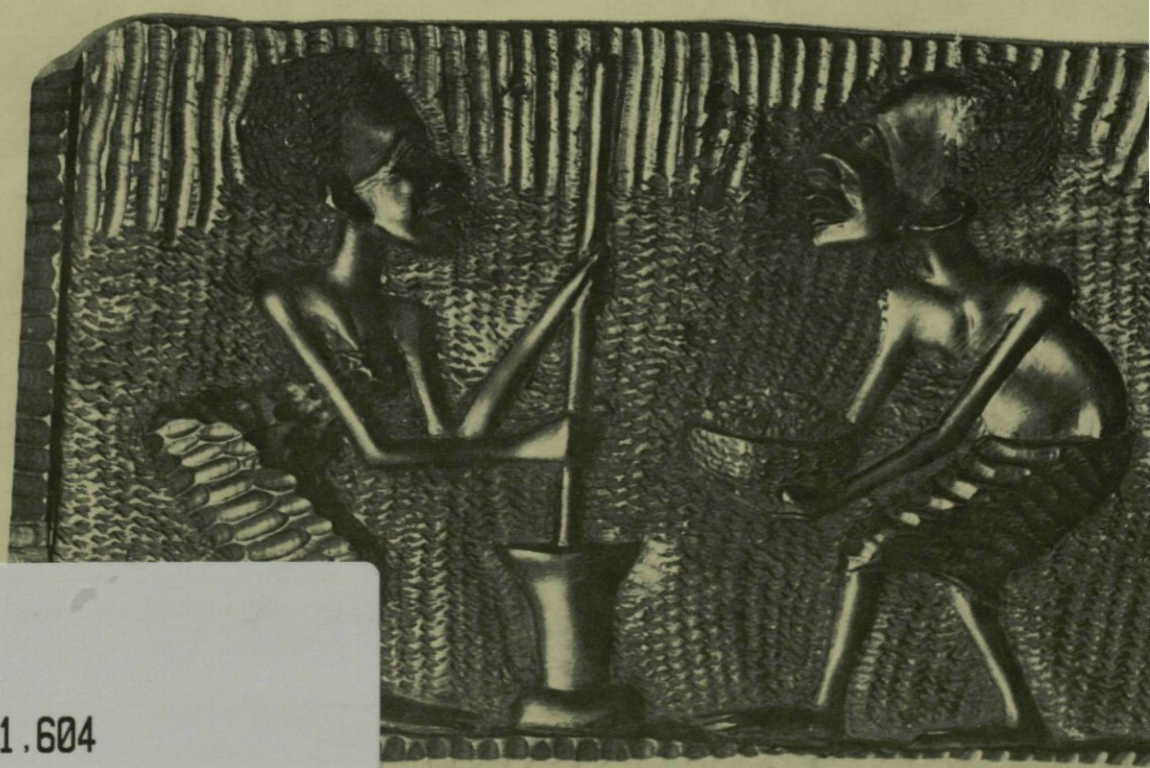
Characterization and ecological aspects of rice yellow mottle virus in Kenya

W. Bakker



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Proefschrift
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Abstract

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Also: Agric. Res. Rep. (Versl. landbouwk. Onderz) 829.

Rice yellow mottle virus (RYMV) is the causal pathogen of yellow mottle of rice in the area around Kisumu near Lake Victoria in Kenya. Affected plants show a yellow or orange discolouration of the leaves, reduced tillering and stunting of the plants, and sterility of the flowers. The results of this study gave the following cryptogram for RYMV: R/1:1.4/23:S/S:S/Cl. RYMV is easily mechanically transmissible. Experimentally a limited number of plants belonging to the family Gramineae only were found to be a host for the virus. Rice was the only host found naturally infected with RYMV. Beetles belonging to the subfamilies Criocerinae, Cryptocephalinae, Galerucinae, Halticinae and Hispinae of the family Chrysomelidae, and the long-horned grasshopper *Conocephalus merumontanus* Sjöstedt (Tettigoniidae) also transmitted the virus .*Chaetocnema pulla* Chapuis (Halticinae) proved to carry the virus in the field.

RYMV is a stable virus with a particle diameter of about 25 nm and sediments as a single component. The base composition of the RNA is similar, but not identical to that of cocksfoot mottle virus. Serologically no relationship nor any other affinity was established between RYMV and a number of other isometric plant viruses of comparable size.

Infected rice is considered to be the main source of the virus in the rice fields. Control of the disease must be sought in cultivation practices and growing of rice with short vegetative periods. Breeding of rice varieties for resistance to RYMV has to be initiated.

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This thesis will also be published as Agricultural Research Reports 829.

Front cover: detail of Makonde relief panel.

Stellingen

I

Bij het onderzoek naar de waardplantenreeks van een virus, ongeacht in welke plant aangetroffen, dienen meer tropische cultuurgewassen betrokken te worden.

II

De term 'lege' (empty) virusdeeltjes gebaseerd op elektronenmicroscopische beoordeling van met fosforwolframaat gecontrasteerde veelkantige virussen, terwijl met andere methoden de aanwezigheid van deze deeltjes niet in de virussuspensie aangetoond is, moet vervangen worden door de term 'inwendig gekleurde' (stain penetrated) virusdeeltjes.

J. A'Brook, 1972. *Lolium* mottle virus. Pl. Path. 21: 118-120.
E. P. Serjeant, 1967. Some properties of cocksfoot mottle virus. Ann. appl. Biol. 59: 31-38.

III

De waarde toe te kennen aan de vergelijkende virologie zal belangrijk toenemen indien virussen onder gelijke omstandigheden worden bestudeerd.

F. Brown & R. Hull, 1973. Comparative virology of the small RNA viruses. J. gen. Virol. 20: 43-60.

IV

Bij de biologische bestrijding van onkruiden door introductie van fytofage kevers zal meer aan het uitgangspunt van Huffaker (het veiligheidsaspect) voldaan moeten worden.

C. B. Huffaker, 1964. Fundamentals of biological weed control. In: DeBach (ed.): Biological control of insect pests and weeds. Chapman and Hall, Londen, p. 631-649.

V

Het onvoldoende aantal genenbanken, die bovendien geografisch slecht gespreid zijn, getuigt van een weinig planmatige aanpak ter verbetering van de huidige wereldvoedsel-situatie.

VI

De sterk stijgende behoefte aan voedsel in de ontwikkelingslanden, maakt een aanvulling van de vaak zeer onvolledige kennis van de aldaar voorkomende planteziekten en plantebeschadigers door een gerichte inventarisatie noodzakelijk.

VII

In Kenya worden bij de bestrijding van planteziekten sanitaire- en cultuurmaatregelen te weinig toegepast.

VIII

De anatomische- en cytologische kennis van de rijstplant is bij vele van haar onderzoekers onvoldoende.

IX

De door de EEG ingestelde invoerbeperkende maatregelen ten aanzien van insekticidenresidus dragende groenten uit ontwikkelingslanden, kunnen bijdragen tot milieubewustwording en verbetering van de tuinbouw in deze landen.

X

Het invoeren van een 'gezondheidsboekje' waarin gegevens over vaccinaties, allergiën, bloedgroep, medische indicaties en doorlichtingen opgenomen kunnen worden, zou een belangrijke vereenvoudiging van een ieders persoonlijke gezondheidsadministratie betekenen en kunnen bijdragen tot een verbetering van de gezondheidszorg.

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Acknowledgements

During this investigation which I carried out while based at the National Agricultural Laboratories at Nairobi, Kenya, and afterwards at the Laboratory of Virology, Agricultural University at Wageningen, the Netherlands, many people helped me in one way or another. I wish to express my sincere gratitude to all of them.

In particular I am most grateful to Professor Dr J. P. H. van der Want for his interest, help and valuable criticism during the course of this work. I am also indebted to Mr J. J. Ondieki, Senior Plant Pathologist, for providing good working conditions in Nairobi, and to the Kenya Ministry of Agriculture and the 'Directie Internationale Technische Hulp' at the Hague for giving me the opportunity to finish this study at Wageningen.

Special thanks are also due to the specialists who identified the biological specimens and to the fellow-workers of the different laboratories and institutes in Kenya and at Wageningen for their helpful discussions. Miss M. de Geus, Mr K. Boekhorst, Mr W. C. Th. Middelplaats and Mr F. J. J. von Planta deserve special mention for their care in preparing the figures, and Miss M. Usmany, Mr J. Groenewegen and Mr H. Lohuis for their instructions and help with the electron microscopy and photography. In thanking Mr G. Looijen and Mr J. Mukoko I want to express my thanks to all who helped me in rearing the plants. I also wish to acknowledge the assistance of Mr G. C. Maan. Finally I thank Mr R. J. P. Aalpol for editing the manuscript and Mrs E. Brouns-Murruy for correcting the English text.

Curriculum vitae

Willem Bakker werd op 26 juli 1937 geboren te Harlingen. In Groningen bezocht hij de Mulo en de Rijks Middelbare Landbouwschool, waarvan hij in 1957 het diploma behaalde. Na de militaire dienst begon hij in 1959 met zijn studie aan de Landbouwhogeschool te Wageningen. In 1963 legde hij het kandidaatsexamen in de richting Planteziektenkunde af en was tijdens zijn praktijktijd werkzaam aan het station van de Servicio Shell para el Agricultor te Cagua in Venezuela. Hij behaalde in 1966 het ingenieursdiploma met als hoofdvak virologie en als bijvakken entomologie, biochemie en plantensystematiek en -geografie van de tropen en subtropen.

In september 1966 werd hij door de Directie Internationale Technische Hulp van het Ministerie van Buitenlandse Zaken uitgezonden naar Kenya waar hij als viroloog werd verbonden aan de National Agricultural Laboratories te Nairobi. Daar onderzocht hij virusziekten van land- en tuinbouwgewassen, waaronder aardappelen, rijst, suikerriet, tarwe, courgette en passievruchten. De meeste aandacht vroeg de 'yellow mottle' van rijst.

Van januari 1973 tot mei 1974 zette hij het onderzoek van het 'rice yellow mottle virus' voort aan het Laboratorium voor Virologie van de Landbouwhogeschool te Wageningen. Sindsdien is hij als leraar in de biologie verbonden aan de Rijks Hogere Landbouwschool te Groningen.

Contents

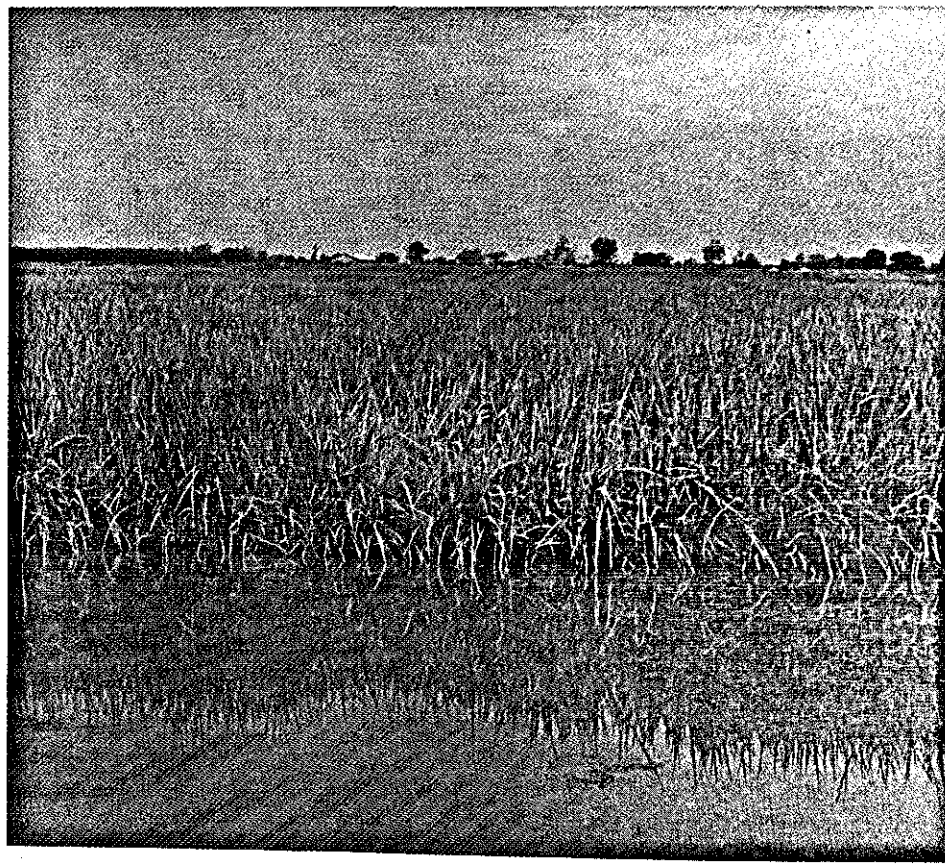


Fig. 1. 'Sindano' rice affected by RYM.

1 Introduction

In November 1966, Mr J. M. Onyango of the District Agricultural Office at Kisumu sent some diseased rice plants from smallholders in the district to the plant pathology department of the National Agricultural Laboratories (NAL) in Nairobi. Mr N. K. Patel isolated a number of fungi from the specimens, but they were not considered to be the cause of the disease, nor was the condition attributed to nematode or insect damage, while Mr A. H. Ramos, bacteriologist, excluded bacteria as causal organisms. Subsequently I was requested to pay a visit to the rice fields at Otonglo near Kisumu along the shore of the Kavirondo Gulf of Lake Victoria, where the disease had been present for a number of years (Fig. 4). After discussions with Mr. Onyango, who had made accurate observations, I suspected that a virus disease was the cause of the affection. From almost full grown 'Sindano' rice collected in the field, I ground leaves and inoculated diluted sap mechanically onto rice, while a number of plants were cut back and planted. The first mechanical inoculation was unsuccessful, but a second attempt with young leaves from the potted plants resulted in systemic symptoms in the inoculated rice. After passage of sap through a Seitz filter, this sap still proved infective, so providing additional evidence that the cause might be of viral nature. An analysis of the soil from the rice fields by the soil chemistry department of the NAL gave no indication that the condition of the soil was responsible for the affection of the rice plants.

In the disease stricken area, the construction of the Ahero Pilot Scheme – a pilot scheme for irrigated crops, rice being planned as the principal crop with two harvests a year – was about to be started and made the study of the disease a first priority.

When sufficient evidence was obtained that the disease was caused by a virus which did not resemble any known virus, I proposed the name rice yellow mottle (RYM) and its causal agent was named rice yellow mottle virus (RYMV), (Bakker, 1970). Additional data about the vectors of RYMV have been published previously (Bakker, 1971).

1.1 Earlier described virus and mycoplasma diseases of rice

In the traditional rice growing countries many diseases, mostly under local names, and then of unknown etiology have been known in rice for many years. Rice dwarf has been studied in Japan since the turn of this century, while in 1957 hoja blanca was recognized as a virus disease in the Americas (in Ou, 1972), being the first disease of viral nature of rice outside Asia. By the work done at, or in co-operation with, the

International Rice Research Institute in the Philippines, the causal pathogens of many of the early recognized disorders of rice have been identified.

Since 1967, when mycoplasmas were found in plants that until then were thought to be affected by a virus, mycoplasma-like bodies have been observed in the phloem of rice yellow dwarf affected plants, as well as in its vectors (Maramorosch et al., 1970). The remission of rice stripe affected plants after tetracycline treatment, indicates that the pathogen may be a mycoplasma, although no electron microscopic study has been reported to prove this hypothesis (Maramorosch et al., 1970). Spherical virus-like particles have, however, been reported as the cause of this disease to (in Ou, 1972).

Leafhoppers (Cicadellidae) and plant hoppers (Delphacidae) transmit most of the rice viruses, mostly in a persistent or transovarial manner, but tungro is unique in the leafhopper-borne viruses by having a non-persistent virus-vector relationship, while these insects are the only known vectors of mycoplasmas in rice.

Mosaic of rice, reported from the Philippines (Martinez et al., 1960) is, however, mechanically transmissible from rice to maize, but further information is lacking. The symptoms induced by this virus in rice are an irregular shaped mottling, varying from greenish dots to elongated yellowish-green lesions which may coalesce to form chlorotic streaks. Mottling is also observed on the leaf sheaths. Severely affected plants are stunted and in later stages of development the infected leaves turn yellow-brown to brown and eventually wither. Affected plants produce few tillers. One of the mosaic inducing viruses of grasses is suspected to be the cause of the disease.

The recently described necrosis mosaic from Japan (in Ou, 1972) is caused by a soilborne virus. Infected plants are slightly stunted and have a reduced number of tillers. Instead of an erect growth, affected plants spread and assume a decumbent growth habit. Mosaic symptoms are first noticed in the lower leaves and later in the upper ones.

A list of the known virus and mycoplasma diseases of rice other than RYMV, abstracted from Ou (1972) and Maramorosch et al. (1970) is summarized in Table 1. For further information about the noted diseases, the reader is also advised to peruse 'The virus diseases of the rice plant' (IRRI, 1969).

Experimentally rice proved to be a host for the following viruses: sugarcane mosaic virus (Anzalone, 1963), barley yellow dwarf virus (in Ou, 1972), barley stripe mosaic virus, brome mosaic virus (Kahn & Dickerson, 1957), maize dwarf mosaic virus (Brambl & Dale, 1967), maize rough dwarf virus, oat pseudo-rosette virus (in Smith, 1972), ryegrass mosaic virus (Mulligan, 1960), African cereal streak virus (Harder & Bakker, 1973), and wheat streak mosaic virus (Podkin & Panarin, 1972).

1.2 Symptoms in field grown RYM affected rice

The characteristic symptoms of RYM are a discolouration and stunting of the plants. The discolouration is noticed about 2 – 3 weeks after transplanting and depending on the variety, the leaves turn yellowish ('Sindano') (Fig. 1), mild yellowish-green

Table 1. Virus and mycoplasma diseases of rice. Abstracted from Ou (1972) and Maramorosch et al. (1970).

Disease	Pathogen	Transmitted by	Pathogen-vector relationship	Form of particle	Distribution	Possible related viruses/diseases
Black-streaked dwarf	virus	leafhoppers	persistent	spherical	Japan	serologically related to maize rough dwarf virus[1]
Dwarf	virus	leafhoppers	transovarial	spherical	Japan, Korea (?), China (?)	
Giallume yellows	mycoplasma			pleomorphic	Italy	
Grassy stunt	mycoplasma	plant hopper		pleomorphic	Philippines, Ceylon, India (?), Indonesia[2]	
Hoja blanca	virus	plant hoppers	transovarial	spherical[3] or flexuous thread[4]	North, Central and South America	
Mosaic	virus	mechanical ways			Philippines	
Necrosis mosaic	virus	soil		rod	Japan	serologically similar to barley and wheat yellow mosaic viruses[6]
Orange leaf	virus	leafhopper	persistent		Philippines, Thailand, Ceylon	
Stripe	virus[5]	leafhoppers	transovarial	spherical	Japan, Korea	
Transitory yellowing	virus	leafhoppers	persistent	bullet-shaped	Taiwan	
Tungro	virus	leafhoppers	non-persistent	spherical	Philippines	Penyakit merah - Malaysia Mentek - Indonesia Yellow-orange leaf - Taiwan
Yellow dwarf	mycoplasma	leafhoppers	persistent	pleomorphic	throughout Asia	

1. Luisoni et al., 1973. 2. Tantera et al., 1973. 3. Herold et al., 1968. 4. Shikata & Galvez-E., 1969. 5. Or mycoplasma (Maramorosch et al., 1970). 6. Ishii, 1973.

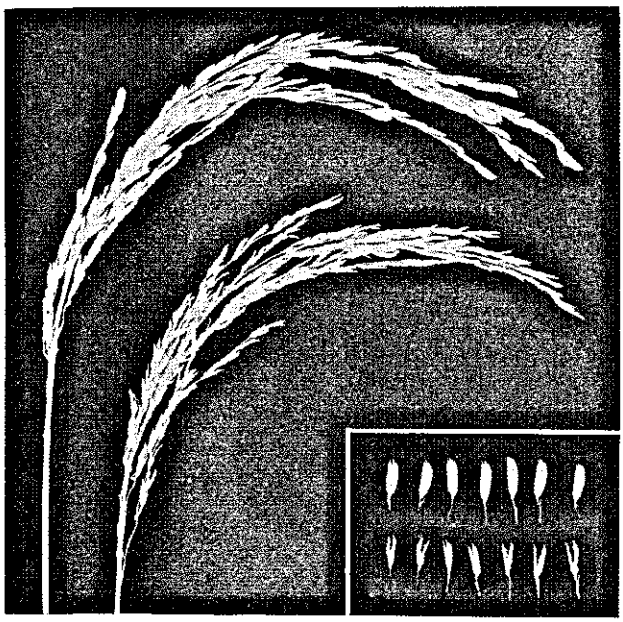


Fig. 2. Panicles and spikelets of field grown 'Sindano' rice before being fully developed. Upper figures from healthy rice, lower figures from RYM affected plants.

('Basmati 217') or orange ('IR 8'). The youngest leaves show mottling. In 'Basmati 217' the symptoms are difficult to distinguish, but are more pronounced in fresh growth of ratoon plants. In some varieties, especially the IRRI varieties, a mostly orange discolouration of the older leaves was noticed in plants whose youngest leaves did not show mottling. These plants proved not to be infected with RYMV and the cause may be damage at transplanting or insect damage (*Locris* spp.?).

Infected plants produce less tillers, especially marked when infection occurs at an early stage of growth. Although diseased plants usually survive, the heads produce grains which are unfilled (Fig. 2). The effect of the disease on yield depends on the time of infection of the rice plant and the variety. In 1966 an estimated yield reduction of 50% occurred in 'Sindano' at Otonglo. When blast (*Pyricularia oryzae* Cav.) occurs too, only very low yields are to be expected in varieties susceptible to these diseases, as is the case with 'Sindano' rice.

1.3 Scope of this study

This study was carried out primarily to find ways of controlling RYM. Therefore I had to become acquainted with the rice cultivation in East Africa, more specifically with the cultivation of this crop in Kenya. Diagnosis and determination of the basic properties of the causal agent as well as its ways of transmission had to be studied.

Thus the host range and the effect of the pathogen on rice and other hosts were determined throughout the study. From the beginning of the study the search for the vector and purification of the virus was undertaken in Nairobi. Soon after the first electron micrographs of purified virus were received from the Laboratory of Virology at Wageningen, a beetle species was found that could transmit the virus. Attention was consequently directed towards these insects. Several beetle species were tested and relations between virus, vector and host plant were determined with common beetles that were able to transmit the virus. Information about these or related insects was collected.

From the observations in the fields, supported by laboratory experiments, information about the ecological aspects of the virus was obtained and ways in which the disease could be controlled were looked for. In Nairobi information about the properties of the virus in crude sap and an antiserum to RYMV were also obtained. With this I continued after my return to Wageningen in 1973. Here the main interest was the intrinsic properties of RYMV and its relation to other simiarly shaped viruses. From the results obtained in Kenya and at Wageningen, RYMV was sufficiently characterized to provide a base for control, but at the same time one could understand the difficulties involved.

Throughout the study phenomena of a more fundamental nature were encountered and these are also noted.

2 Rice in East Africa

Rice, *Oryza sativa* L., is the main staple cereal in tropical areas especially in Southern and Eastern Asia, but it is only of limited importance in Africa. The genus *Oryza* gives its name to the tribe Oryzeae, to which belong several annual or perennial herbs, usually aquatic. The genus *Oryza* comprises about 25 species, which grow in the tropical and subtropical regions of Africa, Asia, Northern Australia and Central and South America. These species are probably derived from two genetical sources, the main one in Asia the other in Africa from which the species in South America have also been said to originate (Grist, 1968). Purseglove (1972) stated however, that Africa with the largest number of endemic species i.e. 9, is usually considered the centre of origin of the genus.

Two *Oryza* species are cultivated: *O.sativa* L., the common rice, evolved in South East Asia and cultivated in the warmer regions throughout the world, and *O.glaberrima* Steud., evolved in Africa and grown in the flood plains of the Sahel and Sudan zones of West Africa where water is largely uncontrolled (Purseglove, 1972).

In East Africa, apart from *O.sativa* L. as minor crop, the following *Oryza* species are found: *O.barthii* A.Chev., *O.eichingeri* Peter, *O.punctata* Steud. and *O.longistaminata* A.Chev. et Roehr. Other genera belonging to Oryzeae occurring in East Africa are *Leersia* represented by *L.denudata* Launert, *L.drepanothrix* Stapf, *L.friesii* Meld., *L.hexandra* Sw. and *L.tisserantii* (A.Chev.) Launert, while *Maltebrunia* is represented by *M.schliebenii* (Pilg.) C. E. Hubbard, according to Clayton (1970).

Little is known about when the cultivation of *O.sativa* L. spread to East Africa, but this crop was certainly grown on the East African coast before the arrival of the Portuguese (Greenway, 1945), i.e. about 1 500.

2.1 Cultivation

In Kenya, Uganda and Tanzania, agriculture dominates the economy. The climate and physical conditions allow the growing of tropical as well as of moderate crops. The main cereal crops are maize, sorghum, finger millet (*Eleusine coracana* (L.) Gaertn.) and wheat, while the acreage under rice when compared to these cereals is small, but expanding. Large areas can be made suitable for irrigated rice growing, providing work and higher income for many families while by diversification of the food production a more stable food supply for the fast growing population would be obtained.

