.W., Fomba, S.N., & Akibbo-Betts, D.T. (1979). Recent advances in knowledge of rice viruses and resistance to a beetle transmitted mottle of rice in West Africa. In: Seminar Proceedings No. 6, Rice breeding and varietal Improvement II. West Africa Rice Development Association PP. 203-212, Sept. 13-17, 1979.

Raymundo, S.A., & Konteh, I.K. (1980). Distribution, importance, screening methods and varietal reaction to rice pale yellow mottle disease. *International Rice Commission Newsletter 29:51-53.*

Reckhaus, P.M., & Adamou, I. (1986). Rice Diseases and Their Economic Importance in Niger. *FAO Plant Protection Bulletin 34:77-82.*

Reckhaus, P.M., & Andriamasintseheno, H.E. (1995). Development of an IPM Strategy to fight RYMV and Constraints to its Implementation in Madagascar. Paper presented at the 1st International Symposium on RYMV, 18-22 Sept., 1995, Mbe, WARDA/ADRAO, Bouake, Cote d'Ivoire.

Reckhaus, P.M., & Randrianangaly, S. (1990). Rice Yellow Mottle Virus (RYMV) on Rice in Madagascar. *IRRN 15:11.*

Rivera, C.T., & Ou, S.H. (1965). Leafhopper transmission of tungro disease of rice. *Plant Disease Reporter 49:127-131.*

Robinson, D.J. (1991). Virus Groups and Genome Strategies. In: Modern Approaches to Plant Virology: Virus Transmission, Detection and Resistance, Speakers' notes 061, The British Council, 17-29 March, 1991, Dundee, Scotland.

Rossel, H.W. (1986). Rice Yellow mottle virus and African Soybean dwarf virus, newly discovered virus disease of economic importance in West Africa. Tropical Agricultural Research Series No. 19. Tropical Agricultural Research Center, Ministry of Agriculture, Japan, P.145-153.

Rossel, H.W., & Thottappilly, G. (1985). Virus Diseases of Important food Crops in Tropical Africa. IITA publication series, IITA, Ibadan, Nigeria, 67p.

Rossel, H.W., Thottappilly, G., Adeoti, A.A., Alam, K.Z. (1982a). A new record of rice yellow mottle virus disease in Nigeria. *International Rice Commission Newsletter 29:51-53.*

Rossel, H.W., Thottappilly, G., & Buddenhagen, I.W. (1982b). Occurrence of rice yellow mottle virus in two important rice growing areas of Nigeria. *FAO-Plant Protection Bulletin 31:137-139.*

Ryder, K., & Gerhardson, B. (1978). Rodklovermosaikvirus sprids med. *Slatter maskiner. Vaextskyddsnotiser 43:112-115.*

Rouw, A. (1993). Influence du raccourcissement de la ja chere sur l'enherbement et la conduite des systemes de culture en zone forestiere. Pages 257-266. In: Proc. International meeting on La Jachere en Afrique de L'ouest, Montpellier, 2-5 Dec., 1991. ORSTOM, Paris.

Sawadogo, A., & Thio, B. 1995. Les nematodes racinaires du riz irrigue au Burkina et a L'Office du Niger au Mali. Paper presented at Irrigated Rice in the Sahel: Prospects for Sustainable Development, WARDA/ADRAO Sahel Research Center Ndiaye, Senegal, March 27-31, 1995.

Seghal, O.P. (1981). Southern Bean Mosaic virus group In: HandBook of Plant Virus infections, comparative diagnosis (E. Kurstak, ed), P91-121. Elsvier/North Holand Biomedical Press, Amsterdam.

Seghal, O.P., Abou-Haidar, M.G., Gellatly, D.L., Ivanov, I., and Thottappilly, G. (1993). An associated small RNA in Rice Yellow Mottle Sobemovirus homologous to the satellite RNA of Lucerene transient streak sobemovirus. *Phytopathology 83:1309-1311*

Selman, B.J. (1973). Beetles-Phytopagous Coleoptera. In: Viruses and Invertebrates (Gibbs, R., ed). North Holland Publishing Company, London P.57-177.

Shoyinka, S.A., Bozarth, R.F., Reese, J., & Rossel, H.W. (1978). Cowpea mottle viruses: a seed-borne virus withd istinctive properties infecting cowpeas in Nigeria. *Phytopathology 68:693-699.*

Singh, B.N. (1995). Breeding for Rice Yellow Mottle Virus resistance. Paper presented at the 1st Inter. Symposium on RYMV, Mbe, 18-22 Sept., 1995, WARDA/ADRAO, Bouake, Cote d'Ivoire.

Slykhuis, J.T. (1955). *Aceria tulipae* Kajer *(Acarina Eryophyidae)* in relation to the spread of wheat streak mosaic. *Phytopathology 45:116-128.*

Slykhuis, J.T. (1967). Virus Diseases of Cereals. *Review of Applied Mycology 46:401-429*

Sy, A.A. (1994). The Integrated Pest Management-Task Force (IPM-TF) Monitoring Tour in Mali, November, 1994 (Available at WARDA library).

Sy, A.A., & Akator, K. (1993). Varietal resistance profiles for rice yellow mottle virus. In: WARDA Annual Report for 1992, WARDA, Bouake, Cote d'Ivoire, pp. 20-21.

Sy, A.A., Alluri, K., & Akator, K. (1993). Selection of RYMV resistant and estimation of RYMV induced yield loss under natural and artificial pressure. In: WARDA Annual Report for 1993, WARDA, Bouake, Cote d'Ivoire, pp. 44-45.

Sy, A.A., Heinrichs, E.A., & Jones, P.M. (1991). Rice yellow mottle virus detection and distribution at Mbe and Gagnoa. In: WARDA Annual Report for 1991. WARDA Bouake, Cote d'Ivoire, pp. 59-60.

Sylvester, E.S. (1956). Beet yellow virus transmission by the green peach aphid. *Journal of Economic Entomology 49:789-800.*

Takata, K. (1896). Results of experiments with dwarf disease of rice plant (In Japanese). *Japanese Journal of Agricultural Society* ***171:1-4, 172:13-32.***

Talwalker, R.T., Garg, N.K., & Khrishna, M.C.R. 1965. Rice bran-A source material for Pharmaceuticals. *Journal of Food Science and Technology* 2: 117-119.

Taylor, C.E., & Robertson, W.M. (1977).Virus vector relationships and mechanics of transmission. *Proceedings of The American Phytopathological Society* ***4:20-29.***

Taylor, D.R. (1989). Resistance of upland rice varieties to pale yellow mottle virus (RYMV) disease in Sierra Leone. *IRRN* ***4:1.***

Taylor, D.R., Fofie, A.S., & Suma, M. (1990). Natural infection of Rice yellow mottle virus on rice in Sierra Leone. *IRRN* ***15(5):9.***

Teakle, D.S. (1986). Abiotic transmission of Southern bean mosaic virus in soil. *Australian Journal of Biological Science* ***39:353-359.***

Thottappilly, G. (1992). Plant Virus Diseases of Importance to African Agriculture. *Journal of Phytopathology* ***134:265-288.***

Thottappilly, G., & Rossel, H.W. (1993). Evaluation of resistance to rice yellow mottle virus in *Oryza* species. *Indian Journal of Virology* ***9(1):65-73.***

Thottappilly, G., Vanlent, J.W.M., Rossel, H.W., & Seghal, O.P. (1992). Rottboellia yellow mottle virus, a new Sobemovirus affecting *Rottboellia cochinchinensis* (Itch grass) in Nigeria. *Annals of Applied Biology* ***1120: 405-415.***

Thresh, J.M. (1989). Insect borne viruses of rice and the green revolution. *Tropical Pest Management* ***35:***264-272.

Thresh, J.M. (1991). The ecology of tropical plant viruses. *Plant Pathology* ***40:324-339.***

Thresh, J.M., & Pitcher, R.S. (1978). The spread of nettlehead and related virus diseases in hop. In: plant disease Epidemiology (P. Scott and A. Bainbridge, eds), PP 291-298. Blackwell, Oxford.

Tinsley, T.W. (1973). Origins of vector borne plant viruses. In: Insect/plant relationships (Van Embden, ed), Blackwell publication, Oxford, UK.pp43-50.

Todd, J.M. (1958). Spread of potato virus x over a distance. *Proceedings of Conference on Potato Virus Disease. 1957, pp 132-143.*

Tomlinson, J.A. (1962). Control of lettuce mosaic by the use of healthy seed. *Plant Pathology* ***11:62-64***

Trudgill, D.L., Brown, D.J.F., & McNamara, D. (1983). Methods and criteria for assessing the transmission of plant viruses by Longidorid nematodes. *Revue de Nematologie 6:133-141.*

Tsuboi, T., Goto, A., Boua, B., & Koto, H. (1995). Outbreak of Rice Yellow Mottle Virus in Paddy Rice in Bandoma Basin of Cote d'Ivoire and its Epidemiological Causes. Paper presented at the 1st International Symposium on RYMV from 18-22 sept., 1995, WARDA, Mbe, Bouaké, Cote d'Ivoire.