Rice is grown for own consumption and in increasing amounts as a cash crop. On

the island Zanzibar, rice is the staple food (Wilson & Tidbury, 1944) but does not usually have this status in the other production areas. Asian and Arabic communities and to a growing extent, the Africans purchase the product (Acland, 1971). Forms of dryland rice growing are practised at Zanzibar and Pemba, the wetter parts of the Southern highlands and the Kilombero escarpment in Tanzania and near Bundibugyo on the Western slopes of the Ruwenzori mountains in Uganda. The bulk of the crop depends on water received from swamps, rivers, lakes or seepage areas. Although direct sowing is applied, most rice is transplanted. The growing of rice in supervised irrigation schemes is applied in Kenya.

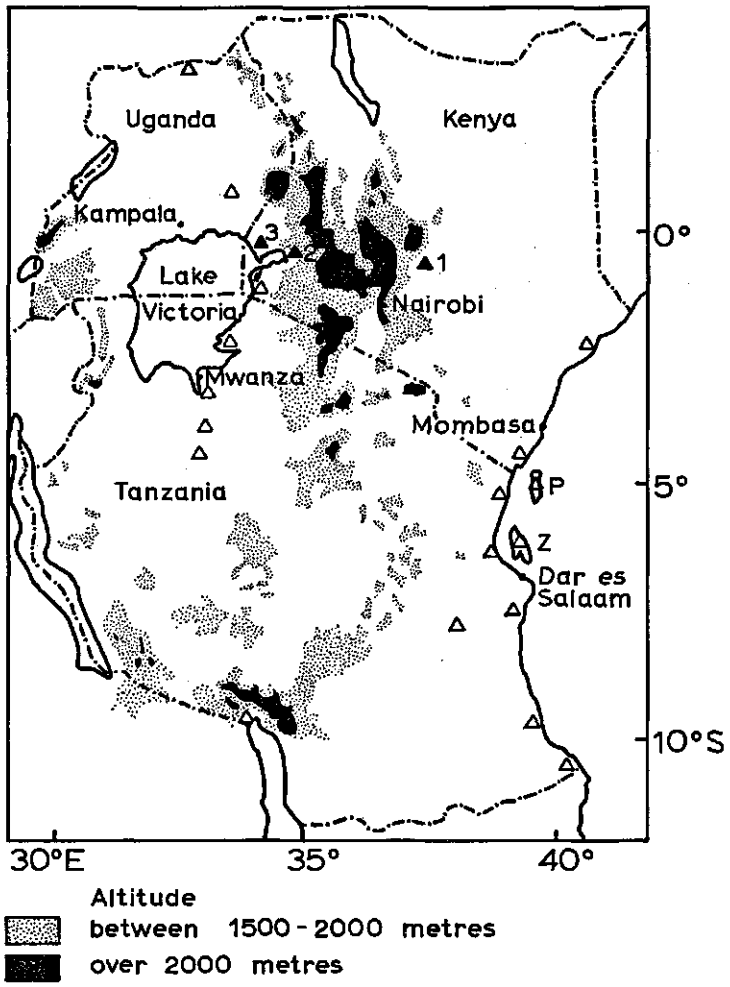


Fig. 3. Areas of rice production in East Africa. P: Pemba, Z: Zanzibar, ▲: rice grown by tenants of irrigation schemes in Kenya, ▲ 1: Mwea Irrigation Settlement, ▲ 2: Ahero Pilot Scheme, ▲ 3: Bunyala Irrigation Scheme. △: rice grown by peasants.

Of the three states, most rice is grown in Tanzania. Here the main rice producing areas are: Western Tanzania, south of Lake Victoria, the northern shores of Lake Nyasa, the Pangani and Rufiji basins and the islands Zanzibar and Pemba.

The main production area in Kenya is the Mwea Irrigation Settlement (in 1972 4800 ha) with one crop a year. The Ahero Pilot Scheme (834 ha), designed to give guidance for further expansion of rice on the Kano Plains, and the Bunyala Irrigation Scheme (212 ha), providing information for irrigated rice cultivation in the Yala swamp, have been in operation since 1968 and 1969, respectively. Here two crops of rice are grown annually. The irrigation schemes are managed by the National Irrigation Board.

A new irrigation scheme, the 1000 ha Kano II Scheme, is planned south of Kisumu near the Kavirondo Gulf.

In addition rice is cultivated by peasants on the banks of the Tana river, on the shores of Lake Victoria, in swamps near Mumias in Western Province and along the coast of the Indian Ocean.

Rice is of little importance in Uganda and is grown as an occasional crop by farmers in the hotter parts where waterlogged ground is available either as swamp margin or in local ground seepages. The principal areas are: Bukedi, Lango, Bwamba (Toro), West Nile and Madi, with smaller areas in cultivation at Busoga, Teso and Acholi (Tiley, 1970).

The main rice growing centres in East Africa are shown in Fig. 3 and in Fig. 4 in more detail for the neighbourhood of Kisumu.

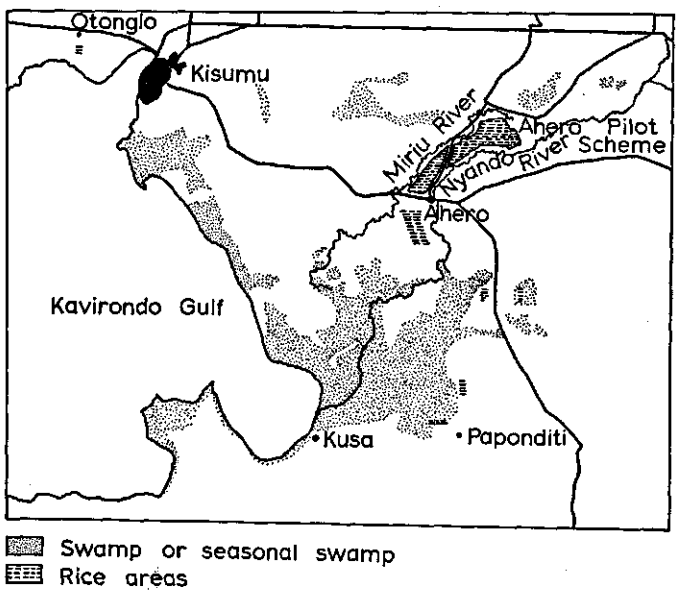


Fig. 4. Areas of rice production near Kisumu.

The area under production by smallholders varies annually, but is influenced by external factors too as demonstrated in the case of Zanzibar and Pemba. Before World War II, only a small part of the fields suitable for rice production was in use. With the money earned in the clove and copra industries imported rice was bought. This situation lasted till 1942 when due to the war this import had to be restricted. A campaign organized by the government resulted in an increase of the planted area from 1000 ha till 4000 ha in 1943 (Wilson, 1944).

Numerous varieties of rice are grown in East Africa. In peasant farming areas mixed stands are common. In the Kenya irrigation schemes 'Sindano', an *indica* type, is the predominant variety, but because of its high disease susceptibility it had to be replaced by 'Basmati 217' in the Ahero Pilot Scheme and in the Bunyala Irrigation Scheme. 'Basmati 217' has quality of higher value but yields normally less than 'Sindano'. 'Mbuyu' and 'Shingo la Majani' are of little importance and are occasionally grown in peasant farming areas. In Tanzania the most popular varieties are 'Afaa' and 'Kahogo'. 'Afaa' differs from place to place and is often given a prefix

Table 2. Rice production and trade figures[1] for East Africa and Africa during 1967 – 1971. Sources: Dhital (1972) and Eruz (1972).

Year	Kenya					Uganda				
	area	prod.	yield	import	export	area	prod.	yield	import	export
1967	3	16	56.3	4	24	3	8	31.2	102	4
1968	3[3]	19	54.5[3]	31	22	3	3	8.0	19	4
1969	5	27	54.3	3	28	4	3	8.0	60	3
1970	5	26	48.4	12	7	8	6	8.0	40	2
1971	6	28	50.5			8[4]	6[4]	8.0[4]		
	Tanzania					Africa				
	area	prod.	yield	import	export	area	prod.	yield	import	export
1967	104[4]	114	11.0[4]	27[2]	5[2]	3088	4486	14.5	6644	4930
1968	128	136	10.6	151	1	3045	4408	14.5	6911	6579
1969	129	136	10.5	80	—	3248	4817	14.8	6911	8462
1970	151[4]	182	12.1[4]	73	5	3296	4912	14.9	8344	7356
1971	153[4]	185[4]	12.1[4]			3364	5082	15.1		

1. area in 1 000 ha, prod(uction) in 1 000 metric tons paddy, yield in 100 kg paddy/ha, imports in 100 metric tons rice and exports in 100 metric tons rice.
2. Tanzania mainland only.
3. Unofficial figure.
4. FAO estimate.

or suffix according to its origin, e.g. 'Afaa Kilombero' (Acland, 1971).

The yield per hectare obtained by smallholders is moderate, but in the Mwea Irrigation Settlement outstanding yields (average 5000 – 6000 kg/ha) are obtained. Production figures for the three states as well as for the whole of Africa are given in Table 2.

The methods of rice cultivation will be dealt with as practised by smallholders along the shores of Lake Victoria and by tenants of the irrigation schemes, both in Kenya.

2.1.1 Practices in use along the shores of Lake Victoria

Rice seeds were probably originally brought from the coast by the Swahili and Arab slave traders before the advent of the white man to the Mumias area of Northern Kavirondo (Anonymus, 1944) about 60 km north-west of Kisumu. 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 (Gamble, 1939). In 1934, this area was expanded and reached by 1938 an estimated 300 ha, an acreage

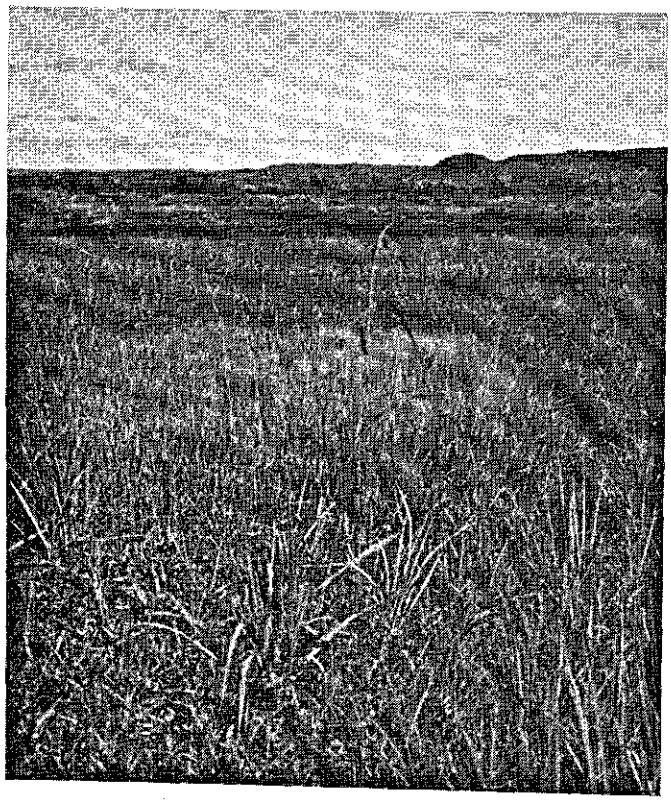


Fig. 5. Rice nursery at Otonglo in 1967. Note RYM affected ratoon rice near nursery and recently transplanted rice.

which was presumably still the same during 1966 – 1970. Rice is grown here under irrigation, or to a lesser extent in fields prepared in or along the swamps. Two growing seasons are known. A long rain-fed crop is grown from December till June and a short rain-fed crop from June till December, but mostly only the short rain-fed crop is planted. Small fields of approximately 0.1 ha are prepared by cutting the weeds and after flooding, hoeing. In a corner of the fields the nursery is prepared (Fig. 5). After sowing the rice, the nursery may be covered with mown weeds to conserve humidity and to prevent depredation by birds. After the seeds have germinated, the grass cover is removed and at transplanting the leaves and roots are often trimmed. Weeding, maintaining the water level and scaring off the birds are the further activities of the farmers till harvest. After the harvest, which begins in December, cattle are allowed to graze, or a ratoon crop is harvested. After the initiation of the Ahero Pilot Scheme, nearby farmers also used the drained water of this scheme so that a relatively large area was planted with rice, mostly during the short rainy season.

At Otonglo a similar pattern is followed. Here a limited amount of drained water from the prison farm was available during the whole year, causing a less strict planting scheme and resulting in vigorous growth of volunteer and ratoon rice as well as of grasses and sedges after the harvest. From 1966 till 1969 about 15 ha was planted with rice annually, often two crops a year. There was no closed season for rice, while water control was insufficient. After 1969 interest in rice diminished at Otonglo and for a couple of seasons no rice was grown here till 1972, when a small area was planted again.

2.1.2 Practices in use at the irrigation schemes

The most important differences in the cultivation of rice in these schemes from the cultivation practized by the smallholders along the shores of Lake Victoria, are: scheduled and supervised work programme, strict water control, fertilizer application, sowing of pregerminated seeds on the nurseries, and when necessary use of pesticides.

The Mwea Irrigation Settlement was initiated in 1954 after field trials proved that the area at an altitude of about 1200 metres was suitable for irrigated rice growing. In 1955 there was 120 ha under rice, but this area was extended to 4800 ha in 1972. Rice is grown in 'black cotton soil', a montmorillonite clay, which is hardly permeable to water, obtained from rivers from Mount Kenya. Each tenant handles 1.62 ha divided in four fields of 0.405 ha. Due to their own initiative some farmers cultivate a larger area. There is only one crop a year in the short rainy season from July till January, and no rotations are practised. The main variety is 'Sindano'. The settlement provides all essential provisions such as water, mechanical cultivation, seed, fertilizers, pesticides, transport and storage. The size of the settlement necessitates an early start of the cultivation. In April the first fields are flooded, occasionally preceded by mowing of the vegetation of weeds and rice. Within 72 hours the fields are cultivated by tractor-driven rotary hoes. After puddling, the fields are kept submerged and new growth, mainly rice, is dealt with by hand (Fig. 6). Nurseries are prepared by the

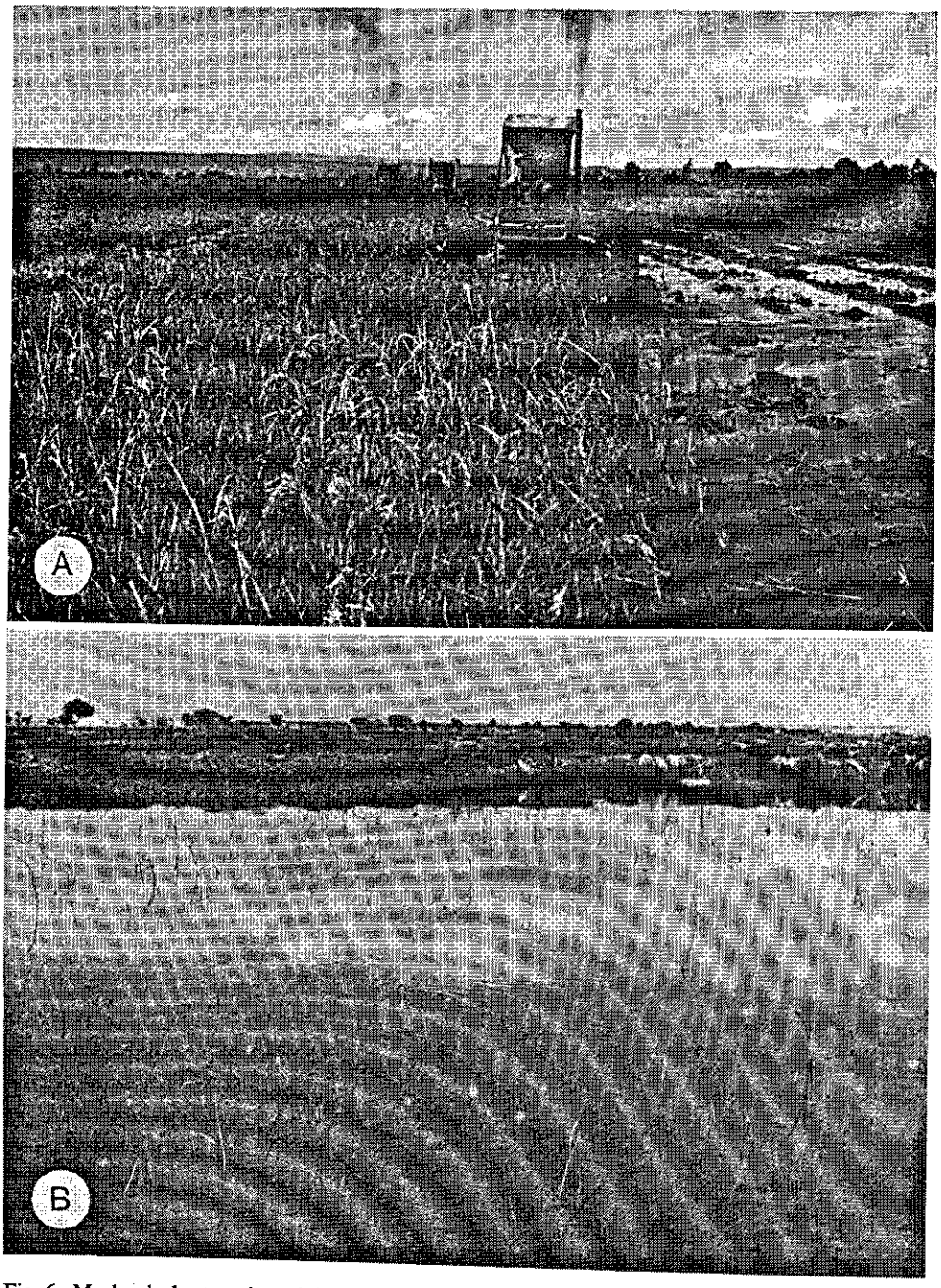


Fig. 6. Mechanical rotavation of rice fields in irrigation schemes.

A. Rotavation of fields at the Ahero Pilot Scheme. The ratoon rice 'Sindano' shows a high incidence of RYM.

B. Ratoon rice which survived the rotavation and which has to be removed by hand at the Mwea Irrigation Settlement.

tenant and these receive ammonium sulphate. Sowing starts at the end of July and transplanting commences at the end of August. Before transplanting the fields receive ammonium sulphate and every other year superphosphate. One month after transplanting the tenants start weeding, which occasionally must be repeated. During weeding the water is not drained off. 'Sindano' and 'Basmati 217' need around 146 days from seed to seed. After the harvest the rice straw is burnt in the field. Because of the low temperature of the irrigation water in the long rainy season, no second crop is grown.

Further information about this successful scheme is to be found in Chambers (1969), Giglioli (1965a, 1965b), Golkowsky (1969), Veen (1969) and in the annual reports of the Mwea Irrigation Settlement and of the National Irrigation Board.

At the Ahero Pilot Scheme, where the Irrigation Research Station Ahero is situated too, 834 ha are leased to tenants to grow rice. The altitude is about 1100 metres and rice is grown here too in 'black cotton soil'. The first crop was grown in the 1968/1969 season. The system of management and cultivation is similar to that of the Mwea Irrigation Settlement. Here two crops a year are grown one in the short rainy season from August till March and the other in the long rainy season from March till August. The water is pumped from the Nyando river. The initial period of the scheme was difficult. Most of the technical problems were overcome, although growing rice twice a year at this scale was new for the area. The effect of diseases resulted in disappointing yields and caused some demoralization of the farmers. The first harvest of 'Sindano' yielded for the 212 ha planted, an average of approximately 5000 kg/ha, but later crops were heavily attacked by blast (*Pyricularia oryzae* Cav.) and RYM. 'Sindano' was therefore replaced by 'Basmati 217', which was less affected by the two diseases. Yields for this variety varied strongly and were between 1200 – 2000 kg/ha, although these figures may be too low.

The growing season from seed to seed for 'Sindano' and 'Basmati 217' are both about 130 days. The growth of volunteer and ratoon rice, and of weeds, mainly grasses, is vigorous and there is a time lapse of about 2 months between cultivation of the first and last fields. Consequently nurseries are found in the neighbourhood of the fields with RYM affected plants that have not yet been tilled.

To illustrate the climate, rainfall, maximum and minimum temperatures for Otonglo, the Ahero Pilot Scheme and the Mwea Irrigation Settlement are given in Table 3.

2.1.3 Research activities

In Kenya, Uganda and Tanzania, rice research is mostly devoted to agronomical aspects such as comparison of varieties, spacing and fertilizer experiments, maintaining and multiplication of seeds. Problems requiring specialist attention, mainly disease, pest or soil problems are generally referred to centrally based officers e.g. the Senior Plant Pathologist. At the East African Agricultural and Forestry Research Station, quarantine of imported seeds and more fundamental problems or those re-

Table 3. Average monthly rainfall, maximum and minimum temperature for Otonglo[1], Ahero Pilot Scheme and Mwea Irrigation Settlement. Figures provided by Ilaco B.V., Arnhem, the Netherlands.

	Otonglo 1960 – 1969			Ahero Pilot Scheme 1963 – 1972			Mwea Irrigation Settlement 1963 – 1971		
	rainfall (mm)	max. temp. (°C)	min. temp. (°C)	rainfall (mm)	max. temp. (°C)	min. temp. (°C)	rainfall (mm)	max. temp. (°C)	min. temp. (°C)
January	60.6	30.7	16.5	72.2	30.9	14.1	25.3	28.4	13.8
February	118.6	30.3	16.8	105.5	30.9	14.7	38.3	30.0	14.7
March	175.5	29.9	17.3	135.7	30.6	15.2	104.1	29.4	16.2
April	237.3	28.8	17.5	198.9	28.9	16.0	287.9	27.8	17.2
May	179.2	28.4	17.2	118.5	28.5	15.7	121.5	26.6	17.0
June	77.9	28.5	16.0	73.7	28.6	14.5	14.9	25.7	16.0
July	56.5	28.2	16.0	64.4	28.8	14.3	12.0	24.5	15.3
August	71.6	28.9	15.8	59.3	29.4	13.9	24.4	25.0	15.0
September	94.0	29.7	16.2	65.8	30.4	14.0	9.9	27.9	15.6
October	93.1	30.2	16.8	78.9	30.7	14.6	92.2	28.8	17.0
November	170.7	29.4	17.0	107.3	30.4	14.7	179.1	27.3	16.6
December	112.6	29.7	16.5	101.4	29.9	14.1	52.2	26.8	14.6

1. Figures for Otonglo are those from Kisumu meteorological station.

quiring specialization, e.g. in nematology are studied.

The Mwabagole Rice Station near Lake Victoria in Western Tanzania was started in 1935 and till 1956, good yielding strains were produced here by selection in local varieties (Doggett, 1965). Varieties originating outside East Africa performed poorly at that time. In Kenya, the work done by the research section of the Mwea Irrigation Settlement played an important role in the success of the scheme. To study rice growing in the Kano Plains and in the Yala swamp, and other crops, an irrigation research station was established at Ahero. This research station was started in 1959 and in the initial period was run by a FAO team. Much work has been done on the diseases encountered in the Ahero Pilot Scheme, while irrigation and drainage are also studied. In Uganda, rice research is centralized at the Kawanda Research Station near Kampala, while in Zanzibar rice was studied at the Kizimbani Experimental Station and its substations.

2.2 Diseases and pests

The fungi recorded on rice are listed by Nattrass (1961) for Kenya, by Hansford (1938) for Uganda and by Riley (1960) for the mainland of Tanzania. Additions to these lists are normally published in the annual reports of the departments of agriculture of the three states.

Blast (*Pyricularia oryzae* Cav.) is present in all areas, but is of importance only in areas of high humidity. It proved to be a limiting factor in the development of rice in Uganda (Hansford, 1938). In the Ahero Pilot Scheme blast caused much damage in the second and successive crops of 'Sindano'. 'Sindano' was replaced by 'Basmati 217' which showed less susceptibility to blast, but during 1972 this variety was also attacked in the Bunyala Irrigation Scheme and to a lesser extent in the Ahero Pilot Scheme too.

Brown spot (*Cochliobolus miyabeanus* (Ito et Kuribayashi) Dastur) common in East Africa occasionally causes serious damage (Bock, 1970); stem rot caused by *Leptosphaeria salvinii* Catt. occurs also in Kenya (Robinson, 1960), but is generally of minor importance. At the Ahero Pilot Scheme (Anonymus, 1973a) the presence of sheath blight (*Corticium sasaki* (Shirai) Matsumoto is suspected but has not yet been confirmed.

No diseases caused by bacteria have been recorded in rice as yet in East Africa. Insufficient water supply at the flowering stage of the rice plants or a low temperature of the water has been reported as the cause of white heads, resembling stem borer damage, at Yatta Furrow in Kenya.