Uyemoto, J.K., & Grogan, R.G. (1977). Southern bean virus: Evidence for seed transmission in been embryo. *Phytopathology 67:1190.*

Van Hoof, H.A. (1959). The Delphacid *Sogata cubana* vector of a virus of *Echinochloa colonum* (in Dutch & English), *Tijdschr Plantenzeikt 65:188-189.*

Wallace, H.R. (1983). Interactions between nematodes and other factors on plants. *Journal of Nematology 15:221-227.*

Walkey, D.G.A., & Whittingham Jones S.G. (1970). Seed transmission of Strawberry latent ringspot virus in celery (*Apium graveolens* Var. *Dulce*). *Plant Disease Reporter 543:802-802.*

Walters, H.J. (1969). Beetle transmission of plant viruses. *Advances in Virus Research 15:339-363.*

WARDA (West AFrica Rice Development Association), (1982). Special Project. Annual Research Report, Freetown, Sierra Leone, WARDA, 1983.

WARDA, (1983). Special Project. Quinquennial Review Provisional Report, February, 1983, Freetown, Sierra Leone.

WARDA, (1993). Rice Trends in Sub-Saharan Africa: A synthesis of Statistics on Rice Production, Trade and Consumption. WARDA, 01 B.P. 2551. Bouake, Cote d'Ivoire.

WARDA, (1996). WARDA research boosts rice production. African Farming Magazine, January/February Issue (1996) p.8.

Watson, M.A., & Roberts, F.M. (1940). Evidence against the hypothesis that certain plant viruses are transmitted mechanically by aphids. *Annals of Applied Biology 27:237-283.*

Webster, R.K. (1992). Introduction. In: Compedium of Rice Diseases (Webster R.K., & Gunnel P.S., eds), APS Press. *The American Phytopathological Society MV. pp. 62.*

Weischer, B. (1993). Nematode-virus interactions In: Nematode interactions (M.M. Khan, ed.). Chapman and Hull publication, London, P. 223-231.

Wetter, C. (1975). Tabakwosaikuirus und para tabakmosaik virus in Zigaretten. *Naturwissenshaftein 62:533.*

Williams, J.T. (1978). Genetics Resources of African Rice. *International Rice Commission Newsletter 27(1):1-3.*

Williams, R.D. (1981). Complimentary interactions between weeds, weed control practices and pests in horticultural cropping systems. *Horticultural Science 16(4):508-513.*

Wilson, F.B. (1944). Emergency food production in Zanzibar. *East African Agriculture Journal 10:93-101.*

Wutscher, H.K., and Shull, A.V. (1975). Machine-hedging of citrus trees and transmission of exocortis and xyloporosis viruses. Plant Disease Reporter *59:368-369.*

Yang, A.F., & Hamilton, R.I. (1974). The mechanism of seed transmission of Tobacco ringspot virus in Soybean virology. *62*:26-37.

Yoboue, W.N. (1989). Screening for resistance to RYMV in Cote d'Ivoire. In: Report on Monitoring tours to West African countries,1988 and 1989, IRTP, Africa, P.50-51.

Zan, K., Perez, A.T., Alluri, K., & Ng, N.Q. (1981). Problems and approaches on genetic improvements of rice in Sub-Sahelian Africa. Mimeographed report.

Appendix 1. Scoring system for rice yellow mottle virus (RYMV)

Score	Resistance[a]	Leaf colour	Stunting	Flowering	Sero-diagnosis[b]	
					ELISA	Agar-gel diffusion
0	HR	Green	Nil	Normal	-	-
1	R	Green	Nil	Normal	+	±
2	R	Green	Nil	Normal	+ +	+
3	R	Green with sparse dots/streaks	Negligible	Normal	+ + +	+
4	MR	Green with visible mottling	Slight	Normal	+ + +	
5	MR	Light green, mottled	25%	Slight delay	Highly Positive	
6	S	Pale green	50%	Delayed	Highly Positive	
7	S	Pale yellow	75%	Delayed	Highly Positive	
8	S	Yellow	>75	No flowering	Highly Positive	
9	HS	Yellow, orange	>75	Death of Plants	Highly Positive	

[a]HR = Highly resistant, R = Resistant, MR = Moderately resistant, S = Susceptible, and HS = Highly susceptible, [b]± = Weak (doubtful), - = Negative, + = Low virus content, + + = High virus content, + + + = Very high virus content

Source: IRRN (1987)

Appendix 2. Rice yellow mottle virus (RYMV) scale for field test at growth stages from 4 weeks and above.