Many species of the insect pests of rice listed by Grist & Lever (1969), do occur in East Africa, although the economic importance of these insects has not been assessed and only a few of them are recorded as pests (Le Pelley, 1959). Pyralid and noctuid stem borers are common and so is *Diopsis thoracica* Westw. (Fam. Diopsidae). Outbreaks of armyworm (*Spodoptera exempta* (Wlk.) are a threat to rice in East Africa as were locusts. The rice hispid (*Trichispa sericea* (Guérin) (Fam. Chrysome-

lidae)) can do considerable damage to young rice plants at the Mwea Irrigation Settlement, and is also present around Mwanza (Tanzania) (Nye, 1960). In Tanzania around the shores of Lake Victoria, *Locris neumanni* Jac. (Fam.Cercopidae) caused the yellowing of young shoots after puncturing, while *Oliarus* sp. (Fam.Cixiidae) caused yellowing of the foliage after feeding on the roots of the rice plant along the coast (Harris, 1937).

Thrips are suspected as being the cause of partly empty heads of 'Basmati 217' during the long rains at the Irrigation Research Station Ahero (Anonymus, 1972).

Nematodes recorded include *Hirschmanniella oryzae* (van Breda de Haan) and the recently recorded white-tip nematode – *Aphelenchoides besseyi* Christie (Taylor et al., 1972).

Of the other pests, birds especially *Quelea* spp., may cause considerable loss at harvesting time (Fuggles-Couchman, 1952), while rats are responsible for damage in experimental plots at the Irrigation Research Station Ahero.

3 General materials and methods

In this chapter only those materials and methods will be described which were used frequently in routine procedures. Details and other methods will be given in the appropriate chapters.

3.1 Visits to the field

From August 1967 till July 1972 I normally visited the Kisumu area once a month during three day trips, while Mwea was regularly paid a visit for a single day. Other rice growing areas in Kenya were visited on a single occasion only.

3.2 Plant material

3.2.1 Origin of plants used

Oryza spp. were provided by the International Rice Research Institute, Los Baños, Laguna, Philippines, while the different rice varieties were obtained from the District Agricultural Officer, Kisumu; the East African Plant Quarantine Station, Nairobi; the Irrigation Research Station Ahero, Ahero; the Mwea Irrigation Settlement, Kerugoya and from the Kawanda Research Station, Kawanda, Uganda.

'Sindano' rice provided by the Mwea Irrigation Settlement and the Irrigation Research Station Ahero was commonly used in the experiments.

Seeds and splits of indigenous Kenya plants, mainly monocotyledons, were obtained from: plants collected from the field which were brought to seed in a screenhouse; the Plant Breeding Station, Njoro (these seeds were originally obtained from the National Agricultural Research Station, Kitale); the UNDP Range Management Project, Nairobi; the National Agricultural Research Station, Kitale; the Katumani Agricultural Research Station, Machakos, while seeds of *Eragrostis tef*, collected in Ethiopia, were obtained from Dr E. Westphal, Department of Tropical Crops, Agricultural University, Wageningen.

The cultivated Kenya grasses were acquired from: samples sent for testing to the Seed Testing Laboratory, NAL, Nairobi; seed merchants in Nairobi; the National Agricultural Research Station, Kitale.

European grasses were obtained from: the Department of Field Crops and Grassland Husbandry, Agricultural University, Wageningen; the Welsh Plant Breeding Station, near Aberystwyth, Wales (*Phleum* spp.).

3.2.2 *Identification of plant species*

The endemic Kenya plants were identified at the East African Herbarium in Nairobi. The large number of Gramineae and Cyperaceae were identified by Miss C. H. S. Kabuye and Miss D. M. Napper. A number of grasses formed no flowers in the screenhouse and the name could therefore not be confirmed. In Table 5 (page 30), these plants are marked by an asterisk. Dried specimens of most of the plant species were placed in the collection of the Department of Plant Taxonomy and Plant Geography, Agricultural University, Wageningen.

3.2.3 *Growing of the plants*

In Nairobi, rice seedlings were grown in pots 10 cm in diameter (5 – 6 seeds/pot) or in a wooden box and subsequently transplanted in the 2 – 3 leaf stage. Steamed compost was used as potting medium. Unsterilized 'black cotton soil' in tins lined by a plastic bag with water added was used only for experiments of long duration. These plants received 150 ml of the standard liquid-nutrient solution at transplanting. The nutrient solution was made of 15 g $(NH_4)_2SO_4$ and 15 g KH_2PO_4 per litre tap water.

The other plants were germinated in a pot or on a Copenhagen table and subsequently transplanted in steamed compost with 2 – 5 plants/pot.

In Nairobi, glasshouse space was limited in the initial period, but this situation improved eventually. A large glasshouse (maximum temperature between 45 and 25°C, minimum temperature between 16 and 22°C), divided in compartments well isolated from each other, was used for growing all plants except rice from seed, and for experiments of short duration. The warmest and brightest compartments were used for the experiments with rice, while the cooler and more shaded ones were kept for other plant species. A small unshaded glasshouse was in use for rearing the rice seedlings, while a similar but shaded glasshouse was used for rice experiments of long duration. A screenhouse (a frame of wood covered with chicken wire of which the roof and most of the sides were covered with plastic) was initially used for rice experiments of long duration, but later for growing of field collected plants only.

The potted rice plants and most grasses were placed in a tray filled with water, while the rice plants in tins were grown in standing water.

In Wageningen, rice was grown in plastic buckets, diameter 18 cm – and height 19 cm with 5 – 10 plants per bucket, or in plastic containers, 46 × 31 × 15 cm with 30 – 35 plants per container. Unsterilized clay (clay fraction 70%) was used here as potting medium for the rice. Rice seeds were germinated in this clay at 26°C and transferred to a glasshouse (temperature about 24°C) in the 1 – 2 leaf stage. Water was added and the plants received 1 g fertilizer per 10 plants. The fertilizer was composed of 20% nitrogen, 8% phosphorus and 5% potash ('Asef' lawn fertilizer). During observation the plants were placed in a glasshouse with a 25 – 20°C day-night temperature, while the rice in use for production of RYMV was grown at a constant temperature of about 25°C.

After transfer of the rice seedlings from the germination box, the seedlings received additional lighting from 'Philips HPI/T' 400 W lamps, hung about 80 cm above soil level (1 per 2 m^2) for 16 hours/day throughout the year. The relative humidity in the glasshouses was about 45% during most of the day.

Only occasionally were the rice seedlings transplanted. Transplanted rice grew in general better than non-transplanted rice.

The other plants were reared in steamed soil, consisting of a mixture of pre-frozen peat and clay.

The properties of RYMV necessitated high standards of sanitation. The rice seedlings were grown in well-isolated glasshouses. Plants used in transmission tests, back inoculation etc. were watered from their own glass beaker. Precautions were taken against pests normally encountered in glasshouses. When necessary plants used in insect transmission experiments were sprayed with insecticide after inoculation feeding of the tested species.

3.3 The virus and mechanical inoculation

Isolate The RYMV isolate used throughout the study was obtained from a young rice plant from Otonglo in 1967. The virus was maintained by mechanical transmission in 'Sindano' rice.

Indicator host plant 'Sindano' served as indicator host. In several experiments this variety was compared with the varieties 'Basmati 217' and 'IR 22', because they may be of value in the schemes near Kisumu.

Standard inoculum The standard inoculum was prepared from young 'Sindano' leaves with clear symptoms of plants inoculated 2 – 3 weeks earlier, or from new growth of rice ratooned 2 – 3 weeks earlier, after the harvest of the diseased leaves. The leaves were squeezed in muslin cloth or cheese cloth with a small pair of pliers. After dropping some 0.01 M phosphate buffer pH 7.0 on the cloth, this process was repeated a number of times. In total 1 – 2 ml buffer per g of leaves was used.

Mechanical inoculation Plants were inoculated at a vigorous stage. After dusting with carborundum 600 mesh, plants were inoculated with the fingers. 'Sindano' was back inoculated 3 – 5 weeks after the inoculation. This inoculum was prepared by the same method as above or by using a pestle and mortar. Only occasionally had buffer to be added. After inoculation the plants were sprayed with tap water. Test plants grown from seed were kept in the glasshouses, while field collected splits were placed in a screenhouse.

3.4 Collecting, handling and identification of invertebrates

Mites were obtained from rice collected in the field and brought to the laboratory. Individual specimens were handled with a single haired brush.

To collect nematodes, soil and roots of diseased rice plants were transported to the laboratory in plastic bags, while care was taken to avoid excessive heat. Nematodes which were ground were collected by Baermann's funnel technique as described by Taylor (1967).

Of the aphids, *Myzus persicae* Sulzer was bred on *Brassica pekinensis* Rupr., while other species were collected from field grown Gramineae.

Other insect species were mostly caught by means of a sweeping net and collected by means of an aspirator. During their transportation from the Kisumu area to Nairobi, the insects were kept on their respective food plants in a plastic bucket closed by a perforated cover with a closable opening, and sealed with sticking plaster. The food plants were generally grasses and rice, but for *Sesselia pusilla* often some blooming flowers of *Cyperus* spp. were added. Excessive humid conditions were prevented by wrapping the roots and soil in a plastic bag and avoiding drastic changes in temperature. When the insects were collected near the laboratory or at Mwea, the specimens were transported in aspirator tubes with some leaves of grass or rice, and a piece of filter paper to absorb liquid. The tubes were closed with muslin cloth.

At the laboratory the small insects except the aphids, were handled individually with an aspirator made from a 25 ml volumetric pipette. The insects were starved in a Petri dish (aphids) or in aspirator tubes.

During the acquisition and inoculation feeding periods of the insects, the aim was to create conditions in which the insects would eagerly feed off the plants. Therefore different methods were applied.

Acquisition feeding was on 'Sindano' rice, inoculated about 3 weeks earlier, or on fresh growth of ratoon rice. Only young leaves clearly showing symptoms were left on the plants. Depending on the number and type of insect used, the insects were placed on:

- 5 – 6 rice plants surrounded by a glass cylinder made of lamp glasses and covered by muslin cloth (Method a);
- a single rice plant surrounded by a glass cylinder, diameter 5 cm, and covered by muslin cloth (Method b);
- detached rice leaves in a glass cylinder. The cut ends of the leaves were kept moist by wet cotton wool wrapped in plastic (Method c);
- a leaf in a micro cage (Method d).

Inoculation feeding was performed on young rice seedlings normally in the 2 – 3 leaf stage, transplanted 1 – 2 days before. Occasionally older test plants were used, for exemple for mite and grasshopper testing. The insects were caged in:

- a glass tube of the size of a test tube;
- a glass tube, diameter 5 cm, length 40 cm;
- a lamp glass;

- a PVC tube, diameter 1½ cm, length 5 or 7½ cm;
- a micro cage.

When the acquisition and inoculation periods were only of short duration (up to 4 hours) the feeding occurred in the dark.

In the experiments in which the retention periods of RYMV by the insects were determined, the insects were transferred to a new test plant for inoculation feeding every day for 10 days.

After the experiments, the insects were preserved and kept for identification purposes.

Per insect, some test plants in which symptoms were incited were tested with antiserum to RYMV in the agar-gel diffusion test. If the serological tests proved to be positive, the insects were considered to be capable of transmitting the virus.

Identification of the species was done by several specialists. Acarina were identified by H. H. Keifer, Sacramento, California, (Eriophydidae), and E. W. Baker and R. L. Smiley, United States Department of Agriculture, Agricultural Research Service, Washington D.C., (Tarsonemidae and Phytoseiidae); Nematoda by D. W. Ngundo, East African Agriculture and Forestry Research Organization, Nairobi; Hemiptera by M. S. K. Ghauri, Commonwealth Institute of Entomology, London, (Homoptera, Aphididae excluded), by D. Hille Ris Lambers, National Council for Agricultural Research (TNO), Bennekom, the Netherlands (Aphididae), and by R. Linnavuori, Somersoje (Berg), Finland, (Heteroptera); Coleoptera by G. Scherer, Museum G. Frey, Tutzing bei München, W. Germany, (Halticinae, Hispinae), by J. A. Wilcox, New York State Museum and Science Service, Albany, New York, (*Sesselia pusilla*), and by N. A. Aslam, E. A. J. Duffy and R. Madge of the British Museum (Natural History) or the Commonwealth Institute of Entomology, London; Hymenoptera by R. D. Eady, Commonwealth Institute of Entomology, London; Lepidoptera by J. D. Bradley, Commonwealth Institute of Entomology, London; Orthoptera and Dictyoptera by F. Willemse, Eygelshoven, the Netherlands; Diopsidae by J. F. Shillito, Old Forge, Staple Cross, Robertsbridge, Great Britain.

Most of the mounted insects are kept at the Laboratory of Entomology, Agricultural University, Wageningen, the Netherlands and by the specialists who identified them.

3.5 Purification of RYMV and determination of its concentration

Purification The procedures as applied by Scott & Moore (1972) for *Desmodium* yellow mottle virus and by Proll & Schmidt (1964) for ryegrass streak virus (= brome mosaic virus (Bancroft, 1970)), both resulted in highly pure RYMV preparations. Because of its ease and the apparently higher yield, a modification of Proll and Schmidt's method was used. This procedure is given in Table 4.

'Sindano' rice was inoculated in the 5 – 6 leaf stage and kept at about 25°C. The first systemic infected leaves were harvested about 10 – 12 days later, before they turned necrotic. The rice plants were then cut and new growth harvested 2 – 3 weeks

Table 4. Procedure for purification of rice yellow mottle virus.

Cut infected rice leaves in ½ cm pieces with scissors; homogenize in 0.1 M phosphate buffer pH 5.0 + 0.2% 2-mercaptoethanol in Waring Blendor, 1 g of leaves/20 ml of buffer (max. 650 ml); squeeze through muslin cloth.	4 °C	
Extract		Discard debris
Add ½ volume chloroform, shake 5 min; centrifuge 15 min at 1500 *g* (MSE High Speed 18, rotor 6 × 250 ml).	4 °C	
Aqueous phase		Discard chloroform phase
Add under stirring with magnetic stirrer 20 g $(NH_4)_2SO_4$/100 ml liquid; centrifuge 15 min at 2500 *g* (MSE High Speed 18, rotor 6 × 250 ml).	room temperature	
Supernatant (S_1)		Discard sediment
Add under stirring with magnetic stirrer 20 g $(NH_4)_2SO_4$/100 ml liquid; let stand for at least 20 min; centrifuge 20 min at 5500 *g* (MSE High Speed 18, rotor 6 × 250 ml).	room temperature	
Sediment (P_2)		Discard supernatant
Homogenize in 10–20 ml 0.1 M phosphate buffer pH 5.0 + 0.2% 2-mercaptoethanol; dialyse against same buffer for 18 h, renew buffer once; centrifuge 20 min at 6600 *g* (Sorvall SS-1).	room temperature 4 °C	
Supernatant (S_3)		Discard sediment
Centrifuge 100 min at 78500 *g* (Spinco L50, rotor R30).	4 °C	
Sediment (P_4)		Discard supernatant
Suspend in 1–2 ml 0.01 M phosphate buffer pH 7.0; dialyse 36 hours against same buffer, renew buffer twice; centrifuge 10 min at 6000 *g* (Sorvall SS-1).	4 °C	
Supernatant (S_5)		Discard sediment
Purified RYMV.		

later. The leaves were processed immediately or deep frozen (−25°C) till use. The phosphate buffers were made of Na_2HPO_4–KH_2PO_4. For small amounts of leaves 20 ml buffer/g leaf was used, but for amounts between 32.5–50 g, 650 ml buffer was used. Dialysis tubing was boiled out for use in 10^{-4} M EDTA, and rinsed with deionized water and buffer.

Although cutting the rice leaves with a razor blade and then grinding in a mortar (Bakker, 1970) gave good results, the use of a Waring Blendor was more convenient. To prevent strong discolouration during the first dialysis, 0.2% 2-mercaptoethanol was added to the buffer used in the first steps of purification. The 2-mercaptoethanol was later removed by dialysing against 0.01 M phosphate buffer pH 7.0. The high speed sediment (P_4) normally had a slightly brownish centre which showed up more clearly when increasing amounts of leaves were used.

Spectrophotometry Measurements were made with a Zeiss PMQ II spectrophotometer using 1-cm quartz cuvets.

Determination of virus concentrations Four times the virus was purified as shown in Table 4. One ml portions of the virus suspensions (S_5) and 10 ml portions of the last used dialysis buffers were freeze-dried under vacuum, further dried above $CaCl_2$ at room temperature, and weighed. From another portion of the same virus suspension the values for $E^{1\,cm}_{260\,nm}$ and $E^{1\,cm}_{260\text{-}290\,nm}$ were determined spectrophotometrically using the last mentioned dialysis buffer as a standard. With less pure virus preparations the absorbance at 260 minus that at 290 nm may be of value (Noordam, 1973).

3.6 Analytical ultracentrifugation

The sedimentation pattern, the sedimentation coefficient and centrifugal analysis of the effect of freezing upon RYMV, were determined with an analytical ultracentrifuge Spinco Model E with the help of Dr H. Huttinga, Institute of Phytopathological Research, Wageningen. A series of different concentrations of RYMV, suspended in 0.01 M phosphate buffer pH 7.0, was spun at 31410 rpm with the rotor temperature kept at 20°C. The displacement of the peaks on the photographs, taken with schlieren optics and under a diaphragm angle of 50°, were measured and with the graphical method of Markham (1960) the S value at each concentration was determined. The sedimentation coefficient at infinite dilution s°, was obtained by extrapolation to a virus concentration of zero.

3.7 Extraction of RYMV nucleic acid

The procedure applied for extracting the nucleic acid from RYMV was based on the method described by van Griensven (1970) for cowpea mosaic virus-RNA. Instead of diethylpyrocarbonate, Macaloid was used. The Macaloid was added to 0.01 M phosphate buffer pH 7.0 (20 mg/ml) and boiled until the suspension was even (2 min).

After cooling the suspension was homogenized and again boiled. When cool the homogenate was centrifuged at low speed (15 min at 2000 *g*) and the supernatant and top of the pellet were discarded. The pellet was redissolved in new buffer and the procedure repeated twice. The final pellet was resuspended in the initial amount of buffer.

The extraction was performed at 4°C with water-saturated phenol kept at room temperature. Afterwards the tubes were placed in ice. The centrifuge used was a Sorvall SS-1 centrifuge.

To a solution of sodium dodecyl sulphate (SDS) in 0.01 M phosphate buffer pH 7.0, containing Macaloid, 40 – 90 mg virus was added so that the final concentrations were: 0.4% RYMV (w/v), 1% SDS (w/v) and 0.1% Macaloid (w/v). This suspension was shaken for 5 min and an equal amount of freshly distilled, water-saturated phenol (8 g phenol : 2 ml water) was added. After shaking for 8 min with a Vortex mixer, the phenol and water phases were separated by centrifuging 10 min at 18000 *g*. The water phases were collected and once again shaken with an equal amount of water-saturated phenol for 8 min and separated by centrifuging. This process was then once again repeated with the water phases. The interphases were collected separatedly suspended in 1% SDS in the same buffer without Macaloid. To this solution an equal amount of water-saturated phenol was added and treated as before. The water phases were combined and shaken four times with peroxide-free ether to remove phenol. To improve separation of the phases low speed centrifugation was applied. The residual ether was subsequently removed by passing nitrogen through the water phase. The nucleic acid was precipitated by adding two volumes of ice cold absolute ethanol and two drops of 3 M sodium acetate per test tube and then storing at −25°C overnight. For use the solution was centrifuged at 8000 *g* for 25 min and the sediment dried in vacuum at 4°C. The sediment was dissolved in the appropriate buffer and centrifuged at 8000 *g* for 10 min. The water clear supernatant contained the RYMV nucleic acid.

3.8 Light microscopy

Young leaves of 'Sindano' rice with clear symptoms, inoculated 2 weeks before and healthy material of the same age, were used fresh or fixed. Epidermal strips were prepared by sticking about 2-cm long pieces of leaf with the upper epidermis onto adhesive tape (Sellotape). With a scalpel the other tissue was carefully removed and the tape with epidermis immersed in water. The tape was then loosened from the epidermis and the remains of the adhesive were carefully removed from the strip with of a water-colour brush.

Epidermal strips and transverse sections from fresh material were examined unstained and stained. The following watery solutions of stains were used: 0.5% phloxine, 0.5% trypthan blue and a combination of these stains. The preparations were immersed in the stain for 15 min and rinsed with water.

For embedding, leaf strips (2 × 5 mm) were fixed in formalin-propionic acid-alcohol (1:1:18), dehydrated in normal butyl alcohol and embedded in Paraplast.

Transverse serial sections were cut 5 – 12 μm thick, stained with the above named stains or with Giemsa in methanol for 15 min and subsequently rinsed in water, or methanol.

3.9 Electron microscopy

Negative staining of purified virus was done with 1% sodium phosphotungstate (PTA) pH 4.5 or with 1% uranyl acetate pH 4.3, on 150 mesh copper grids coated with Formvar and carbon. The dimensions of the particles were calculated from photographs of twice enlarged negatives obtained at 80000 ×. Particles in a mixture of RYMV and tobacco mosaic virus (TMV), stained with PTA were measured on the negatives with a stereo microscope, assuming the width of the TMV particles to be 16 nm (Taylor et al., 1968).

For the study of RYMV in leaf tissue, young leaves of mechanically infected 'Sindano' rice with clear symptoms were harvested at different times after inoculation. Leaves were collected from plants which were:
– inoculated at the 3 leaf stage and harvested 11 days later at 36 days after sowing – the leaf taken was very young and dotted (Stage A);
– inoculated at the 4 leaf stage and harvested 23 days later at 44 days after sowing – the leaf taken was incompletely developed and showed clear mottling (Stage B);
– inoculated at the 6 leaf stage and harvested 33 days later at 71 days after sowing – the leaf taken was mature and mottled with pronounced chlorotic areas (Stage C).

For comparison healthy leaves of plants of the same age and stage of development were used. Due to the retardation in growth caused by a RYMV infection in 'Sindano' rice, leaves from a younger plant but of the same stage of development had to be taken for Stage C control.

The procedure for preparation of the tissue was proposed by Dr G. A. de Zoeten, Department of Plant Pathology, University of Wisconsin, Madison, USA, and was as follows: the night prior to harvesting, the plants were placed in the dark. At harvest leaf tissue from the lower part of the leaves was cut in small pieces and fixed at 0°C in 5% glutaraldehyde in 0.08 M cacodylate buffer pH 7.4 for 2 hours. Vacuum from a waterpump was applied till the pieces had sunk. After rinsing the leaf tissue three times for 20 minutes in 0.08 M cacodylate buffer pH 7.4, it was cut in 1 × 2 mm pieces, the original sides discarded, and postfixed in Pallade's fixative pH 7.4 (with 1% OsO_4) (Pallade, 1952) at 4°C for 16 hours. This fixative was changed once. After washing in bidistilled water the tissue pieces were dehydrated in a graded series of acetone and embedded in the standard medium of a low viscosity epoxy resin (Spurr, 1969), while uranyl acetate staining was done in the 70% dehydration step (70% acetone saturated with uranyl acetate, overnight).

The sections were cut with an LKB ultratome III using a glass knife, mounted on 150 mesh Formvar coated copper grids and stained with Reynolds's lead citrate (Reynolds, 1963) for 8 min. The specimens were examined and photographed in a Siemens Elmiskop 101 electron microscope at an accelerating voltage of 80 kV.

3.10 Serology

The author prepared antiserum to RYMV in Kenya (Bakker, 1970) as well as in the Netherlands. In the Netherlands, purified virus (S_5) was administered to rabbits according to the following scheme:

- 1 ml virus preparation (6.3 mg virus) injected intravenously;
- 3 days later 1 ml virus preparation (12.7 mg virus) injected intravenously also;
- 15 days after the last intravenous injection, 1 ml virus preparation (28.5 mg virus), emulsified with 1 ml Freund's incomplete adjuvant (Difco-Bacto) injected intramuscularly.

Serum was obtained 15 days, 35 days and 48 days after the last injection, while sodium azide (0.01%) was added as preservative prior to storage at −25°C.

Serological tests were done with the Ouchterlony agar double-diffusion test by using 1% agar in 0.85% NaCl. Sodium azide in a concentration of 0.02% was added to prevent development of bacteria. The tests were performed at room temperature (about 20°C). Dilutions of sap and antisera were made with 0.85% NaCl.

4 Transmission by mechanical means and effects of RYMV on plants

4.1 Transmission with expressed sap

To prove the presence of RYMV in its hosts, inoculum was routinely prepared from the leaf blades and subsequently inoculated onto 'Sindano' rice. By the same method, the virus was also recovered from the leaf sheaths and roots of 'Sindano', and from green sterile seeds of unripened panicles of rice 'IR 22'.

The virus was not recovered from yellow sterile seeds of ripened 'IR 22'. Neither was RYMV recovered from ripened sterile seeds, nor from the husks and grains separately, from 'Basmati 217' inoculated 3 months before harvest and tested 8 months after harvest. From 'Sindano' that had died prematurely because of the disease, no virus was recovered from the leaves, which had turned yellow.

4.1.1 Experimental host range and symptoms

By determining the host range of RYMV, information useful for identification purposes was obtained and possible natural hosts other than rice could be assigned. Another aim was to find tolerance or resistance to the virus in *Oryza* spp. and rice varieties. By sap inoculation followed by back tests to 'Sindano', plants susceptible to RYMV (i.e. plants in which inoculation results in infection whether they show symptoms or not) were differentiated from plants which were (apparently) immune to the virus. Plants resistant to RYMV are not affected because they possess qualities which hinder the development of the virus.