Scale	Descriptions/Symptoms
1	No symptoms
3	Leaves green but with spare dots or streaks and less than 5% of height reduction
5	Leaves green or pale green with mottling and 6 to 25% of height reduction, flowering slightly delayed
7	Leaves pale yellow or yellow and 26-75% of height reduction, flowering delayed
9	Leaves turn yellow or orange, more than 75% of height reduction, no flowering or some plants dead

Source: IRRI (1988)

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Appendix 3

Date: June 28, 1996

Our ref.: SEA/lc/280696

Dr. A. A. Sy
West Africa Rice Development Association
WARDA
01 B.P. 2551
Bouaké
Ivory Coast

Dear Dr Sy

This in to inform you about the partial result of the testing of the rice seed sample no IR 1529-680-3 (our acc.no. 39154) for seed-transmitted (ST) RYMV.

Out of 750 seeds planted, 460 seedlings developed. No symptoms were observed after 2 - 4 weeks of growth. All 460 seedlings, in groups of 2 seedlings, were tested by ELISA against anti-RYMV-serum obtained from ATCC, USA (no. PVAS515). The negative control material was known healthy rice seedling extract. The positive control was ATCC (no. PV515) received as a purified RYMV and used in ELISA at 1:100 and 1:1000 dilutions.

All tested seedlings reacted clearly negatively with absorption values not exceeding 0.02 while positive controls at the same time showed 0.5 - 0.7, ie at least 25 x higher values than the test material.

Since, so far, no ST viruses in rice have been reported, a possible RYMV ST may occur only in traces. To examine that we have to continue with the work testing many more seeds of the above sample. The next series of tests will be started soon. If you have more similar seed samples of other cvs (cvs may differ in ST) you could, perhaps, send them with Henrik.