The pathological effect in a susceptible plant depends upon its sensitivity to RYMV. In a highly sensitive host an infection seriously affects the plant. A tolerant plant endures an infection but shows no or only mild symptoms of the disease.

Methods

Most test plants were grown from seed; occasionally vegetative material was used. Dicotyledons commonly used to determine a virus host range were inoculated at the usual stage, vegetatively propagated plants at a vigorous stage of growth, while the other plants, mainly grasses, were inoculated in the 3 – 10 leaf stage, depending on the size of the leaves and the growth habitat of the plant. At each inoculation 5 – 10 'Sindano' seedlings were also inoculated.

Back inoculations were performed with sap from the inoculated leaves if possible

as well as with sap from new growth of the tested plants. If no symptoms had been noticed in a plant species, and the back inoculation proved the presence of the virus in this species, two separate back inoculations were performed one with sap obtained from the remaining inoculated leaves and the other with sap from new growth. Also new test plants were grown and rubbed either with inoculum prepared from the inoculated leaves or from new growth, to determine whether a local or a systemic infection had occurred. It was aimed to test each time at least 5 plants of each species.

Results

The results of the mechanical inoculations are given in Table 5. Only in a few tribes of the Gramineae were hosts of RYMV found, but none of them showed symptoms on the inoculated leaves. All tested rice varieties (*O.sativa* L.), listed in Table 6, proved to be systemic hosts in which symptoms resembling those in 'Sindano', were incited, while the percentage of infection was 100%. Other systemic hosts in which symptoms were incited were:

Eragrostideae: *Dinebra retroflexa* (Vahl) Panz., *Diplachne caudata* K. Schum., *Eragrostis aethiopica* Chiov., *E.ciliaris* (L.) R.Br., *E.namaquensis* Nees var. *namaquensis*

Oryzeae: *Oryza australiensis* Domin, *O.barthii* A.Chev., *O.brachyantha* A.Chev. et Roehr., *O.glaberrima* Steud., *O.nivara* Sharma et Shastry, *O.punctata* Steud., *O.ridleyi* Hook f., *O.rufipogon* Griff., '*O.spontanea*'

Phalarideae: *Phleum arenarium* L.

A systemic host which developed no symptoms was:

Eragrostideae: *Eragrostis tenella* (L.) Roem. et Schult.

Hosts from which the virus was regularly recovered from the inoculated leaves only were:

Bromeae: *Bromus hordeaceus* L.

Eragrostideae: *Eragrostis chapelieri* (Kunth) Nees, *E. cilianensis* (All.) Lut., *E.macilenta* (A.Rich.) Steud., *E.tef* (Zucc.) Trotter

Oryzeae: *Oryza alta* Swallen, *O.eichingeri* Peter, *O.grandiglumis* (Doell) Prodh., *O.latifolia* Desv., *O.minuta* C. B. Presl, *O.officinalis* Watt

Paniceae: *Setaria viridis* (L.) P. Beauv.

Irregular recovery of RYMV from the inoculated leaves only occurred with:

Eragrostideae: *Dactyloctenium aegyptium* (L.) P. Beauv., *Eleusine coracana* (L.) Gaertn., *Eragrostis aspera* (Jacq.) Nees, *E.pilosa* (L.) P. Beauv.

Table 6. Rice (*Oryza sativa* L.) varieties susceptible to RYMV when mechanically inoculated.

Afaa	Introduction 1/324	S.C. 70	IR 5
Afaa Kilombero	Kahogo 1/146	Shimokita	IR 8
Afaa Kilombero 0/906	Kangala	Shingo la Majani	IR 12-178-2-3
Afaa Kilombero 1/196	Kialangawa	Sialkot 16	IR 20
Afaa Kilombero 2/214	Kibawa Chanzi	Sindano	IR 22
Afaa Mwanza 0/746	Kibawa Chekundu	Sindano 0/606	IR 24
Afaa Mwanza 1/133	Kibawa Cheupe	Sindano Nsemevu	IR 52-18-2
Afaa Mwanza 1/159	Kosura	SML 128/4	IR 127-80-1-10
Basmati	K + C 270/20	SML 140/5	IR 154-61-1-1
Basmati 217	K + C 803/6	SML 140/10/4	IR 520-1-26-3-3
Bayu	Lindi Safari	SML 242	IR 532E537
Bitamatami	Madevu	SML Apura	IR 661-1-127-3-1
Blue Belle	Mbuyu	SML Temerin	IR 661-1-170-1-3-3
Bungala	Mialakuna	Taichung Native 1	IR 662-2-7-2-2
Demarara Creole	Milfor 6 (2)	Uchuki	IR 665-24-1
Demarara Creole 2/100	Mkarafuu	Ungambi	IR 665-29-2
Faya S.L.	Perolla	Yonechiro	IR 665-40-1
Fujiminori	Portuguese	YRL I	IR $773A_1$-36-2-1
Gamiti	Radin Goi	Zira	IR 790-28-6
Gamti			IR 822-347
			IR $878B_2$-62-2

Table 5. Susceptibility and sensitivity of plant species mechanically inoculated with RYMV.

Plant species	Grown from seed or split[1]	Number of plants tested	Number of tests	Symptoms[2]	Type of host plant[3]
Angiospermae					
MONOCOTYLEDONEAE					
Gramineae					
Acroceras macrum Stapf	sp*	3	1	—	—
Agrostis canina L., 'Montana'	se	15	1	—	—
Agrostis canina L., 'Novobent'	se	10	1	—	—
Agrostis stolonifera L.	se	20	1	—	—
Agrostis tenuis Sibth.	se	12	2	—	—
Aira caryophyllea L.	se	15	1	—	—
Aira praecox L.	se	46	4	—	—
Alloteropsis semialata (R.Br.) Hitchc. var. *semialata*	sp	3	2	—	—
Alopecurus myosuroides Huds.	se	15	1	—	—
Alopecurus pratensis L.	se	15	1	—	—
Andropogon amethystinus Steud.	se	18	1	—	—
Andropogon canaliculatus Schumach. var. *fastigiens* Stapf	sp	2	2	—	—
Andropogon distachyos L.	se	15	2	—	—
Andropogon gayanus Kunth var. *squamulatus* (Hochst.) Stapf	se	6	2	—	—
Andropogon pratensis Hack.	sp	4	1	—	—
Anthephora hochstetteri Nees	se	25	3	—	—
Apera spica-venti (L.) P.Beauv.	se	9	2	—	—
Aristida adoensis Hochst.	se	43	5	—	—
Arrhenatherum elatius (L.) J.S. et C.B.Presl	se	8	2	—	—
Arthraxon quartinianus (A.Rich.) Nash	se	5	2	—	—
Arundinella nepalensis Trin.	se	8	2	—	—
Avena fatua L.	se	7	1	—	—
Avena sativa L., 'Condor'	se	9	1	—	—
Avena sativa L., 'Lampton'	se	10	1	—	—
Avena sativa L., 'M.Fc. 15/67'	se	20	2	—	—
Beckeropsis uniseta (Nees) K.Schum.	sp	2	2	—	—
Bothriochloa glabra (Roxb.) A.Camus	se	31	3	—	—
Brachiaria brizantha (A.Rich.) Stapf	sp	3	1	—	—

Brachiaria eminii (Mez) Robyns	se	10	1	—	—
Brachiaria eruciformis Griseb.	se	13	3	—	—
Brachiaria jubata (Fig. et De Not.) Stapf	se	5	2	—	—
Brachiaria lachnantha (Hochst.) Stapf	sp*	2	2	—	—
Brachiaria radicans Napper	sp	14	2	—	—
Brachiaria ruziziensis Germain et Evrard, 'Congo Signal'	se	8	2	—	—
Brachiaria umbratilis Napper	se	5	4	—	—
Brachypodium pinnatum (L.) P.Beauv.	se	8	2	—	—
Brachypodium sylvaticum (Huds.) P.Beauv.	se	1	1	—	—
Bromus arvensis L.	se	15	1	—	—
Bromus carinatus Hook. et Arn.	se	15	1	—	—
Bromus commutatus Schrad.	se	15	1	—	—
Bromus erectus Huds.	se	10	3	—	—
Bromus hordeaceus L.	se	79	6	—	L
Bromus marginatus Nees	se*	10	1	—	—
Bromus racemosus L.	se	20	2	—	—
Bromus secalinus L.	se	15	1	—	—
Bromus sterilis L.	se	25	2	—	—
Bromus tectorum L.	se	17	1	—	—
Calamagrostis epigejos (L.) Roth	sp*	3	1	—	—
Capillipedium parviflorum (R.Br.) Stapf	se	6	1	—	—
Catapodium marinum (L.) C.E.Hubbard	se	5	1	—	—
Cenchrus ciliaris L.	se*	4	1	—	—
Cenchrus setigerus Vahl	se	26	3	—	—
Chloris gayana Kunth, 'Mbarara'	se	4	1	—	—
Chloris gayana Kunth, 'Pokot'	se	16	1	—	—
Chloris pycnothrix Trin.	se	12	2	—	—
Chloris roxburghiana Schult.	se*	18	1	—	—
Chrysochloa orientalis (C.E.Hubbard) Swallen	se	13	1	—	—
Cymbopogon excavatus (Hochst.) Stapf	se	3	2	—	—
Cynodon dactylon (L.) Pers.	se	25	2	—	—
Dactylis glomerata L., 'S.37'	se	6	1	—	—
Dactylis glomerata L., 'S.143'	se	6	1	—	—

1. sp: split, se: seed, *: name of species not confirmed.
2. –: no symptoms incited, +: symptoms incited.
3. –: no host of RYMV; L: local host, RYMV regularly recovered; Lo: local host, RYMV occasionally recovered; S: systemic host.

Plant species	Grown from seed or split[1]	Number of plants tested	Number of tests	Symptoms[2]	Type of host plant[3]
Dactylis glomerata L., 'Danish cocksfoot'	se	16	2	—	—
Dactyloctenium aegyptium (L.) P.Beauv.	se	19	5	—	Lo
Dactyloctenium geminatum Hack.	sp	2	1	—	—
Dichanthium annulatum (Forsk.) Stapf	sp	3	1	—	—
Digitaria ciliaris (Retz.) Koel.	se	14	2	—	—
Digitaria gazensis Rendle	sp	3	1	—	—
Digitaria scalarum (Schweinf.) Chiov.	se	30	3	—	—
Digitaria ternata (A.Rich.) Stapf	se	16	1	—	—
Diheteropogon amplectens (Nees) W.D.Clayton var. *amplectens*	sp	1	1	—	—
Dinebra retroflexa (Vahl) Panz.	se	80	11	+	S
Diplachne caudata K.Schum.	se	30	4	+	S
Echinochloa colona (L.) Link	se	58	9	—	—
Echinochloa crus-galli (L.) P.Beauv.	se	23	1	—	—
Echinochloa haploclada (Stapf) Stapf	se	12	2	—	—
Echinochloa pyramidalis (Lam.) Hitchc. et Chase	sp	4	1	—	—
Eleusine sp.	se	4	2	—	—
Eleusine sp.	se	23	3	—	—
Eleusine coracana (L.) Gaertn.	se	86	10	—	Lo
Eleusine floccifolia (Forsk.) Spreng.	se*	31	3	—	—
Eleusine indica (L.) Gaertn. subsp. *africana* (Kenn.-O'Byrne) Phillips	se	25	2	—	—
Eleusine multiflora A.Rich.	se	19	3	—	—
Enneapogon schimperanus (A.Rich.) Renv.	se	5	2	—	—
Enteropogon macrostachyus (A.Rich.) Benth.	se	15	1	—	—
Entolasia imbricata Stapf	se	21	2	—	—
Eragrostis aetiopica Chiov.	se	2	2	+	S
Eragrostis aspera (Jacq.) Nees	se	58	12	—	Lo
Eragrostis atrovirens (Desf.) Steud. var. *congesta* Robyns et Tournay	se	24	3	—	—
Eragrostis barbinodis Hack.	se	24	6	—	—
Eragrostis braunii Schweinf.	se	24	6	—	—
Eragrostis caespitosa Chiov.	se	43	5	—	—
Eragrostis capensis (Thunb.) Trin.	se	26	4	—	—
Eragrostis chapelieri (Kunth) Nees	se	29	5	—	L

Eragrostis cilianensis (All.) Lut.	se	32	9	—	L
Eragrostis ciliaris (L.) R.Br.	se	44	3	+	S
Eragrostis curvula (Schrad.) Nees	se	53	3	—	—
Eragrostis exasperata Peter	se	43	5	—	—
Eragrostis heteromera Stapf	se	30	4	—	—
Eragrostis humidicola Napper	se	14	4	—	—
Eragrostis macilenta (A.Rich.) Steud.	se	48	7	—	L
Eragrostis minor Host	se	37	7	—	—
Eragrostis namaquensis Nees var. *namaquensis*	se	40	3	+	S
Eragrostis paniciformis (A.Br.) Steud.	se	40	3	—	—
Eragrostis pilosa (L.) P.Beauv.	se	43	8	—	Lo
Eragrostis racemosa (Thunb.) Steud.	sp	2	2	—	—
Eragrostis schweinfurthii Chiov.	se	39	5	—	—
Eragrostis superba Peyr.	se	43	6	—	—
Eragrostis tef (Zucc.) Trotter, accession no. W.18	se	11	1	—	L
Eragrostis tef (Zucc.) Trotter, accession no. W.51	se	20	2	—	L
Eragrostis tef (Zucc.) Trotter, accession no. W.8556	se	19	2	—	L
Eragrostis tenella (L.) Roem. et Schult.	se	40	3	—	S
Eragrostis tenuifolia (A.Rich.) Steud.	se	31	6	—	—
Eriochloa meyeranum (Nees) Pilg.	se	6	2	—	—
Eriochloa nubica (Steud.) Thell.	se	17	1	—	—
Eriochloa parvispiculata C.E.Hubbard	se	18	1	—	—
Eriochloa procera (Retz.) C.E.Hubbard	se	21	4	—	—
Eulalia ferruginea Stapf	sp	3	1	—	—
Eulalia geniculata Stapf	sp*	3	1	—	—
Eulalia polyneura (Pilg.) Stapf	sp*	1	1	—	—
Eustachys paspaloides (Vahl) Lanza et Mattei	sp	3	3	—	—
Exotheca abyssinica (A.Rich.) Anderss.	sp	1	1	—	—
Festuca gigantea (L.) Vill.	se	15	1	—	—
Festuca pratensis Huds.	se	20	1	—	—
Festuca rubra L.	se	15	1	—	—
Harpachne schimperi A.Rich.	se	38	5	—	—
Hemarthria natans Stapf	sp	6	3	—	—
Hordeum marinum Huds.	se	19	1	—	—
Hordeum vulgare L., 'Cambrinus'	se	10	1	—	—
Hordeum vulgare L., 'Proctor'	se	20	2	—	—
Hyparrhenia hirta (L.) Stapf	se*	8	1	—	—

Plant species	Grown from seed or split[1]	Number of plants tested	Number of tests	Symptoms[2]	Type of host plant[3]
Hyparrhenia rufa (Nees) Stapf	se	6	1	—	—
Koeleria cristata (L.) Pers.	se	5	1	—	—
Leersia hexandra Sw.	sp	28	5	—	—
Leptochloa obtusiflora Hochst.	se*	38	2	—	—
Lolium multiflorum Lam.	se	15	1	—	—
Lolium perenne L.,	se	20	1	—	—
Lolium perenne L., 'N3-H9'	se	6	1	—	—
Lolium perenne L., 'N.Z. perennial'	se	6	1	—	—
Lolium perenne L., 'N.Z. Italian'	se	6	1	—	—
Lolium perenne L., 'Ariki'	se	6	1	—	—
Lolium perenne L., 'Westerworlds'	se	6	1	—	—
Lolium remotum Schrank	se	10	1	—	—
Lolium temulentum L.	se	11	1	—	—
Loudetia flavida (Stapf) C.E.Hubbard	se*	6	2	—	—
Loudetia phragmitoides (Peter) C.E.Hubbard	se	7	3	—	—
Melinis minutiflora P.Beauv.	se	18	2	—	—
Melinis tenuissima Stapf	se	15	1	—	—
Oryza alta Swallen, IRRI accession no. 100 952	se	8	2	—	L
Oryza australiensis Domin, IRRI accession no. 101 397	se	5	1	+	S
Oryza barthii A.Chev., IRRI accession no. 100 122	se	5	1	+	S
Oryza brachyantha A.Chev. et Roehr., IRRI accession no. 101 235	se	5	1	+	S
Oryza eichingeri Peter, IRRI accession no. 101 419	se	4	1	—	L
Oryza glaberrima Steud., IRRI accession no. 100 160	se	5	1	+	S
Oryza grandiglumis (Doell) Prodh., IRRI accession no. 100 161, 101 405	se	4, 8	1, 2	—, —	L, L
Oryza latifolia Desv., IRRI accession no. 100 891, 100 914	se	3, 5	1, 1	—, —	L, L
Oryza minuta C.B.Presl, IRRI accession no. 100 887, 101 094	se	5, 5	1, 1	—, —	L, L
Oryza nivara Shara et Shastry, IRRI accession no. 101 512	se	5	1	+	S
Oryza officinalis Watt, IRRI accession no. 100 896, 101 118	se	8, 5	2, 1	—, —	L, L
Oryza punctata Steud., IRRI accession no. 100 125	se	5	1	+	S
Oryza ridleyi Hook f., IRRI accession no. 100 821	se	5	1	+	S
Oryza rufipogon Griff., IRRI accession no. 100 946, 101 156	se	5, 5	1, 1	+, +	S, S
Oryza sativa L., (see Table 6)	se	-	-	+	S

Oryza 'spontanea' (T.T.Chang's temporary designation for taxa of uncertain classification or hybrids of an intermediate nature), IRRI accession no 101 145	se	5	1	+	S
Panicum coloratum L.	se	15	1	—	—
Panicum infestum Anderss.	sp	3	1	—	—
Panicum infestum Anderss.	se	2	1	—	—
Panicum maximum Jacq., 'Makueni'	se	11	2	—	—
Panicum repens L.	sp	4	1	—	—
Panicum repens L.	se	5	3	—	—
Paspalidium desertorum (A.Rich.) Stapf	se*	30	2	—	—
Paspalum auriculatum J.S.Presl	se	4	2	—	—
Paspalum notatum Fluegge	se	7	3	—	—
Paspalum orbiculare Forst.	se	16	1	—	—
Pennisetum americanum (L.) K.Schum.	se	3	1	—	—
Pennisetum clandestinum Chiov.	sp*	4	1	—	—
Pennisetum mezianum Leeke	se	15	4	—	—
Pennisetum polystachion (L.) Schult.	se	10	1	—	—
Pennisetum purpureum Schumach.	sp*	4	1	—	—
Perotis patens Gand.	se	17	1	—	—
Phleum arenarium L.	se	55	4	+	S
Phleum bertolonii DC.	se	33	3	—	—
Phleum pratense L.	se	43	4	—	—
Phragmites australis (Cav.) Steud.	sp	2	1	—	—
Poa compressa L.	se	15	1	—	—
Poa nemoralis L.	se	35	3	—	—
Poa trivialis L.	se	15	1	—	—
Pogonarthria squarrosa (Roem. et Schult.) Pilg.	se	17	2	—	—
Pucinella distans (L.) Parl.	se	15	1	—	—
Rhynchelytrum repens (Willd.) C.E.Hubbard	se	22	3	—	—
Rhynchelytrum scabridum (K.Schum.) Chiov.	se	25	3	—	—
Roegneria canina (L.) Nevski	se	18	1	—	—
Rottboellia exaltata L. f.	se	12	5	—	—
Saccharum officinarum L., 'NCo 310'	sp	1	1	—	—
Saccharum officinarum L., 'Q 45'	sp	6	1	—	—
Schmidtia pappophoroides J.A.Schmidt	sp*	1	1	—	—
Secale cereale L.,	se	10	1	—	—
Secale cereale L., 'Zelder'	se	5	1	—	—
Sehima nervosum (Willd.) Stapf	se	4	3	—	—

Plant species	Grown from seed or split[1]	Number of plants tested	Number of tests	Symptoms[2]	Type of host plant[3]
Setaria atrata Hack.	sp*	1	1	—	—
Setaria chevalieri Stapf	se*	10	1	—	—
Seteria glauca (L.) P.Beauv.	se	16	2	—	—
Setaria holstii Herrm.	se*	5	2	—	—
Setaria holstii Herrm.	sp	3	2	—	—
Setaria longiseta P.Beauv.	sp	4	1	—	—
Setaria pallidifusca (Schumach.) Stapf et C.E.Hubbard	se	2	2	—	—
Setaria phragmitoides Stapf	sp	3	1	—	—
Setaria plicatilis (Hochst.) Hack.	sp	3	1	—	—
Setaria sphacelata (Schumach.) Stapf et C.E.Hubbard	se	16	2	—	—
Setaria splendida Stapf	sp*	3	1	—	—
Setaria verticillata (L.) P.Beauv.	se	6	2	—	—
Setaria viridis (L.) P.Beauv.	se	69	5	—	L
Sorghum bicolor (L.) Moench, 'H 726'	se	9	1	—	—
Sorghum bicolor (L.) Moench, 'H 6060'	se	12	2	—	—
Sorghum bicolor (L.) Moench, (field collected)	se	6	1	—	—
Sorghum brevicarinatum Snowden	se	11	1	—	—
Sorghum verticilliflorum (Steud.) Stapf	se	10	1	—	—
Sporobolus africanus (Poir.) Robyns et Tournay	se	9	2	—	—
Sporobolus agrostoides Chiov.	se	9	4	—	—
Sporobolus confinis (Steud.) Chiov.	se	1	1	—	—
Sporobolus consimilis Fres.	se	16	3	—	—
Sporobolus helvolus (Trin.) Dur. et Schinz	se	15	1	—	—
Sporobolus pyramidalis P.Beauv.	se	11	1	—	—
Themeda triandra Forsk.	se	2	1	—	—
Tragus berteronianus Schult.	se	8	2	—	—
Triticum aestivum L., 'Kenya Kudu'	se	20	2	—	—
Triticum aestivum L., 'Stella'	se	10	1	—	—
Triticum aestivum L., 'Wisconsin'	se	13	1	—	—
Triticum durum Desf.	se	14	1	—	—
Urochloa bolbodes (Steud.) Stapf	se*	2	2	—	—
Vulpia myuros (L.) C.C.Gmel.	se	10	1	—	—

Zea mays L., 'Caldera'	se	5	1	—	—
Zea mays L., 'Hybrid 611B'	se	5	1	—	—
Zea mays L., 'Hybrid 612'	se	10	2	—	—
Zea mays L., 'Hybrid 613B'	se	5	1	—	—
Zea mays L., 'Katumani CA'	se	8	1	—	—
Zea mays L., 'Synthetic III'	se	11	2	—	—
Cyperaceae					
Cyperus sp.	sp	6	1	—	—
Cyperus immensus C.B.Cl.	se	13	1	—	—
Cyperus papyrus L.	sp	3	1	—	—
Fimbristylis quinquangularis (Vahl) Kunth	se	15	1	—	—
Mariscus sieberianus Steud.	se	19	2	—	—
Pycreus flavescens (L.) Reichenb.	se	17	1	—	—
Typhaceae					
Typha domingensis Pers.	sp	8	2	—	—
Araceae					
Pistia stratiotes L.	sp	5	1	—	—
Commelinaceae					
Commelina diffusa Burm. f.	se	6	1	—	—
Commelina diffusa Burm. f.	sp	7	1	—	—
Commelina reptans Brenan	sp	3	1	—	—
DICOTYLEDONEAE					
Amaranthaceae					
Gomphrena globosa L.	se	5	2	—	—
Chenopodiaceae					
Beta vulgaris L.,	se	12	1	—	—
Beta vulgaris L., 'Corona'	se	4	1	—	—
Chenopodium amaranticolor Coste et Reyn.	se	9	3	—	—
Chenopodium quinoa Willd.	se	6	2	—	—
Compositae					
Carthamus tinctorius L.	se	12	1	—	—

Plant species	Grown from seed or split[1]	Number of plants tested	Number of tests	Symptoms[2]	Type of host plant[3]
Helianthus annuus L., 'Grey Striped'	se	7	1	—	—
Zinnia elegans Jacq., 'Californische Reuzen'	se	4	1	—	—
Cruciferae					
Brassica napus L.	se	10	1	—	—
Brassica chinensis L., 'Granaat'	se	4	1	—	—
Cucurbitaceae					
Cucumis sativus L., 'Lange Gele Tros'	se	3	1	—	—
Leguminosae					
Arachis hypogaea L., 'Natal Common'	se	5	1	—	—
Glycine max (L.) Merr.	se	2	1	—	—
Phaseolus vulgaris L., 'Master Piece'	se	3	1	—	—
Pisum sativum L., 'Onward'	se	12	1	—	—
Vigna unguiculata (L.) Walp.,	se	6	1	—	—
Vigna unguiculata (L.) Walp., 'Blackeye Early Ramshorn'	se	12	1	—	—
Onograceae					
Ludwigia stolonifera (Guill et Perr.) Raven	sp	7	1	—	—
Pedaliaceae					
Sesamum indicum L.	se	4	1	—	—
Solanaceae					
Datura stramonium L.	se	2	1	—	—
Nicotiana clevelandii Grah.	se	10	3	—	—
Nicotiana glutinosa L.	se	6	2	—	—
Nicotiana tabacum L., 'White Burley'	se	9	3	—	—
Petunia hybrida, 'Pink Beauty'	se	4	1	—	—
Pteridophyta					
Azollaceae					

In general mechanically inoculated *O.sativa* developed more severe symptoms in the glasshouse, than was observed in the field. Depending on growth circumstances, in 'Sindano', inoculated in the 3 – 4 leaf stage, the first symptoms were generally noticed 5 – 7 days after inoculation and consisted of small yellow dots at the base of the youngest leaves. Soon after the whole leaf was dotted and the dots became elongated parallel to the veins, so that the leaves looked a yellow-green. Later formed leaves were mottled and often spirally twisted (Fig. 7). After turning yellowish the leaves soon became necrotic. The plants were severely stunted, formed few tillers and no panicles. A partial recovery of the rice seedlings inoculated at this stage could occur, but mostly the plants died prematurely. When the plants were ratooned 2 – 3 weeks after inoculation, the newly formed leaves showed clear symptoms but did not become necrotic so soon.

Light microscopy was used to study whether there are internal symptoms under-

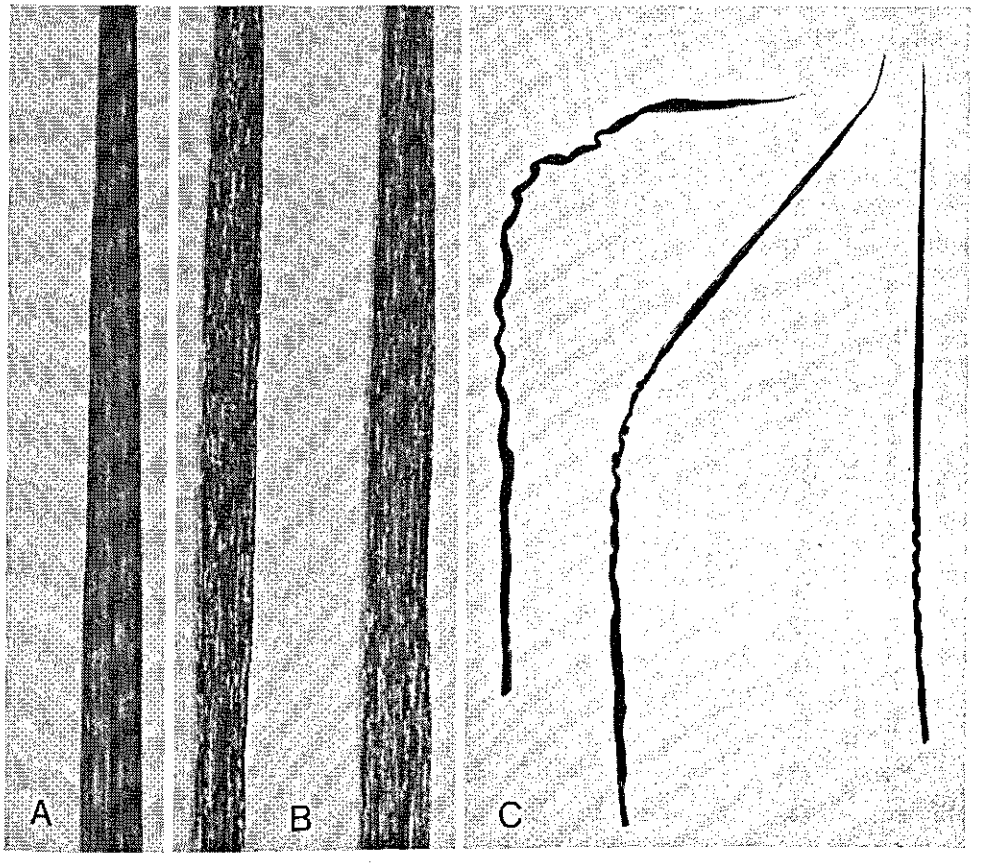


Fig. 7. Symptoms induced in 'Sindano' rice leaves after mechanical inoculation with RYMV.
A. Yellowish dots which elongate.
B. Mottling.
C. Crinkling.

lying the external symptoms in a RYMV-infected rice leaf. The most obvious difference between healthy and RYMV-infected 'Sindano' leaves noticed in transverse sections, was the difference in colour: green for healthy material and yellowish-green for RYMV-infected leaf tissue. The mesophyll cells of infected leaves appeared to have a reduced number of chloroplasts. No inclusions or structures attributable to the RYMV infection were noticed in the infected leaf.

In the other rice varieties similar symptoms were induced, although differences in sensitivity were noticed. In the variety 'Basmati 217' it was difficult to discern the first symptoms.

The other reacting hosts all showed mottling of the leaves which were often lighter in colour too (Fig. 8). Appearance of the first symptoms was noticed 7 – 14 days after inoculation. For the other *Oryza* spp., symptoms ranged between mottling of the whole leaves to only a few elongated dots like in *O.punctata.* In *Dinebra retroflexa,*

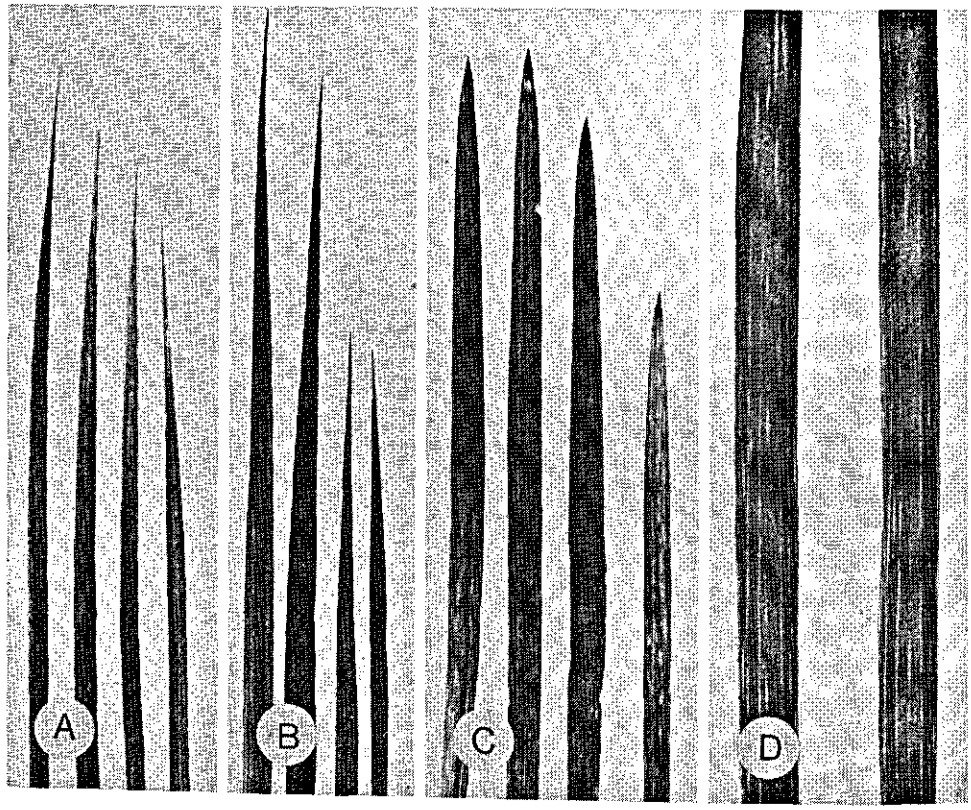


Fig. 8. Leaf symptoms of grasses after mechanical inoculation with RYMV.
A. *Dinebra retroflexa.*
B. *Eragrostis ciliaris.*
C. *Phleum arenarium.*
D. *Oryza punctata.*

symptoms were easily noticeable by the yellowish-green discolouration and mild mottling of the newly formed leaves. The clearly mottled leaves of *Phleum arenarium* turned yellow and died off later.

4.1.2 Factors influencing symptom development

In general virus multiplication depends on the condition of the host plant. Day-length, temperature, light intensity, humidity, potting medium and nutrition influence the growth of the plant, while symptom expression may be strongly influenced by light. During the experiments in Nairobi and Wageningen the following observations with regard to RYMV in rice and other hosts were made:

1. 'Sindano', inoculated in 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. Prolonged periods of temperatures below 20°C delayed symptom appearance up to 10 – 12 days.

   At 20°C the yellow dots enlarged more slowly parallel to the veins than at 25°C, while at higher temperatures the period in which only yellow dots were seen was very short.
2. Inoculation of 'Sindano' at increasing age, delayed symptom appearance.
3. In the glasshouse spiral twisting of the leaves was seen regularly, but not in the field. After the first leaves showed symptoms, new growth was inhibited and newly formed leaves appeared to meet difficulties in emerging. In the glasshouse, plants were also more stunted than in the field. Conditions of growth might have influenced these phenomena.
4. Rice leaves with symptoms turned necrotic earlier in the glasshouse than in the field. The low relative humidity is thought to be responsible for this difference.
5. Many of the non-rice host plants, e.g. *Eragrostis aethiopica, E.namaquensis* var. *namaquensis* and *Diplachne caudata,* showed more pronounced symptoms in the glasshouse in Nairobi than in Wageningen. A possible cause might have been the daylength.

4.1.3 Influence of time of infection on performance of three rice varieties

The occurrence of blast and RYM together in field grown rice made it difficult to assess the damage caused by RYM alone. To obtain information on the growth, yield, germination capacity and sensitivity to RYMV at various ages of the rice plants, plants of the varieties 'Sindano', 'Basmati 217' and 'IR 22' were mechanically inoculated in the laboratory at different stages of development.

Methods

Uniform seedlings were transplanted at a 3 leaf stage to a tin, lined with a plastic bag, and filled with 'black cotton soil' in water. The first group was inoculated at a

4 – 5 leaf stage and subsequent groups, each group consisting of 20 plants, at intervals of about two weeks. Of each variety six groups were inoculated with the standard inoculum, while a seventh group served as control.

Just before transplanting each tin received 150 ml of the standard fertilizer solution. When the panicles appeared in the control, each plant was given 5 ml of a solution which contained 30 g $(NH_4)_2SO_4$ per litre tap water. At harvest each panicle was put in a paper bag for readings later. Height was recorded by measuring the distance between ground level and a ruler held above the top of a group of plants.

In recording the number of panicles, those panicles just emerging from the flag leaf-sheath were included, and the number of spikelets included the filled as well as the empty ones. Germination capacity was determined at the Seed Testing Laboratory at the NAL 5 months after harvest.

The growing season of 'Sindano' and 'Basmati 217' was in the warmer period of the year, but the flowering of 'IR 22' occurred in the cooler period of the year and consequently no seeds were formed in this variety.

Results

Leaf symptoms were only noticed in new growth and appearance of the first symptoms was delayed at increasing age of the inoculated plants. In 'Sindano' symptoms were as described in Section 4.1.1; the first inoculated group recovered partly. In 'Basmati 217', leaf symptoms were masked and difficult to notice in full light. By hand shading symptoms were better visible. In this variety the last two inoculated groups did not show symptoms of the disease, but when ratooned after harvest, the new growth of these plants showed symptoms. Clear symptoms appeared in 'IR 22'. The influence of the time of infection of RYMV on height is given in Figs. 9 and 10, while the other performance characteristics of the three rice varieties are given in Table 7.

Height After the first symptoms appeared, growth of the plants was retarded. At later stages of development this retardation was still noticeable especially with 'Sindano', to a lesser extent with 'IR 22' and least with 'Basmati 217'.

Number of panicles The number of panicles depended on the time of infection. When the plant was inoculated at an early stage, this number was strongly reduced in the three varieties. Infection at a later stage had little influence on tillering and panicle formation in 'Basmati 217' but was more pronounced in 'IR 22' (the control showed many late formed tillers, which would not have given any seed yield). In 'Sindano' panicle formation was strongly reduced in all groups.

Number of spikelets and percentage empty grains In 'Sindano' and 'Basmati 217' the number of spikelets per plant followed the trend shown by the number of panicles per plant. In 'Sindano' all inoculated groups showed a very high percentage of empty

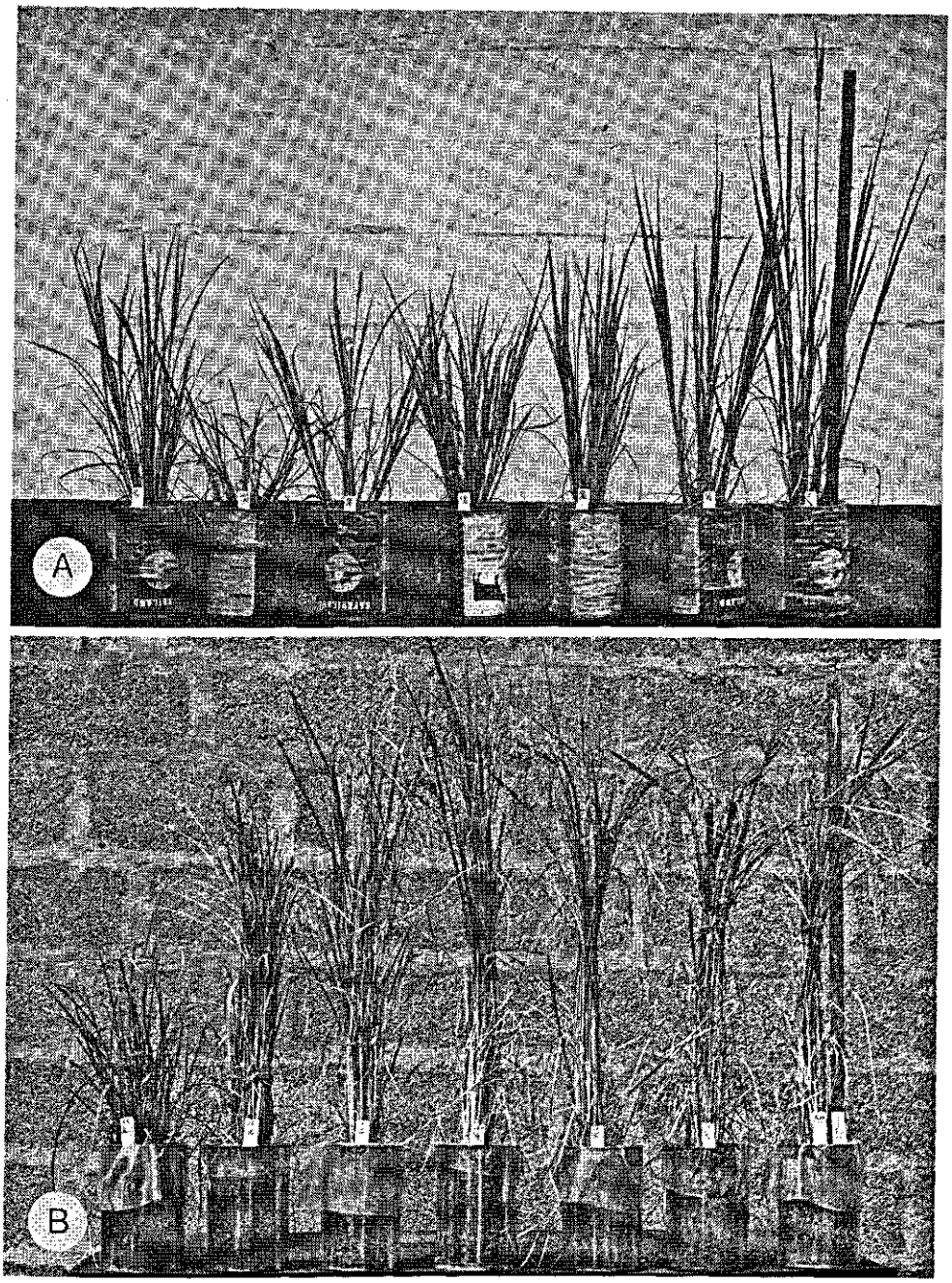


Fig. 9. Influence of the time of infection with RYMV on performance of rice.
A. Rice 'Sindano'. From left to right, plant inoculated at 34, 49, 64, 79, 93, 110 days after sowing and control.
B. Rice 'Basmati 217'. From left to right plant inoculated at 25, 39, 53, 67, 81, 95 days after sowing and control. (Plants are tied up).

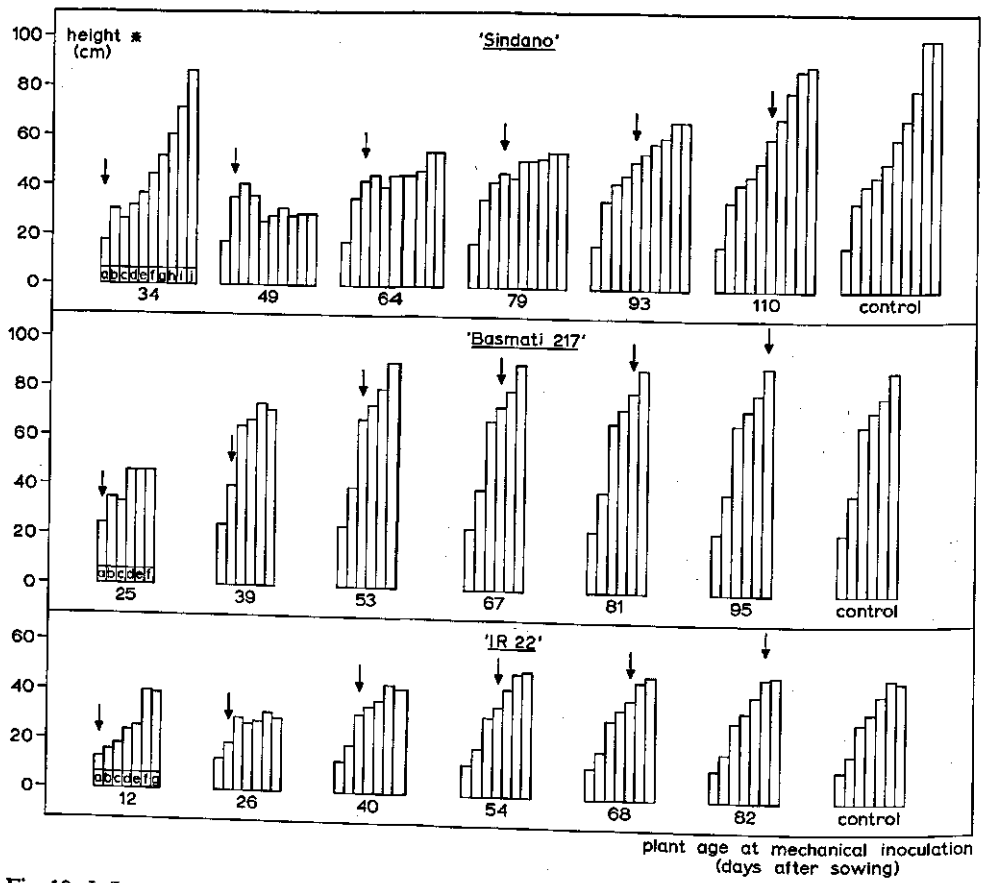


Fig. 10. Influence of the time of infection with RYMV on height of the rice varieties 'Sindano', 'Basmati 217' and 'IR 22'. Arrow indicates time of mechanical inoculation.
*Recording data in days after sowing.

	a	b	c	d	e	f	g	h	i	j
'Sindano'	34	49	64	79	93	110	123	138	153	169
'Basmati 217'	25	39	53	67	81	95				
'IR 22'	12	26	40	54	68	82	96			

grains. In 'Basmati 217' the percentage of empty grains was less at each later inoculation.

Yield Apart from the first inoculated group in 'Sindano' which yielded per plant less than half of the control, the other groups in this variety produced still more strongly reduced yields. In 'Basmati 217' the yield per plant increased with increasing age of the plant at inoculation. The last infected group even produced better than the control. The seeds of the infected plants were in general lighter, which was more pronounced in 'Sindano' than in 'Basmati 217'.

Table 7. Influence of the time of infection of RYMV on performance of the rice varieties 'Sindano', 'Basmati 217' and 'IR 22'.

Variety[1]	Plant age at inoculation (days after sowing)	Appearance of symptoms (days after inoculation)	Number of panicles/ plant	Number of spikelets/ plant	Percentage empty grains	Weight of filled grains/plant (g)	1000 grain weight (g)	Germination capacity after 5 days	14 days
Sindano	34	7	6.6	497	25.5	5.8	15.7	60	68
	49	9	—	—	—	—	—	—	—
	64	11	0.5	29	100	—	—	—	—
	79	16	3.9	204	99.6	0.1	15.2	68	79
	93	20	4.8	228	99.8	0.1	22.2	56	56
	110	17	5.6	418	89.7	0.7	16.1	60	62
	control		9.3	715	8.3	13.9	21.3	95	97
Basmati 217	25	6	0.3	5	100	—	—	—	—
	39	9	9.8	593	95.5	0.4	14.6	85	96
	53	17	18.0	1262	61.6	9.4	19.5	92	99
	67	20	20.8	1464	47.1	13.9	18.0	87	99
	81	—	18.3	1282	22.4	18.8	18.9	92	98
	95	—	19.4	1383	17.9	22.0	19.3	94	99
	control		19.0	1313	17.5	19.8	18.3	89	98
IR 22[2]	12	5	1.5						
	26	8	0.3						
	40	9	6.9						
	54	17	16.0						
	68	21	25.4						
	82	25	24.6						
	control		32.9						

1. Particulars of growing of the rice varieties.

Variety	Grown in	Growing season	Heading (days after sowing)	
			control	harvest
Sindano	screenhouse	Sept-March	129	190
Basmati 217	glasshouse	Sept-March	87	116
IR 22	glasshouse	Febr-August	96	178

2. No seeds were obtained because of the low temperatures during flowering.

Germination capacity In 'Sindano' the germination capacity of the filled grains was negatively influenced by the disease, while in 'Basmati 217' no influence of the virus infection of the plants was noticed.

4.2 Transmission by other ways

Irrigation water In February and March 1969 water samples were collected from a rice field at Otonglo. The rice had been harvested about a month earlier and the ratoon rice had formed well developed leaves. The incidence of RYM was very high. The water level was low while cattle was grazing in the field. Both times four water samples were taken and inoculated without any treatment onto 'Sindano'. Three of the samples taken in February and all samples from March proved to be infectious. The presence of RYMV in the inoculated plants was serologically confirmed by the agar-gel diffusion method.

Guttation fluid About $\frac{1}{4}$ ml guttation fluid collected from 'Sindano' plants mechanically inoculated 3 months earlier, was inoculated onto 9 'Sindano' seedlings. After two weeks RYM symptoms were observed in all plants.

Seed Seeds collected from several mechanically infected rice varieties were planted in boxes or pots six weeks after harvest. The varieties used and the number of germinated seeds were: 'Basmati 217' (110), 'Blue Belle' (24), 'Fujiminori' (23), 'IR 661-1-127-3-1' (2), 'IR 822-347' (35), 'Kibawa Chanzi' (340), 'Shimokita' (12), 'Sindano' (114), and 'YRL I' (341). None of the seedlings showed symptoms of the disease after two months.

Dodder When *Cuscuta subinclusa* Dur. et Hilg. was trained from *Vicia faba* L. to young leaves of ratoon 'Sindano' with clear RYM symptoms, the dodder did not establish on the rice. No transmission experiments could therefore be performed.

Contact By inoculating only a part of the 5 – 10 'Sindano' seedlings in a bucket, only the inoculated plants developed symptoms of the disease. Occasionally a non-inoculated plant became infected after 1 – 2 months, possibly caused by contact of the leaves or roots of the healthy and diseased plants. Afterwards when all seedlings were cut together with a sterile knife, symptoms of RYM were noticed in a large percentage of the new growth of the non-inoculated plants.

4.3 Conclusions

RYMV proved to be easily transmissible by mechanical means. Slightly diluted sap from 'Sindano' leaves affected with RYMV was highly infectious. In all tested rice varieties mechanical inoculation of this sap caused complete infection. Apart from rice, a limited number of plants in the Gramineae were found to be a host for the

virus. Systemic hosts belonged mainly to the tribes Oryzeae and Eragrostideae, providing an indication which plants apart from rice may be a natural host for the virus. *Phleum arenarium* (Phalarideae) proved to be a systemic host in which symptoms were also incited. Hosts from which the virus was recovered from the inoculated leaves only, were found in the tribes Oryzeae and Eragrostideae, but also in genera of the tribes Bromeae and Paniceae. Possible systemic hosts may exist in these genera too. A possible cause for the irregular recovery of RYMV from some of these plants may be that leaves were often dying off when collected.

An infection with RYMV caused more pronounced effects in 'Sindano' in the laboratory than was noticed in the field. The effects of an infection with the virus on seed yield of 'Sindano' was still noticed when infection took place about three weeks before heading of the plants. For the rice variety 'Basmati 217' this effect was restricted to those plants infected at a young stage. From the influence of the time of infection of RYMV on height in 'IR 22', it may be concluded that yield depression might be severe in this variety too.