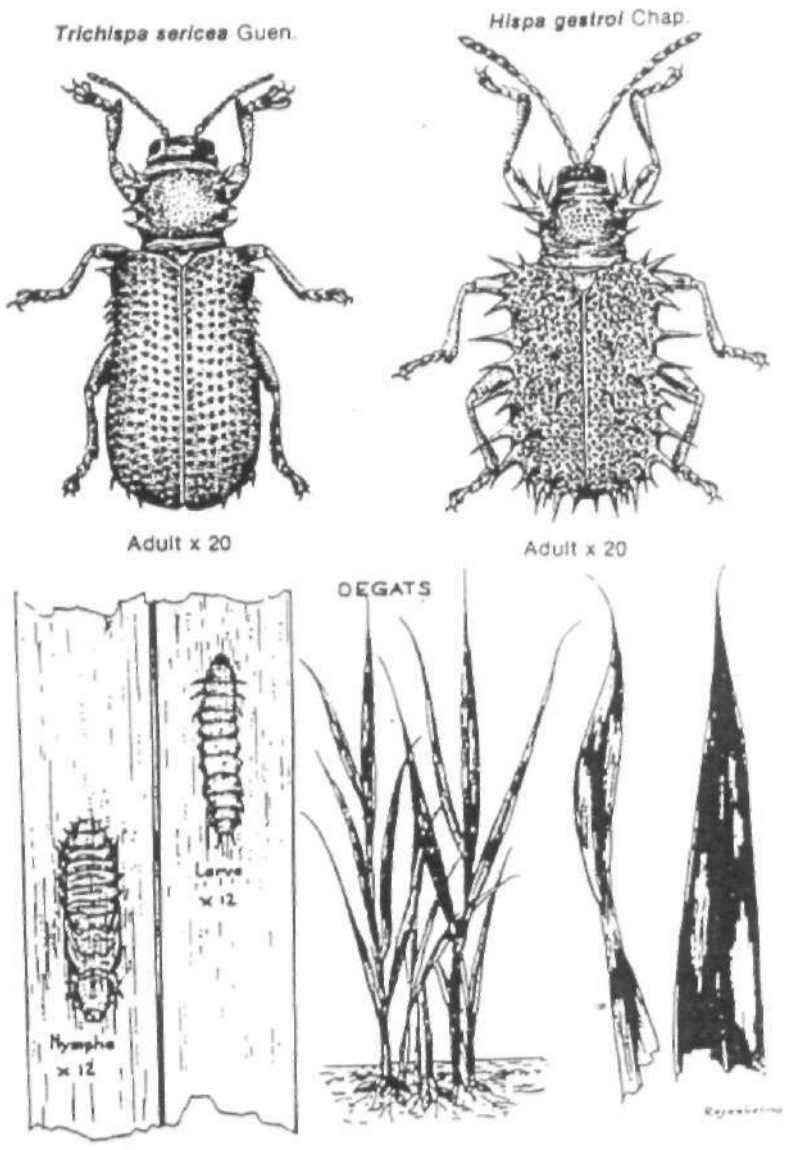
With best regards

Yours sincerely

Sven Erik Albrechtsen
Associate Professor

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Appendix IV Hispidae



The life cycle of the Hispidae (Chrysomelidae-Hispinae):
Life span: egg: 3 to 4 days; larva: 9 to 17 days; pupa: 4 to 5 days.
Preoviposition: 7 days. Complete development takes 4 to 5 weeks in the dry season and 7 to 8 weeks in the rainy season.
Source : Breniere (1983).

BIOGRAPHY

1. NAME: ABO, Myimaorga Emmanuel

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3. PLACE OF BIRTH: Kendev/Ngenev, Nigeria

4. LOCAL GOVERNMENT OF ORIGIN: Ukum

5. STATE OF ORIGIN: Benue

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7. MARITAL STATUS: Married

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(ii) National Youth Service Corps (NYSC), Wudil, Kano, Kano State, Nigeria	1985-1986
(iii) Educational Officer, Ministry of Education, Kano State, Nigeria	1986-1988
(iv) Research Officer, National Cereals Research Institute (NCRI), Baddeggi, Niger State, Nigeria	1990-

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(iii)	University of Jos, Jos, Nigeria.	1981-1985

(iv)	University of Jos, Jos, Nigeria.	1988-1990
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(iv)	M.Sc.-Applied Microbiology & Plant Pathology	1990
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15. PUBLICATIONS/CONTRIBUTIONS TO KNOWLEDGE:

(A) THESES:

(1) Abo, M.E. (1985)
Influence of rosette virus disease on growth and productivity in groundnut (*Arachis hypogea* L). B.Sc. Dissertation, University of Jos, Jos, Nigeria.

(2) Abo, M.E. (1990)
The Effects of Bean yellow mosaic virus on growth and yield of cowpea (*Vigna unguiculata* (L) Walp. M.Sc. Dissertation, University of Jos, Jos, Nigeria.

(3) Abo, M.E. (In View)
Studies on The Mode of Transmission of Rice Yellow Mottle Virus. Ph.D Thesis, Ahmadu Bello University, Zaria, Nigeria.

(B) SCIENTIFIC JOURNALS (NATIONAL/INTERNATIONAL):

(1) Abo, M.E. (1985)
Sex Determination in *Drosophila melanogaster*. *Euphorbia* **5**:45-48

(2) Abo, M.E., & Akueshi, C.O. 1992. Reactions of Cowpea Cultivars to Bean yellow mosaic virus isolate under greenhouse conditions in Nigeria. *East Afr. Agric. & Forestry Journal* **58(1)**:49-53.

(3) Abo, M.E., & Alegbejo, M.D. 1997.
Strategies for Sustainable Control of Virus Diseases of Some Crops in Nigeria. *Journal of Sustainable Agriculture* **10 (2/3)**:57-80.

(4) Abo, M.E., Alegbejo, M.D., & Kashina, B.D. 1996.
The Control of plant Viruses and Virus Diseases. (*Noma News Magazine* **13** (Accepted for publication: 18/3/1996-In Press).

(5) Abo, M.E., & Okusanya, B.A. 1996.
Incidence and Variability Reaction of Sugarcane smut (*Ustilago scitaminea* Syd.) Isolates in greenhouse and laboratory tests in Nigeria. *Discovery & Innovations* **8(3)**:227-231

(6) Abo, M. E., & Sy, A. A. 1998. Rice Virus Diseases: Epidemiology and Management Strategies. *Journal of Sustainable Agriculture* $11(^2/_3)$:113-134.

(7) Abo, M.E., Sy, A.A., & Alegbejo, M.D. 1998. Rice Yellow Mottle Virus (RYMV) in Africa: Evolution, Distribution, Economic Signifance on Sustainable Rice Production and Management Strategies. *Journal of Sustainable Agriculture* $1(^2/_3)$*:85-111.*

17. RESEARCH INTERESTS:

Plant Virus Epidemiology and Integrated Pest & Disease Management.