RYMV was not found to be seed transmissible. That the irrigation water collected at Otonglo proved to be infective, must be considered as an exceptional case. There was only shallow water, and cattle grazed here too. Apart from crushing rice plants, they possibly contaminated the water through their faeces. It is not believed that this source of contamination has practical implications for newly planted fields. Infection of rice plants in the field by contact cannot be excluded. This form of infection is however not believed to be responsible for the fast spread of the virus in fields which have been planted recently.

5 Transmission by invertebrates

Initially during the search for the vector of RYMV, transmission experiments were performed with leafhoppers. These insects were present abundantly in and around the rice fields. The fact that RYMV proved to be mechanically transmissible made it unlikely, however, that the vector would be found in this group of insects. After negative results other possible vectors were tested. The regular occurrence of mites on diseased rice at Otonglo necessitated close examination of these organisms as possible transmitting agents. After electron micrographs had been obtained from purified RYMV, revealing polyhedral virus particles, attention was directed towards beetles of which several species were tested.

As each group of possible vectors required different handling, supplementary techniques are given under the respective headings.

5.1 Nematoda

Although the spread of RYM in the field did not resemble that of a nematode-transmitted virus, samples of rice, root and soil were sent twice from Otonglo for investigation for nematodes. The nematodes extracted, mostly identified to genus only, were: *Aphelenchoides* sp., *Criconemoides* sp., *Helicotylenchus* sp., *Hemicycliophora* sp., *Hirschmanniella oryzae* (van Breda de Haan), *Meloidogyne* sp., *Pratylenchus* sp., *Trophurus* sp. Of these nematodes *H.oryzae* had not yet been recorded in East Africa (B. W. Ngundo – pers. commun., 1968).

Two lots of one hundred 'Sindano' seedlings were transplanted in soil collected around diseased rice plants at Otonglo and observed in the glasshouse for two months. None of the seedlings became infected with RYMV.

Mechanical inoculation of plants with an inoculum prepared by grinding nematodes, extracted from rice root samples from Otonglo, in some 0.01 M phosphate buffer pH 7.0, did not result in infection of the 'Sindano' seedlings.

5.2 Arachnida - Acarina

Most of the mites recorded on rice belong to the family Tetranychidae (Grist & Lever, 1969), but from Madagascar a deformation of rice, initially attributed to *Tarsonemus oryzae* Targ.-Toz. (Bouriquet, 1946), proved to be caused by *Steneotarsonemus madecassus* Gutierrez (Fam. Tarsonemidae) (Gutierrez, 1967). This author reported that the panicles emerged before being fully grown, while the axes were

twisted like a corkscrew. The seeds were aborted or developed poorly. The described damage shows some resemblance to the effect of RYMV on the panicle of rice.

At Otonglo, rice plants were frequently colonized by mites. These mites belonged to the following species:

- *Aceria bakkeri* K. (Fam. Eriophydidae), also collected from the grass *Paspalum orbiculare* Forst. *A.bakkeri* is very close to *A.tulipae* K. (Keifer, 1969), the vector of wheat spot mosaic virus and wheat streak mosiac virus (Slykhuis, 1967).
- *Steneotarsonemus spinki* Smiley (Fam. Tarsonemidae) (Smiley, 1967), also collected from the grass *Brachiaria radicans* Napper.
- *Amblyseius* sp. (Fam. Phytoseiidae), a predator mite.

Tests were performed with *A.bakkeri* and *S.spinki* of which large colonies were present in humid conditions under the leaf sheaths, while *S.spinki* was also found on the panicle. Because of its size and the humid conditions in which these pests lived, difficulties were encountered in experimentation.

Methods

For the transmission experiments mites were collected from the leaf sheaths of infected rice from Otonglo. Sometimes an additional acquisition feeding period of 1 – 3 days on mechanically infected 'Sindano' was given at the laboratory. Therefore mites were placed under the leaf sheath, singly transferred or on a small piece of leaf sheath of the field collected plant. To allow loosening of the leaf sheath more developed rice plants were used. The mites were transferred to the test plants by the same methods, while a number of control plants received pieces of leaf sheath of a diseased plant without mites. The plants were covered by a glass cylinder of appropriate size, covered by muslin cloth. The mites were not removed from the plants, which were examined to see whether a colony had been established.

The inoculum from ground mites was prepared from 50 *A.bakkeri* and 50 *S.spinki* in $\frac{1}{2}$ ml 0.01 M phosphate buffer pH 7.0 separately. To prove the presence of RYMV in the liquid under the leaf sheath of diseased rice, which was also transported at transfer of the mites, the single hair brush was dipped in this liquid and subsequently in $\frac{1}{2}$ ml of the buffer 100 times. The suspension so obtained was inoculated mechanically on 'Sindano'.

Results

Singly transferred *A.bakkeri*, 4 – 50 mites/test plant, infected none of the 14 'Sindano' plants on which they were placed, while with *S.spinki*, 50 – 200 mites/test plant, 2 out of 35 plants showed symptoms of the disease.

Transfering 20 – 30 mites on pieces of RYMV-infected leaf sheath resulted with *A.bakkeri* in infection of 3 out of 6 plants and with *S.spinki* in infection of all 4 plants used. When similar pieces of RYMV-infected leaf sheath without mites were placed under the leaf sheath of the test plants, symptoms developed in 6 out of 10 plants.

Often both mite species formed colonies on the test plants.

Inoculation of the suspension, obtained by dipping the single hair brush in buffer after a preceding dip in the liquid present under the leaf sheath of an infected rice plant, resulted in 3 out of 5 plants becoming infected. The ground suspensions of the two mite species proved infective for all 10 plants in both cases.

5.3 Insecta

5.3.1 Hemiptera

Most viruses and mycoplasma-like organisms of rice are transmitted by insects of this order. The first insect to be tested as a possible vector of RYMV was a cicadellid (*Nephotettix afer* Ghauri) a recently described species and common in the Kenya rice fields. According to Ghauri (1968) the genus *Nephotettix* is only represented by *N.afer* and *N.modulatus* Melichar in Africa, Malagasy and Palestine. This genus, to which several vectors of rice viruses and mycoplasma belong, has been reviewed by Ghauri (1971).

Other species of this order tested included:

- *Cicadulina mbila* Naude, a vector of maize streak virus, common in a large part of Africa.
- *Locris auripennis* Dist. which occurs abundantly in the swampy fields near Kisumu and *L.cardinalis* Gerst. Harris (1937) reported yellow discolouration of the rice shoots after feeding of *L.neumanni* Jac.
- Aphid spp., most of them naturally occurring on Gramineae. Of these aphids *Rhopalosiphum rufiabdominalis* Sasaki was regularly found at the base and the roots of non-flooded rice at Otonglo. This rice root aphid is distributed over a large part of the world (Tanaka, 1961).

Methods

Apart from *C.mbila*, which was bred in the laboratory at the East African Agriculture and Forestry Research Organization and kindly supplied by Dr K. R. Bock, and *Myzus persicae* which was bred at the NAL, the insects were field collected. Of the aphids, only *R.rufiabdominalis* was collected from diseased rice plants at Otonglo, while the others were collected from Gramineae at Nairobi. The *Locris* species were caught at Otonglo and at Nairobi. Often mixtures of adults and larvae were used in the experiments. In the experiments with *N.afer*, the rice plants were ratooned occasionally after 2 – 4 months.

Results

None of the insects tested transmitted RYMV (Table 8). The only rice plant in which symptoms were noticed, 7 months after the inoculation feeding of *N.afer*,

Table 8. Transmission of RYMV by Hemiptera from mechanically infected rice 'Sindano' to 'Sindano'.

Insect	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Results[2]
Cicadellidae							
Cicadulina mbila Naude	3 – 4	—	3	a	5	E	0/3
Nephotettix afer Ghauri	1 – 4	—	1 – 6	a	2 – 5	E	1/26[3]
Cercopidae							
Locris auripennis Dist.	10	—	4	a	3 – 4	B	0/5
	2	—	4	a	2	B	0/10
Locris cardinalis Gerst.	3	12	5	a	3	B	0/1
Aphididae							
Kugegania ageni Eastop	10	½	2	d	4	E	0/4
Metopolophium dirhodum Wlk.	15	½	4	d	4	E	0/3
Myzus persicae Sulzer	5 – 7	½	1	d	1	E	0/2
Rhopalosiphum maidis Fitch	9 – 20	½	1 – 2	d	2 – 4	E	0/9
Rhopalosiphum rufiabdominalis Sasaki	5 – 60	—	1	d	2 – 3	E	0/16
Sitobion sp.	14	1½	2	d	1	E	0/3
Sitobion chanikiwiti Eastop	17 – 40	½	1 – 3	d	3	E	0/3

1. a: on 5 – 6 plants, d: in micro cage, B: in large glass tube, E: in micro cage.
2. Ratio infected to tested plants.
3. Plant showed symptoms after 7 months.

was probably infected by contamination. *Locris* spp. caused the death of the acquisition plants as well as of many of the test plants after 4 weeks.

5.3.2 Coleoptera

Superficial inspection of rice revealed little damage attributable to beetles in the Kisumu area. When the RYMV particles proved to be isometric, as is the case with beetle transmitted viruses, attention was directed towards these insects, which were abundantly present on ratooned rice and on the grasses growing on the bunds. Several species mostly belonging to the Chrysomelidae, but also to other families were tested. When beetles could be collected in high numbers, more thorough experiments were performed.

5.3.2.1 CHRYSOMELIDAE

5.3.2.1.1 Transmission experiments

Criocerinae

Of the Criocerinae, *Oulema oryzae* (Kuwayama) causes severe damage to the rice leaves as larvae and adults in northern Japan, Korea and China, while *O.tibialis* (Lap.) attacks millet as well as paddy in Senegal and Mali (Grist & Lever, 1969). Heinze & Pinsdorf (1963) have revised the African Criocerinae.

O.melanopa L. and *O.lichenis* (Voet.) are vectors of viruses in Gramineae like cocksfoot mottle virus (Serjeant, 1967) and *Phleum* mottle virus (Catherall, 1970). The Criocerinae were therefore of special interest for the study of the transmission of RYMV. In Kenya however, only low numbers of members of this subfamily were caught. Therefore additional tests were performed with *O.melanopa* and *O.lichenis* in the Netherlands.

The insects tested were: *Lema* spp., *L.chalcoptera* Lac., *L.chalcoptera* Lac. var. *sanguinicollis* Lac., *L.diversicola* Hze., *L.mulangensis* Hze., *Oulema dunbrodiensis* Jac. f. *nigripennis* Hze., *O.lichenis* (Voet.) and *O.melanopa* L.

Methods

In Kenya the insects were collected on a vegetation mainly consisting of grasses. *O.melanopa* and *O.lichenis* were collected on wheat at Wageningen. For the transmission experiments the generally adopted procedures were followed. For *O.melanopa* and *O.lichenis* mechanically infected *Phleum arenarium* served also as acquisition virus source, while this plant was also used as test plant.

Results

The results of the transmission experiments are given in Table 9. Most insects caused no or only little feeding damage to the rice. Of the insects indigenous in Kenya, only *O.dunbrodiensis* f. *nigripennis* was able to transmit RYMV. This insect made short strip incisions along the rice leaves. At Wageningen, *O.melanopa* and *O.lichenis* hardly fed on rice. The feeding damage was also in the form of short strip incisions. Although these insects caused more feeding damage on *P.arenarium* than on rice 'Sindano', much more feeding damage was caused on wheat. No transmission of RYMV with *O.melanopa* and *O.lichenis* was obtained.

Galerucinae

Several Galerucinae are known to be vectors of plant viruses (Walters, 1969), but there are only few reports of damage to rice by insects of this subfamily. In the experiments with RYMV a number of Galerucinae, some of which have only been partly identified, were tested. The insects tested for transmission ability of RYMV were: insects belonging to a genus near *Apophylia* (Fig. 11), *Lamprocopa* sp.?, insect belonging to a genus near *Leptaulaca*, *Luperodes quaternus* Fairm., *Monolepta flaveola* Gerst., *M.haematura* Fairm., *M.intermedia* Rits., *M.irregularis* Rits. and *Sesselia pusilla* Gerst. (Fig. 12).

Of these insects *S.pusilla* was collected most. *Sesselia* belongs to a group of Galerucinae, which Wilcox (pers. commun., 1969) tentatively called a tribe 'the Metacyclini' although he stated 'there is considerably doubt as to the validity of considering this group a distinct tribe and doubt concerning its relationship to the other tribes in the Galerucinae'. Little is known about the biology of these insects and to Wilcox's knowledge no larvae of any of the 30 genera in this group have been found.

Sesselia flavicincta Jac. has been reported from Zaire (Congo) (Pelerents, 1957) and *Monolepta bifasciata* Hornst. from Malaysia (Yunus & Rothschild, 1967) both feeding from rice pollen. Other *Monolepta* spp. recorded from rice are: *M.signata* (Oliv.) a major pest in Nepal, *M.suturalis nigrobilineata* Motsch. occurring in Japan, while *M.elegans* All. and *M.goldingi* Bryant occur in Nigeria (Grist & Lever, 1969).

S.pusilla was common in the swampy areas near Kisumu and present throughout the year at Otonglo where at times very large numbers could be caught. But the insect was not so numerous at the Ahero Pilot Scheme. Initially the insects were caught in fields of ratoon rice, but later the beetles were found eating pollen of *Cyperus* spp., especially *C.latifolius* Poir. and *C.articulatus* L. Other sedges or grasses on which the insects were found feeding from pollen too, are: *C.longus* L., *C.laevigatus* L., *Leersia hexandra* Sw., *Digitaria scalarum* (Schweinf.) Chiov., *Panicum repens* L. and *Sporobolus pyramidalis* P.Beauv.

Table 9. Transmission of RYMV by Criocerinae (Chrysomelidae).

Insect	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	(method)[1]	source[2]	Inoculation period (days)	(method)[1]	test plant[2]	Feeding damage to test plant	Results trans-mission[3]	ser.test
Lema sp.	2	—	3	a	rice	7	C	rice	none	0/1	
	1	—	3	a	rice	2	A	rice	none	0/1	
Lema sp.	1	—	3	a	rice	3	D	rice	none	0/1	
	1	1	3	a	rice	3	D	rice	none	0/1	
Lema chalcoptera Lac.	1	—	3	b	rice	3	D	rice	none	0/1	
Lema *chalcoptera* Lac. var. *sanguinicollis* Lac.	1	—	5	a	rice	3	D	rice	little	0/2	
Lema diversicola Hze.	1	2	3	a	rice	3	D	rice	none	0/4	
	1	1	3	a	rice	3	D	rice	none	0/1	
Lema mulangensis Hze.	1	—	3	b	rice	3	D	rice	none	0/1	
Oulema dunbrodiensis Jac. f. *nigripennis* Hze.	1	—	5	a	rice	3	A	rice	moderate	0/1	
	1	—	3	a	rice	3	D	rice	moderate	1/1	positive
Oulema lichenis (Voet.)	3	17	1	a	rice	3	D	rice	little	0/2	
	3	17	1	a	*P.arenarium*	3	D	rice	little	0/2	
Oulema melanopa L.	1	19	1	a	rice	4	D	rice	little	0/8	
	2	19	1	a	rice	4	D	rice	little	0/7	
	3	17	1	a	rice	3	D	rice	little	0/9	
	3	17	1	a	*P.arenarium*	3	D	rice	little	0/10	
	5	24	4	a	*P.arenarium*	4	D	*P.arenarium*	little	0/9	

1. a: on 5 – 6 rice plants, b: on 1 rice plant, A: in small glass tube, C: in lamp glass, D: in PVC tube.
2. Rice: rice 'Sindano'.
3. Ratio infected to tested plants.

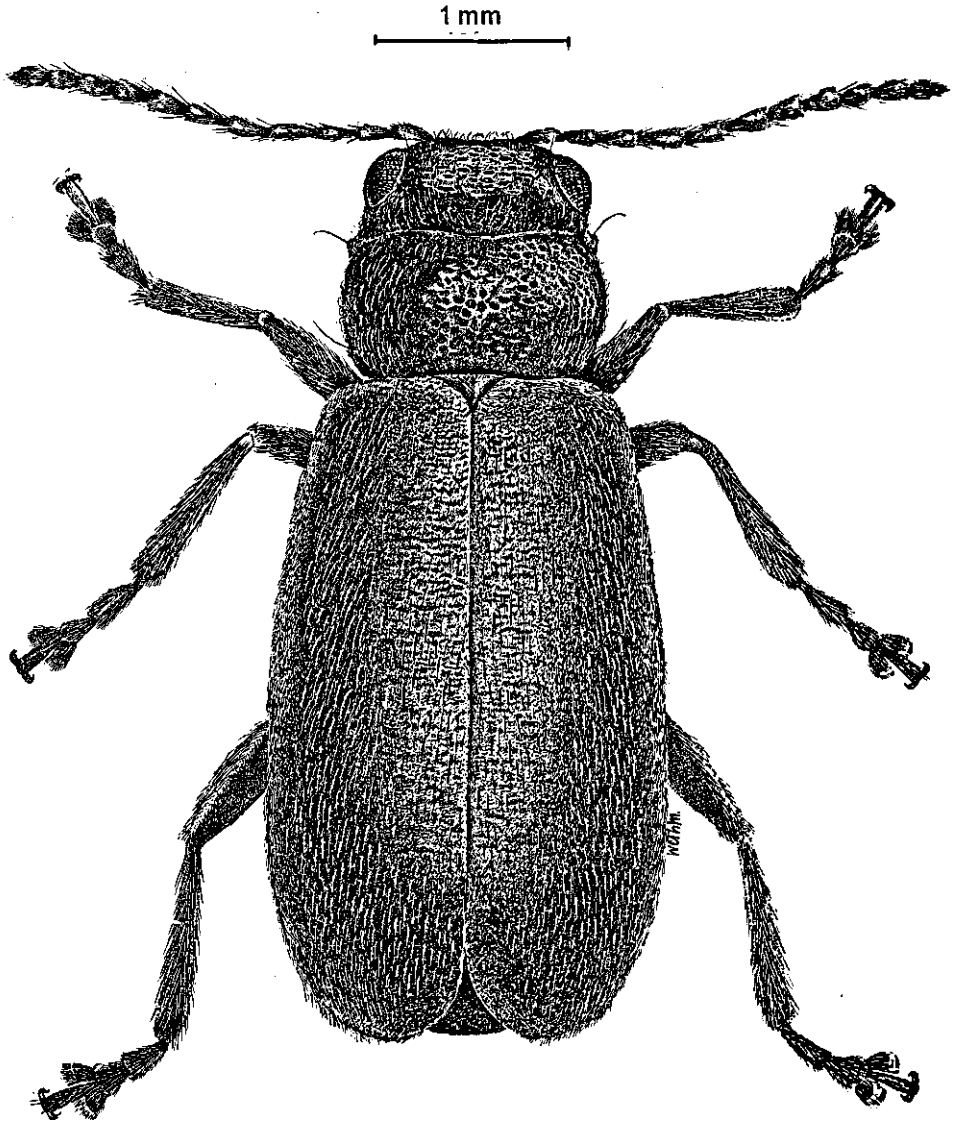


Fig. 11. Beetle of a genus near *Apophylia* (Chrysomelidae, Galerucinae).

Methods

The insects were field collected and the generally adopted procedures were followed. *S.pusilla* was caught at Otonglo. Initially collections were made on ratoon rice, but later the flowers of *Cyperus* spp. were shaken above the net. To test the presence of RYMV in *S.pusilla*, unstarved beetles were placed for 4 days on diseased rice (Method

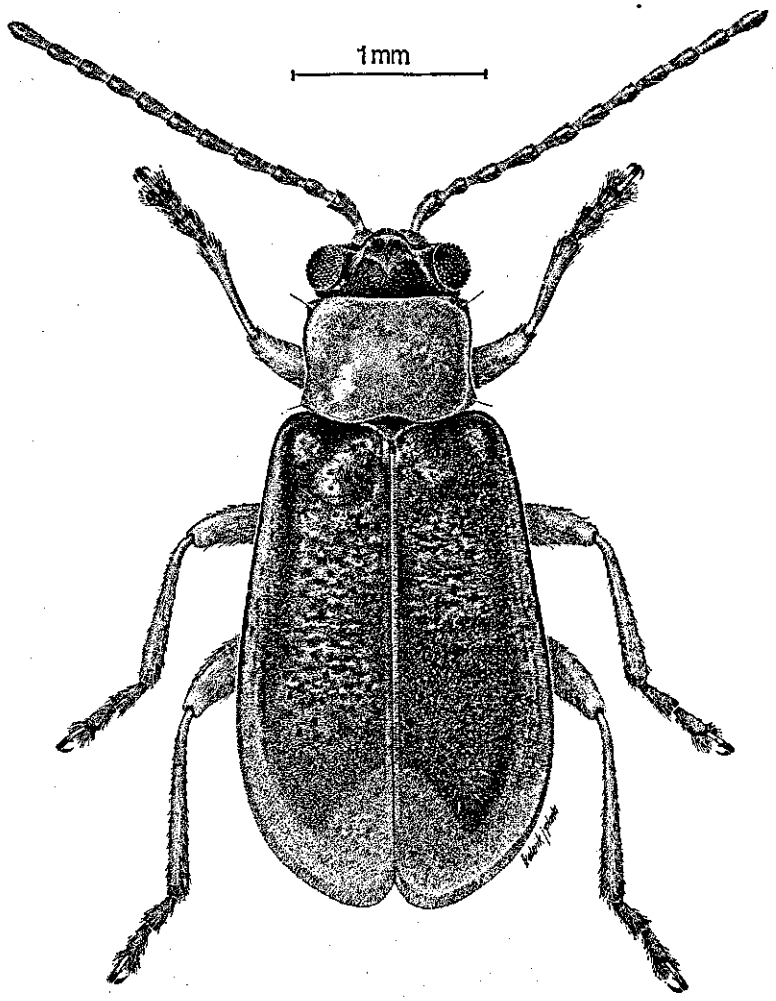


Fig. 12. *Sesselia pusilla* Gerst. (Chrysomelidae, Galerucinae).

a). Twenty insects were decapitated and the heads and the rest of the bodies ground separately in 1 ml 0.01 M phosphate buffer pH 7.0. The liquid was inoculated on 6 'Sindano' seedlings. To test the infectivity of the faeces, ten insects which were given the same acquisition period were placed on a healthy leaf in a test tube. After 4 hours the faeces was collected and suspended in 1 ml buffer and subsequently inoculated on 6 rice seedlings. Breeding of *S.pusilla* was attempted on *C.articulatus* and 'Sindano'.

The three insects belonging to a genus near *Apophylia* were caught with a net on grasses at Otonglo and Ahero on different occasions. The *Monolepta* spp. were collected mainly on flowers of *Cyperus* spp. at Otonglo and at Mwea, while the other insects were collected on grasses.

Results

The results of the transmission experiments are given in Table 10. Transmission of RYMV was effected by insects of the genus near *Apophylia, Monolepta flaveola, M.haematura* and *Sesselia pusilla.*

Insects of the genus near *Apophylia* caused slightly frayed sides of the rice leaves (Fig. 19A). The *Monolepta* spp. caused similar damage but with most damage at the tip of the leaves. *S.pusilla* caused frayed damage (Fig. 19B) while these insects gnawed at the stem too.

The insects of the genus near *Apophylia,* transmitting the virus in all three tests with a single insect per plant, and *S.pusilla* proved to be efficient transmitters of RYMV from 'Sindano' to 'Sindano'. *S.pusilla* also transmitted RYMV from 'Sindano' to the following rice varieties: 'Basmati 217', 'Faya SL', 'IR 5', 'IR 8', 'IR 22', 'IR 24', 'IR 12-178-2-3', 'IR 52-18-2', 'Madevu', 'Portuguese', 'Radin Goi', 'Shingo la Majani' and 'Uchuki'.

RYMV was recovered from the head – 3 out of 6 plants gave symptoms –, the rest of the body (6/6), as well as from the faeces of *S.pusilla* (6/6).

Attempts to breed *S.pusilla* failed. The beetles mostly died within 3 weeks and no larvae were noticed.

Halticinae

Several members of the Halticinae are known to be vectors of plant viruses, e.g. viruses affecting Cruciferae such as turnip yellow mosaic virus, turnip crinkle virus and radish mosaic virus (Walters, 1969). A number of insects belonging to this subfamily, with *Chaetocnema* spp. being collected in the highest numbers, were tested for ability to transmit RYMV.

The insects of the genus *Chaetocnema* Stephens are characterized by a tooth on the dorsal side on the intermediate and posterior tibiae (Jourdheuil, 1963). Many species are known throughout the world and several of them damage crops (Bryant, 1928; Jourdheuil, 1963). Plants belonging to the families Polygonaceae and Chenopodiaceae are often preferred as food plants, but several species do feed on cereals. The following have been recorded as being minor pests of rice: *C.obesula* Lec., *C.basalis* (Baly), *C.gregaria* Weise (Grist & Lever, 1969), *C.concinnipennis* Baly (Bryant, 1928). Reveche (1922) reported a *Chaetocnema* sp. damaging rice in the Philippines. *C. pulicaria* Melsh. is the principal vector of Stewart's disease, or bacterial wilt (*Aplanabacter stewarti* (E.F.Sm.) McC.) of maize in the USA (Poos, 1955). *C.pulla* Chapuis (*C.zeae* Bryant) has been reported seriously attacking maize and millets in Sierra Leone (Hargreaves, 1936).

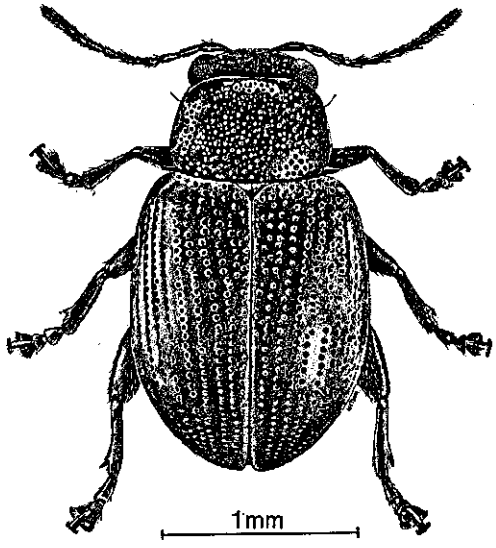
Morphologically the species are difficult to separate, while of the African species too little is known to devise a good key as yet (Bryant, 1928; Scherer, pers. commun., 1973).

Chaetocnema spp. tested for transmission of RYMV were: *Chaetocnema* sp., *C.*

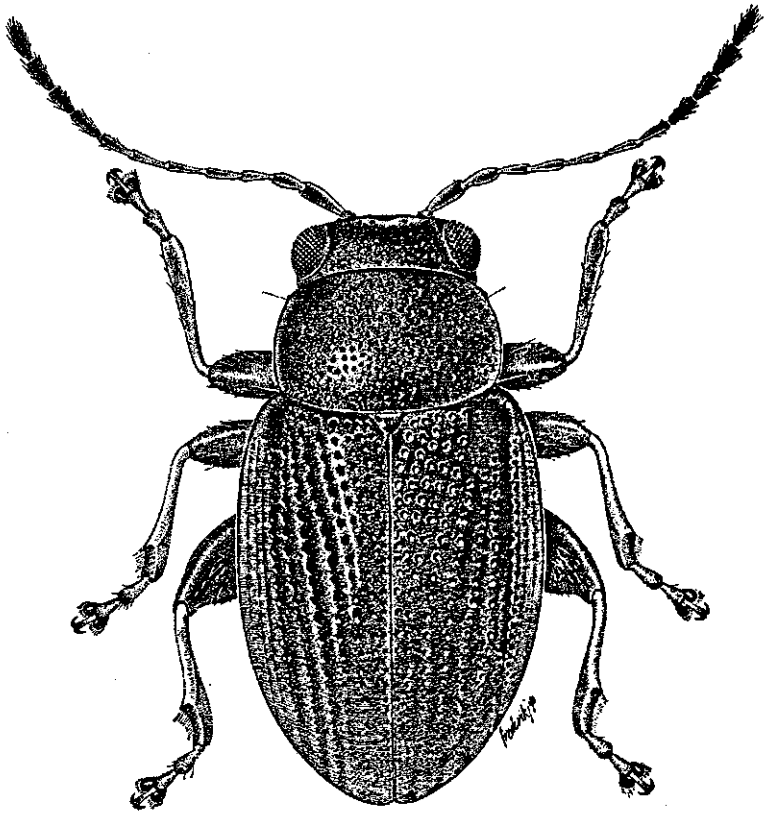
Table 10. Transmission of RYMV by Galerucinae (Chrysomelidae) from mechanically infected 'Sindano' to 'Sindano'.

Insect	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Feeding damage to test plant	Results transmission[2]	Results ser.test
Genus near *Apophylia*	1	—	3	a	2	A	moderate	1/1	not perf.
	1	12	3	a	3	D	moderate	1/1	positive
	1	2	3	a	3	D	moderate	1/1	positive
Lamprocopa sp.?	1	—	4	a	6	D	none	0/1	
Genus near *Leptaulaca*	1	—	5	a	2	D	none	0/1	
Luperodes quaternus Fairm.	4	—	3	a	3	B	little	0/4	
Monolepta flaveola Gerst.	7	—	4	a	5	C	moderate	2/3	not perf.
	3	—	3	a	3	D	moderate	3/13	positive
Monolepta haematura Fairm.	10	—	4	a	3	C	moderate	0/1	
	1	2	3	a	3	D	moderate	3/25	not perf.
Monolepta intermedia Rits.	6	—	4	a	3	C	none	0/2	
	9	—	4	a	3	C	none	0/2	
Monolepta irregularis Rits.	1	2	3	a	3	D	moderate	0/5	
Sesselia pusilla Gerst.	5	—	3	a	3	C	moderate	4/7	positive
	5	—	4	a	3	C	moderate	11/15	positive
	5	1	3	a	3	C	moderate	18/21	not perf.
	1	1	3	a	3	D	moderate	16/25	not perf.

1. a: on 5 – 6 rice plants, A: in small glass tube, B: in large glass tube, C: in lamp glass, D: in PVC tube.
2. Ratio infected to tested plants.



A



B

Fig. 13. A. *Chaetocnema abyssinica* Jac. (Chrysomelidae, Halticinae).
B. *Chaetocnema kenyensis* Bryant (?) (Chrysomelidae, Halticinae.)

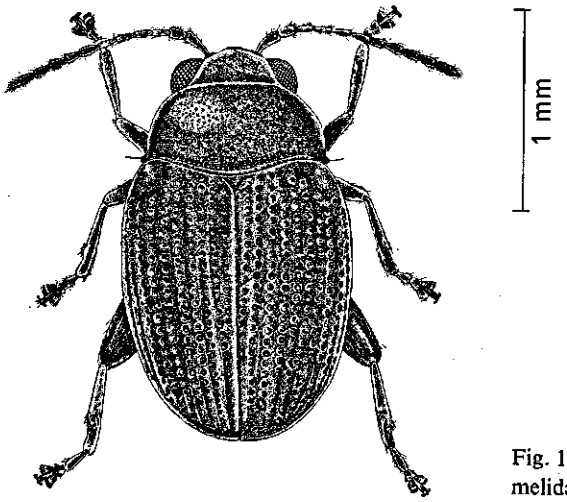


Fig. 14. *Chaetocnema pulla* Chapuis (Chrysomelidae, Halticinae).

abyssinica Jac. (Fig. 13A), *C.kenyensis* Bryant (?) (Fig. 13B), *C.pulla* Chapuis (Fig. 14) and *C.pallidipes* Fairm. (Fig. 15A). Sometimes more than one species was tested at the same time as revealed at identification afterwards. Preliminary results of the transmission experiments with *C.pulla* have already been published (Bakker, 1971). Other Halticinae tested included: *Altica* spp., *A. malvernensis* Jac., *Apthona* sp., *A.bamakoensis* Bech., *A.friguiagbensis* Bech., *Longitarsus* sp., *Podagrica nigriceps* Bryant and *P.*sp.? *puncticollis* Weise.

Little is known of the biology of the tested species, but for the *Chaetocnema* spp. it may be assumed from the places of collection, that they mainly feed on Gramineae and perhaps on Cyperaceae. Hargreaves (1937) listed *Arachis*, maize, millet, rice and other grasses, and ginger attacked by *C.pulla* (*C.zeae*). The life history of some *Chaetocnema* spp. on cereals is known (Jourdheuil, 1963) and this may provide a base for more knowledge of the species used here. Very large numbers of *C.pulla* were collected at Mwea on fresh growth mainly consisting of the grass *Echinochloa colona* (L.) Link and the sedge *Scirpus confuses* N.E.Br. (Fam. Cyperaceae) in a rice field tilled some weeks earlier. At the Irrigation Research Station Ahero these insects were also collected on grasses on the bunds and on fresh growth of ratoon rice; it was also seen on *Dinebra retroflexa*. Feeding damage on rice and *D.retroflexa* caused by *Chaetocnema* spp. was easy to note.

Methods

The insects were field collected. The *Altica* spp. mainly on vegetation in ditches or on rice, where they did not feed, at Mwea, Ahero and Papondíti; *Apthona* spp. at Otonglo and Ahero on grasses; *Chaetocnema* spp. at Mwea, Ahero and Otonglo on grasses and on plants in ratoon rice fields; *Longitarsus* sp. and *Podagrica* spp. at

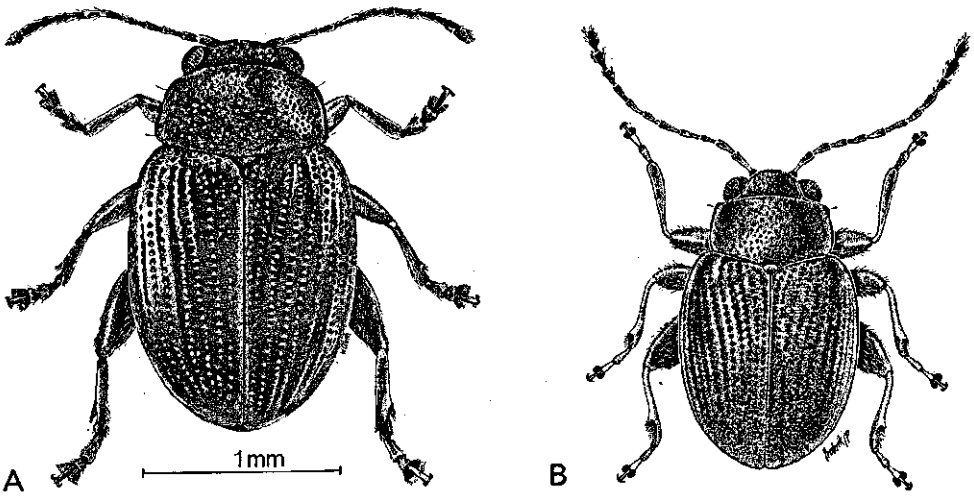


Fig. 15. A. *Chaetocnema pallidipes* Fairm. (Chrysomelidae, Halticinae).
B. *Chaetocnema pusilla* Lab. (Chrysomelidae, Halticinae).

Otonglo on a vegetation mainly existing of grasses. The general adopted testing procedures were followed.

To test if *C.pulla* was field infected with RYMV, 'Sindano' seedlings, grown in a small container with a PVC tube in position, were brought to the Irrigation Research Station Ahero. *C.pulla* caught on fresh growth of 'Sindano' ratoon rice with a high incidence of RYM, were placed on the test plants immediately. A number of seedlings, which did not receive insects, served as control. Plants with the insects were transported to the laboratory, where the seedlings were transplanted into a pot after the inoculation period of the insects.

Breeding of *C.pulla* was attempted on 'Sindano' and on *E.colona* in the laboratory.

Results

The results of the transmission experiments are given in Table 11. *Chaetocnema abyssinica, C.kenyensis* (?), *C.pulla* and mixtures of *Chaetocnema* sp. + *C.pallidipes* and *C.pallidipes* + *C.kenyensis* (?) transmitted RYMV. In general higher rates of transmission were obtained with 5 insects per test plant than with single insects. *C.pulla* proved to be naturally infected with the virus at the Irrigation Research Station Ahero (Table 12).

The adults of the tested *Chaetocnema* spp. are leaf eaters. They eat the surface of the rice leaves parallel to the veins (Fig. 19C). *Apthona bamakoensis* caused feeding damage similar to that of *Chaetocnema* spp.

Attempts to breed *C.pulla* failed.

Table 11. Transmission of RYMV by Halticinae (Chrysomelidae) from mechanically infected 'Sindano' to 'Sindano'.

Insect	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Feeding damage to test plant	Results transmission[2]	Results ser.test
Altica sp.	10	—	4	a	5	B	none	0/1	
Altica sp.	3	—	3	a	3	D	none	0/4	
Altica malvernensis Jac.	8	—	3	a	4	D	none	0/5	
Altica sp. + *A.malvernensis*	5	—	3	a	3	D	none	0/16	
Apthona sp.	2	—	3	a	2	D	none	0/1	
Apthona sp.	1	2	3	a	3	D	none	0/3	
Apthona bamakoensis Bech.	4	—	3	a	2	D	moderate	0/1	
Apthona friguiagbensis Bech.	5	—	3	a	2	D	none	0/8	
Chaetocnema abyssinica Jac.	2	—	3	b	3	D	moderate	0/1	
	1	—	3	b	3	D	moderate	2/5	positive
Chaetocnema kenyensis Bryant (?)	1	—	3	a	3	D	moderate	1/1	positive
Chaetocnema pulla Chapuis	5	—	3	a	2	A	moderate	11/25	positive
	5	—	3	a	2	B	moderate	4/6	not perf.
	5	—	3	a	3	B	moderate	31/38	not perf.
	3	—	3	a	2	B	moderate	1/1	not perf.
	1	—	3	a	2	A	moderate	1/5	positive
	1	—	4	a	3	A	moderate	8/40	positive
C.abyssinica + *C.kenyensis* (?)	3	—	3	b	3	D	moderate	3/3	positive
C.pallidipes Fairm. + *C.* sp.	5	—	3	a	3	D	moderate	17/27	positive
C.pallidipes + *C.kenyensis* (?)	2	—	3	a	3	D	moderate	1/2	positive
Longitarsus sp.	1	—	3	a	3	D	none	0/1	
	1	3	3	a	3	D	none	0/1	
Podagrica nigriceps Bryant	1	—	3	a	3	D	none	0/1	
P. sp. ? *puncticollis* Weise	2	—	3	a	4	A	none	0/1	
	1	—	3	a	2	D	little	0/1	

1. a: on 5 – 6 rice plants, b: on 1 rice plant, A: in small glass tube, B: in large glass tube, D: in PVC tube.
2. Ratio infected to tested plants.

Table 12. Transmission of RYMV by naturally infected *Chaetocnema pulla* Chapuis to 'Sindano'.

Experiment no.	Number of insects per plant	Inoculation period (days)	(method)[1]	Results transmission[2]	ser.test	control
1	1	4	D	1/32	positive	
	5	4	D	0/46		0/50
2	1	4	D	6/46	positive	0/40

1. D: in PVC tube.
2. Ratio infected to tested plants.

Hispinae

The name of this subfamily refers to the spines arising from the thorax and the body that many species possess. Most Hispinae are found in the warmer regions of the world where they feed on palms and to a lesser extent on Gramineae; a few species live on crops like sweet potato, capok, coffee and some other plants (Risbec, 1950).

Grist & Lever (1969) listed the following Hispinae as pests of rice: *Asamangulia wakkeri* (Zehnt.), *Dactylispa echinata* (Gyll.), *D.spinulosa* (Gyll.), *Dicladispa armigera* (Oliv.), *D.boutani* (Weise), *D.gestroi* (Chapuis), *D.paucispina* (Weise), *D.viridicyanea* (Kraatz), *Dorcathispa bellicosa* (Guérin), *Hispa stygia* (Chapuis), *Hispellinus moestus* (Baly), *Leptispa pygmaea* Baly, *Oediopalpa guerini* (Baly), *Onchocephala gestroi* Weise, *Polygonia spinicornis* (Kraatz), *Rhadinosa lebongensis* Maulik, *R.parvula* (Motsch.) and *Trichispa sericea* (Guérin).

The larvae of these species mine in the leaves, while the adults eat the surface of the leaves parallel to the veins. The biology of some of these species are given by Grist & Lever (1969) and Risbec (1950), while Uhmann (1957, 1958 and 1964) classified the Hispinae recently.

Species tested for transmission of RYMV were: *Dactylispa bayoni* Gest. (Fig. 16), *D.spinigera* Gyll., *Dicladispa quadrifida* Gerst., *D.(Chrysispa) paucispina* (Weise), *D. (C.) viridicyanea* (Kraatz) (Fig. 17) and *Trichispa sericea* (Guérin) (Fig. 18).

Differentiation between *D. (C.) paucispina* and *D. (C.) viridicyanea* is difficult (Uhmann, 1960).

Methods

T.sericea was collected by means of an aspirator from rice at Mwea, while the other Hispinae were caught with a sweeping net, mainly on grasses on the bunds of the rice fields and on the plains near Kisumu. For the adult insects the generally adopted procedures were followed.

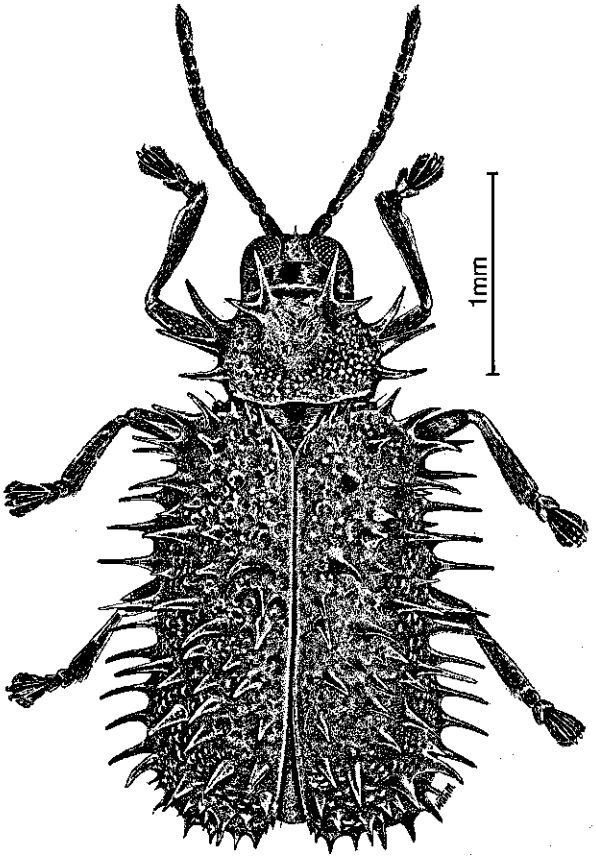


Fig. 16. *Dactylispa bayoni* Gest. (Chrysomelidae, Hispinae).

Larvae of *T.sericea* obtained from rice leaves collected at Mwea were given a pre-acquisition starvation period of two hours under humid conditions and subsequently placed on detached diseased 'Sindano' leaves. The larvae were given an acquisition period of 2 days (Method c, in the dark). A number of larvae mined into the leaves immediately, while those which did not do so, died. After the acquisition period the larvae in the leaves had passed into pupal instars and could not be used for transmission experiments.

Breeding of *T.sericea* was done by placing adult insects collected at Mwea in September, on 'Sindano'.

Results

Dactylispa bayoni, Dicladispa (*Chrysispa*) *paucispina, D.* (*C.*) *viridicyanea* and *Trichispa sericea* transmitted RYMV from and to rice 'Sindano' (Table 13). The feeding damage to rice leaves caused by the Hispinae consisted of incisions parallel to the veins (Fig. 19D).

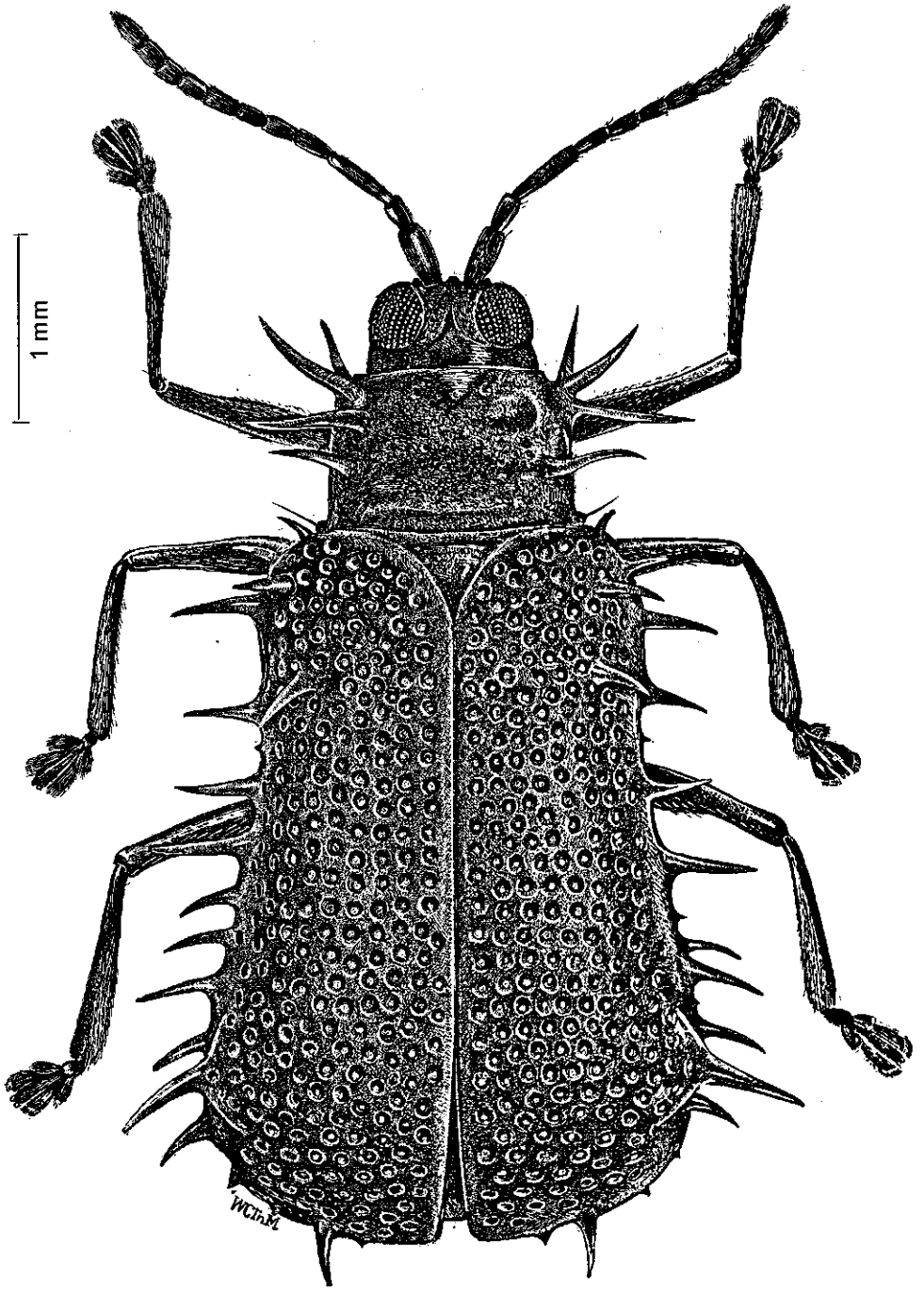


Fig. 17. *Dicladispa* (*Chrysispa*) *viridicyanea* (Kraatz) (Chrysomelidae, Hispinae).

Table 13. Transmission of RYMV by Hispinae (Chrysomelidae) from mechanically infected 'Sindano' to 'Sindano'.

Insect	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Feeding damage to test plant	Results transmission[2]	Results ser. test
Dactylispa bayoni Gest.	10	—	4	a	2	C	moderate	3/10	not perf.
	5	—	4	a	2	C	moderate	4/18	not perf.
	3	—	5	a	3	C	moderate	0/4	
	2	—	3	a	3	D	moderate	5/18	positive
Dactylispa spinigera Gyll.	1	—	3	a	3	A	moderate	0/1	
	2	—	4	a	6	C	moderate	0/3	
	1	2	3	a	3	D	moderate	0/2	
	1	—	3	a	3	D	moderate	0/1	
Dicladispa quadrifida Gerst.	2	—	3	a	2	D	moderate	0/3	
Dicladispa (*Chrysispa*) *paucispina* (Weise)	1	—	3	a	2	A	moderate	0/1	
	1	—	4	a	6	C	moderate	1/1	not perf.
Dicladispa (*Chrysispa*) *viridicyanea* (Kraatz)	2	—	4	a	3	C	moderate	2/2	positive
	1	—	4	a	3	A	moderate	1/3	not perf.
Trichispa sericea (Guérin)	5	17	2	a	3	C	moderate	12/15	positive
	1	17	2	a	3	C	moderate	4/15	positive

1. a: on 5 – 6 rice plants, A: in small glass tube, C: in lamp glass, D: in PVC tube.
2. Ratio infected to tested plants.

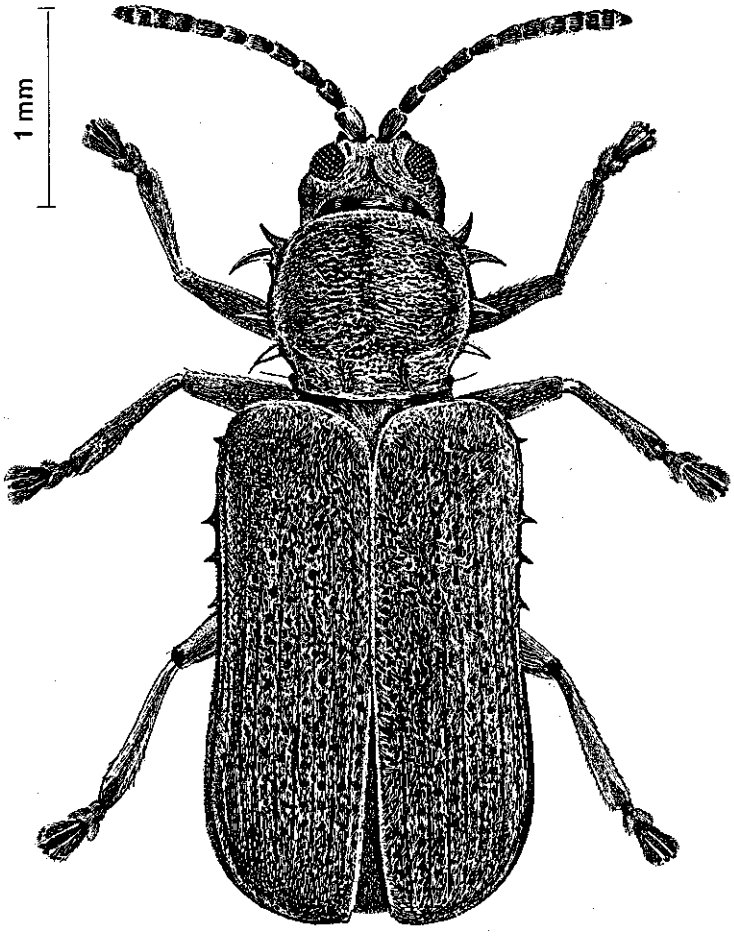


Fig. 18. *Trichispa sericea* (Guérin) (Chrysomelidae, Hispinae).

In the breeding experiments with *T.sericea*, larvae were noticed after two weeks and the first adults after about four weeks.

Other Chrysomelidae

A number of chrysomelids belonging to subfamilies other than the Criocerinae, Galerucinae, Halticinae and Hispinae were caught occasionally, mostly in small numbers only. Tests for ability in transmitting RYMV from mechanically infected 'Sindano' to 'Sindano' were performed with the generally adopted procedures. The subfamily, insect, number of tests performed and the results of the experiments were:
- Chrysomelinae; *Phaedonia areata* (F.), 2, no transmission, no feeding damage.
- Clytrinae; *Melitonoma* sp., 4, no transmission. *Melitonoma* sp., 1, no transmission. Both species caused little feeding damage.

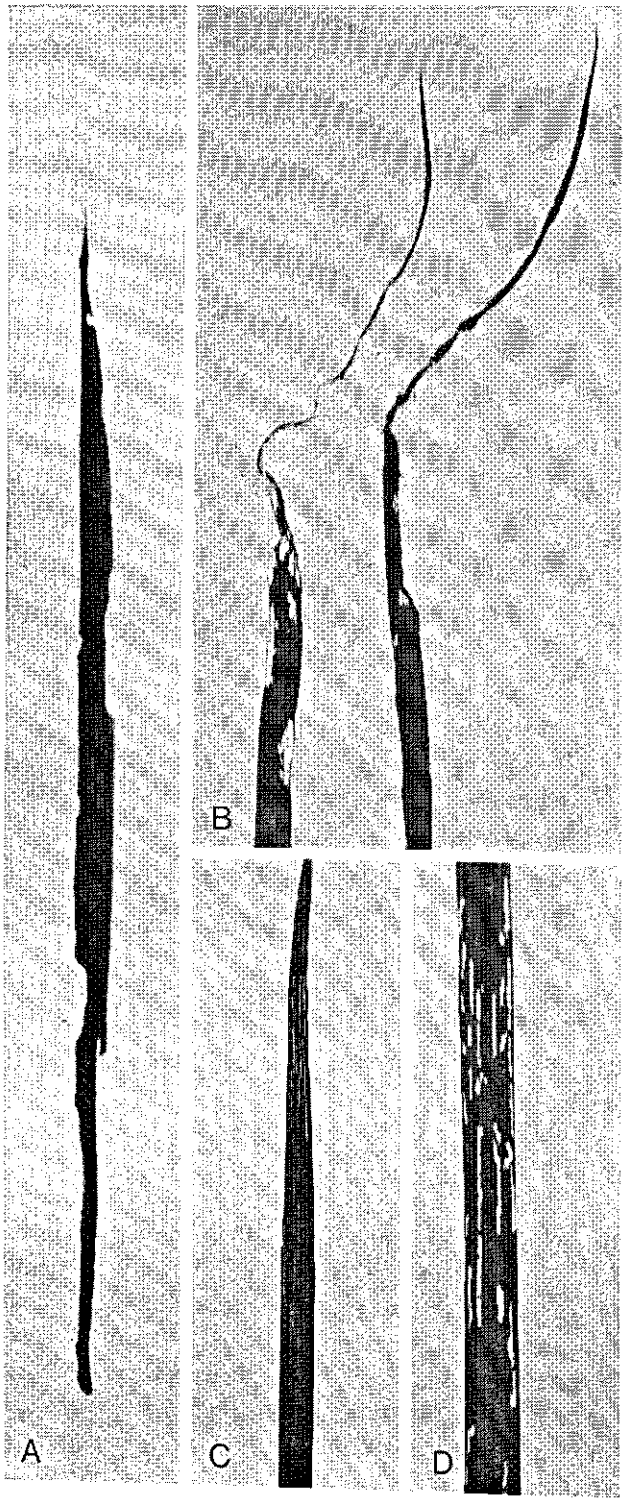


Fig. 19. Feeding damage of rice 'Sindano' leaves caused by caged chrysomelid beetles. Damage caused by:
A. insect of a genus near *Apophylia;*
B. *Sesselia pusilla;*
C. *Chaetocnema pulla;*
D. *Trichispa sericea.*

- Cryptocephalinae; *Cryptocephalus* sp., 2, no transmission. *Cryptocephalus callias* Suffr., 4, no transmission. *Cryptocephalus* sp. *?chalybeipennis* Suffr., 9, one plant infected. *Cryptocephalus* sp. ? W – *nigrum* Suffr., 2, one plant infected. The transmissions of RYMV were serologically confirmed. The *Cryptocephalus* spp. took small bites from the sides and the middle of the rice leaves.

5.3.2.1.2 Relations between virus, vector and host plant

The chrysomelids, *Sesselia pusilla*, *Chaetocnema pulla* and *Trichispa sericea* as representatives of the Galerucinae, Halticinae and Hispinae respectively, were used to study some relations between these insects, RYMV and some host plants of this virus.

The relationships determined with these three insects were: (1) the minimum acquisition period, (2) the minimum inoculation period, (3) the ability to transmit RYMV when given a combination of short acquisition and short inoculation periods, (4) the period of retention of RYMV, (5) the rate of transmission of the virus to 'Sindano', 'Basmati 217' and 'IR 22', and (6) the ability to transmit the virus to a number of hosts other than rice.

Methods

The generally adopted procedures for insect transmission experiments were followed. *S.pusilla* was collected at Otonglo on the flowers of *Cyperus* spp. at times when no rice was present. For the more crucial tests a number of these insects were tested for the presence of RYMV by placing them on rice 'Sindano' for 2 – 3 days on arrival at the laboratory. *C.pulla* and *T.sericea* were collected at Mwea where RYM did not occur.

In the experiments performed to determine the minimum acquisition and inoculation periods, a period of 15 min was the shortest time in which the insects were allowed on a plant. 'Sindano' was used as test plant both for these tests and the determination of the retention period, in which the insects were transferred daily. After the short inoculation period the insects were placed on a new test plant for 2 – 3 days. Unless otherwise stated, mechanically infected 'Sindano' was used as acquisition source of RYMV. In the transmission experiments to non-rice hosts, 'Sindano' was back inoculated after 4 – 5 weeks.

Results

In some experiments with *C.pulla*, some specimens of another insect of the same genus, *C.pusilla* Lab. (Fig. 15B), proved to be present when the insects were identified afterwards. This insect might well prove to be an additional vector of RYMV.

Minimum acquisition period When all three insect species were starved before the acquisition period, they were able to acquire RYMV in 15 min (Table 14). With *S.pusilla* given a pre-acquisition starvation period of 24 hours, about the same percentages of transmission of the virus were obtained as with prolonged acquisition periods (Table 10).

For *C.pulla* and *T.sericea* prolonged acquisition periods resulted in an increase of infected plants, while this was less clearly demonstrated for *S.pusilla.*

Minimum inoculation period The results of the experiments are given in Table 15. In this experiment *S.pusilla* transmitted the virus after a 30 min inoculation period while the same insects infected more plants when placed for longer periods on the test plants.

A period of 15 min on the test plant by individual viruliferous *C.pulla* resulted in infection of one test plant with RYMV. There was hardly an increase in the number of infected plants when the inoculation period was extended to 4 hours. When *C.pulla* was transferred after the short inoculation period to a new test plant for 3 days, the number of infected plants strongly increased.

T.sericea proved able to infect a rice seedling in the 15 min period also, but prolonging this period up to 4 hours resulted in an increase in the number of infected plants.

Combinations of short acquisition and inoculation periods *S.pusilla* given an acquisition period of 15 min followed by 10 successive inoculation periods of 15 min, each on a new test plant, infected only 3 out of 196 plants (Table 16). In experiments with periods of 30 min and 1 hour, the percentage of transmission increased.

Of 20 *T.sericea*, given an acquisition and inoculation period of 15 min, none caused the infection of a rice seedling (Table 17). When the insects were transferred to a new test plant afterwards for 3 days, 5 plants showed symptoms of the disease. In an experiment with 10 insects which were given an acquisition feeding period of 15 min, followed by 10 successive inoculation periods of 15 min, each on a new test plant, only 1 plant out of 88 plants became diseased. Placing the beetles after the last short inoculation period on a new test plant for 3 days resulted in infection of 1 out of 7 plants. This infection was caused by another insect than during the short inoculation period.

Retention of RYMV The results of the experiments on the retention of RYMV by the three beetles, tested by daily transfer to a new test plant for 10 days, are given in Table 18.

The maximum retention period of RYMV by *S.pusilla* was 8 days, often causing infection of seedlings on 3 or more consecutive days. For *C.pulla*, this period was 5 days, but the beetles did not transmit the virus for more than 3 consecutive days. Daily transfer of *T.sericea* to a new test plant resulted only in infection of plants tested on the first day.

Table 14. Transmission of RYMV by individual *Sesselia pusilla* Gerst., *Chaetocnema pulla* Chapuis, and *Trichispa sericea* (Guérin) given varied acquisition periods, to 'Sindano'.

Insect	Exp. no.	Pre-acquisition starvation (hours)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Transmission results[2]: acquisition period ¼ h	½ h	1 h	2 h	4 h	Control number of insects per plant	Control transmission results[2]
S.pusilla	1	3	c – d	1	A		10/15	9/14	8/15		10	0/6
	2	3	c – d	2	A		1/15				1	0/20
	3	24	c – d	3	D	19/25	16/25	8/25	20/25	23/24	1	0/50
C.pulla	1[3]	16	c – d	2	D	1/50	2/50	5/49	6/50	1/49		
	2	19	c – d	3	D	2/30	7/30	3/30	8/30	8/30	some *Chaetocnema pusilla* Lab. present	
T.sericea	1	12	c – d	2	D	7/50	11/50	11/50	10/50	14/49		

1. c – d: on detached leaves in the dark, A: in small glass tube, D: in PVC tube.
2. Ratio infected to tested plants.
3. Performed in cool season.

Table 15. Transmission of RYMV by individual *Sesselia pusilla* Gerst., *Chaetocnema pulla* Chapuis and *Trichispa sericea* (Guérin) given varied inoculation periods, to 'Sindano'.

Insect	Exp. no.	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (method)[1]	Days on new plant after transfer (tr)	Transmission results at tested period and after transfer to a new test plant[2]										Control	
							¼ h	tr	½ h	tr	1 h	tr	2 h	tr	4 h	tr	number of insects per plant	trans-mission results[2]
S.pusilla	1	—	3	a	C (–d)	3			1/9	5/9	0/10	3/10	1/10	5/10				
	2	—	3	a	A (–d)	3			1/6	4/6	0/6	3/6					8	0/5
	3	—	3	a	A (–d)	2	0/20	11/20	1/20	12/20							10	0/6
	4	2	3	a	D	3	0/25	19/25	0/25	21/25	0/25	16/25	5/25	15/25	2/25	17/25	1	0/50
C.pulla	1	5	3	a	D	2	0/22	3/24	0/25	3/24	0/24	1/24	2/21	0/19	1/24	2/23		
	2	5	3	a	D	3	1/25	10/25	2/25	14/25	1/25	13/24	3/25	14/25	2/25	14/25		
T.sericea	1	24	3	a	D	3	2/24	13/25	4/25	14/25	3/25	15/25	7/25	13/25	8/25	10/23		

1. a: on 5 – 6 rice plants, A: in small glass tube, C: in lamp glass, D: in PVC tube, (–d): ¼ h – 4 h inoculation period in the dark.
2. Ratio infected to tested plants.

Table 16. Transmission of RYMV to 'Sindano' by individual *Sesselia pusilla* Gerst., given combinations of varied acquisition and inoculation periods.

Exp. no.	Number of insects tested	Pre-acquisition starvation (hours)	Acquisition period (method)[2]	Inoculation period (method)[2]	Days on new plant after last transfer (tr)	Transmission results[3] at acquisition – inoculation combinations of									
						15 min			30 min			1 h			control[3]
						10 × 15 min	tr	i[1]	10 × 30 min	tr	i[1]	10 × 1 h	tr	i[1]	(1 insect/pl.)
1	20	18	c – d	A	2	3/196	3/17	5	4/193	12/20	14				0/20
2	20	12	c – d	A	2							6/188	3/18	6	0/20

1. Number of transmitting insects.
2. c – d: on detached leaves in the dark, A: in small glass tube.
3. Ratio infected to tested plants.

Table 17. Transmission of RYMV to 'Sindano' by individual *Trichispa sericea* (Guérin) with an acquisition and inoculation period of 15 min.

Exp. no.	Number of insects tested	Pre-acquisition starvation (hours)	Acquisition period (method)[1]	Inoculation period (method)[1]	Days on new plant after last transfer	Transmission results[2] at 15 min inoculation periods and after transfer of insects			
						1 × 15 min	transfer	10 × 15 min	transfer
1	20	24	c – d	A	3	0/20	5/20		
2	10	24	c – d	D	3			1/88	1/7

1. c – d: on detached leaves in the dark, A: in small glass tube, D: in PVC tube.
2. Ratio infected to tested plants.

Table 18. Retention of RYMV by individual *Sesselia pusilla* Gerst., *Chaetocnema pulla* Chapuis and *Trichispa sericea* (Guérin) tested on 'Sindano' at daily transfer.

Exp. no.	Insect[1] no.	Transfer (day)[3] 1	2	3	4	5	6	7	8	9	10
S.pusilla[2]											
1	6	+	+	−	−	−	−	−	−	−	−
	8	+	+	+	+	+	−	−			
	9	+	+	−	−	−	−	−	−	−	−
	total[4]	5/10	3/10	1/10	1/10	1/10	0/10	0/10	0/8	0/7	0/6
2	2	−	+	−	+						
	4	+	+	+	−	−	−	−	−		
	5	+	−	+	−						
	7	−	+	+	−	−	−	−	−	−	−
	8	−	+	+	−						
	9	+	+	−	+	−	−	−	−	−	−
	11	+	−	+	+	+	−	−			
	13	−	+	+	+	−	−				
	14	O	+	−	+	−	−	−	+	−	−
	15	−	+	+	−	−	−	−	−	−	
	17	+	+	+	+	+	−				
	18	+	+	+	+	−	−	−	−	−	−
	total[4]	8/19	10/18	9/15	6/15	2/11	0/10	0/9	1/7	0/6	0/5
3	2	+	+	+	+						
	4	−	+	−	+	+					
	6	+	+	−	−	−	−	−	−	−	−
	7	−	+	−	+	+	−	−	−		
	8	+	+	−							
	10	−	+	+	+	−	+	+			
	11	+	+	+	−	+	−	−	+	−	−
	12	+	+	+	−	−	−	−			
	13	+	+	+	−	−	−	−	−	−	−
	14	+	+	−	−	−	+	+	−	−	−
	15	+	+	−							
	16	+	−	−	+						
	18	+	+	+	+	−	+	−	+	−	−
	20	+	+	+	−	−	−				
	22	+	+	−							
	23	+	−	+	−	−	−	−	−	−	−
	total[4]	19/25	14/25	9/23	5/17	3/16	3/13	2/12	2/9	0/8	0/7
C. pulla											
1	5	+	−								
	7	+	+	−	+	−	−	−	−	−	−
	11	+	−	−	−	−	−	−	−	−	−
	13	−	−	−	−	+	−	−			
	16	+	−	−	−	−	−	−	−	−	
	21	+	−	−	−						
	22	+	−	+	−	−	−	−	−	−	−
	30	+	+	−	−	−	−	−	−	−	−
	33	−	−	−	+	−	−	−	−	−	−
	49	−	+	+	+	−	−	−	−	−	−
	total	7/50	3/48	2/46	3/46	1/45	0/44	0/44	0/43	0/42	0/41
T.sericea											
1	total[4]	2/15	0/15	0/15	0/15	0/15	0/15	0/14	0/15	0/14	0/14
2	total[4]	9/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21	0/21

Footnotes Table 18.

1. Treatment of the insects

Insect	Exp no.	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[5]	Inoculation period (method)[5]
S.pusilla	1		4	a	C
	2		3	a	A
	3	1	3	a	D
C.pulla	1	12	3	a	D
T.sericea	1	24	2	a	A
	2	24	4	a	D

2. *S.pussilla* which transmitted RYMV on the first day only are omitted, but are included in total figures.
3. +: transmission of RYMV, −: no transmission of RYMV, O: plant died.
4. Ratio infected to tested plants.
5. a: on 5 – 6 rice plants, A: in small glass tube, C: in lamp glass, D: in PVC tube.

Transmission of RYMV to 'Sindano', 'Basmati 217' and 'IR 22' The results of the experiments in which the rice varieties were tested under the same conditions for each insect species, are given in Table 19.

For the three insect species the lowest number of infected seedlings was recorded in the variety 'Basmati 217'. The number of infected seedlings of 'IR 22' and 'Sindano' was about twice as high as for 'Basmati 217'. The highest number of seedlings affected was in the variety 'Sindano' with *S.pusilla* and *C.pulla* as vectors, while in a single experiment with *T.sericea*, this insect infected about the same percentage of 'IR 22' and 'Sindano' seedlings.

Ability to transmit RYMV to non-rice host plants of the virus Virus transmission experiments from mechanically infected 'Sindano' to a number of non-rice hosts were performed with *S.pusilla* and *C.pulla* only. The results are given in Table 20. The experimental hosts *Bromus hordeaceus*, *Eragrostis chapelieri* and *Setaria viridis* in which infection remained localized when inoculated with sap and *Phleum arenarium* which was systemically infected by this method, were not infected by *S.pusilla*. Apart from *P.arenarium* the tested grasses showed considerable feeding damage.

Transmission of RYMV by *C.pulla* to *E.chapelieri* and *E.cilianensis*, also a local host for the virus, was not demonstrated. The systemic host *Dinebra retroflexa* ,however, became infected after inoculation feeding of this insect, while RYMV was transmitted

Table 19. Transmission of RYMV by individual *Sesselia pusilla* Gerst., *Chaetocnema pulla* Chapuis and *Trichispa sericea* (Guérin) to 'Sindano', 'Basmati 217' and 'IR 22'.

Insect	Exp. no.	Pre-acquisition starvation (hours)	Acquisition period (days)	(method)[1]	Inoculation period (days)	(method)[1]	Transmission results[2] 'Sindano'	'Basmati 217'	'IR 22'
S.pusilla	1	2	3	a	3	D	19/50	13/50	19/50
	2	2	3	a	3	D	26/50	6/50	20/50
percentage infected plants							45%	19%	39%
C.pulla	1[3]	12	3	a	3	D	1/50	2/50	2/49
	2	12	3	a	3	D	26/48	13/50	19/49
percentage infected plants							28%	15%	21%
T.sericea	1	24	3	a	3	D	8/50	4/50	9/49
percentage infected plants							16%	8%	18%

1. a: on 5 – 6 rice plants, D: in PVC tube.
2. Ratio infected to tested plants.
3. Performed in cool season.

from *D.retroflexa* to 'Sindano' by the same beetle species together with some *C.pusilla* (Table 21). The feeding damage caused by *C.pulla* on *D.retroflexa* was similar to that caused on rice.

5.3.2.2 OTHER COLEOPTERA

A number of beetles not belonging to the family Crysomelidae were tested for ability to transmit RYMV too. Apart from *Apalochrus* spp. only low numbers were tested with the generally adopted methods. Family, insect species and number of tests performed were:

- Buprestidae; *Spenoptera* (*Haplistura*) sp., 1.
- Coccinellidae; *Brumoides nigrifrons* Gerst., 1. *Isora anceps* Muls., 1.
- Melyridae; *Apalochrus* sp., 11. *Apalochrus elgonensis* Champ., 2.
- Phalacridae; *Stilbus dollmani* Champ., 1. *Phalacrus* sp., 1.
- Scarabaeidae; *Leucocelis plebeja* Klb., 1.

Only *Sphenoptera* (*Haplistura*) sp. caused severe feeding damage comparable to that caused by grasshoppers, while with the other insects no feeding damage was observed. None of the insects transmitted the virus.

5.3.3 Orthoptera

Grasshoppers were common in the rice fields and on the bunds. With juvenile species belonging to the family Acrididae and adult *Conocephalus conocephalus* L.

Table 20. Transmission of RYMV by *Sesselia pusilla* Gerst. and *Chaetocnema pulla* Chapuis from mechanically infected 'Sindano' to non-rice hosts of the virus.

Insect	Test plant	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Results transmission[3]	Results ser.test
S.pusilla	*Bromus hordeaceus* L.	3	—	3	a	2	A	0/5[2]	
	Eragrostis chapelieri (Kunth) Nees	5	—	3	a	3	C	0/15[2]	
	Phleum arenarium L.	1	—	4	a	3	A	0/5	
	Phleum arenarium L.	3	—	3	a	3	A	0/6	
	Setaria viridis (L.) P.Beauv.	3	—	3	a	1	A	0/5[2]	
C.pulla	*Dinebra retroflexa* (Vahl) Panz.	5	12	4	a	4	D	6/15	positive
	Dinebra retroflexa (Vahl) Panz.	10	12	4	a	4	D	6/7	positive
	Eragrostis chapelieri (Kunth) Nees	10	—	4	a	4	A	0/5[2]	
	Eragrostis chapelieri (Kunth) Nees	5	—	4	a	4	A	0/10[2]	
	Eragrostis cilianensis (All.) Lut.	2	12	4	a	2	D	0/50[2]	

1. a: on 5 – 6 rice plants, A: in small glass tube, C: in lamp glass, D: in PVC tube.
2. After back inoculation to 'Sindano'.
3. Ratio infected to tested plants.

Table 21. Transmission of RYMV by *Chaetocnema pulla* Chapuis from mechanically infected *Dinebra retroflexa* (Vahl) Panz. to 'Sindano'.

Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Results transmission[2]	
1	12	3	a	2	D	1/20	some *C.pusilla*
5	12	3	a	2	D	2/10	Lab. present

1. a: on 5 – 6 rice plants, D: in PVC tube.
2. Ratio infected to tested plants.

and *C.merumontanus* Sjöstedt (Tettigoniidae) transmission experiments with RYMV were performed.

Methods

Apart from *C.merumontanus* which was collected at Nairobi, the insects were caught near Kisumu in or near the rice fields. Initially the same procedures were followed as with the coleopterous insects, but because of severe feeding damage of the test plants, the procedures had to be modified. With *C.merumontanus* the acquisition feeding period was one day, while the inoculation feeding period was restricted to a few bites from the test plant only, after which the insects were removed. After 3 hours the insects which had not eaten from the plant were also removed.

Results

Feeding damage was severe and consisted mainly of large bites from the leaves, while much plant material was spoiled. The results of the transmission experiments are given in Table 22. Transmission of RYMV was only obtained with *C.merumontanus* on those plants where feeding damage was relatively small. Two out of the 25 insects tested transmitted the virus in this experiment.

5.4 Conclusions

The question whether nematodes and mites are able to transmit RYMV has not been answered conclusively. Although purified virus suspensions revealed particles resembling polyhedral viruses transmitted by nematodes the occurrence and spread of RYMV in the field show that nematodes are not responsible for this. Experimentation with mites was difficult. The liquid under the leaf sheath in which the mites were

Table 22. Transmission of RYMV by Orthoptera from mechanically infected 'Sindano' to 'Sindano'.

Insect	Stage	Number of insects per plant	Pre-acquisition starvation (hours)	Acquisition period (days)	Acquisition period (method)[1]	Inoculation period (days)	Inoculation period (method)[1]	Results transmission[2]	Results ser.test
Acrididae spp.	juvenile	10	—	3	a	1	C	0/1	
Acrididae spp.	juvenile	9	—	3	a	1	C	0/1	
Conocephalus conocephalus L.	adult	3	—	3	a	1	C	0/4	
Conocephalus conocephalus L.	adult	2	—	5	a	4	C	0/10	
Conocephalus merumontanus Sjöstedt	adult	1	24	1	a	1-3 hours	C	2/25	positive

1. a: on 5 – 6 rice plants, C: in lamp glass.
2. Ratio infected to tested plants.

present, contains the virus. The few 'Sindano' seedlings infected by placing singly transferred *S.spinki* under the leaf sheath, and so transferring some of the liquid also, are therefore believed to have been contaminated by this liquid. This can be compared with the effect of placing pieces of leaf sheath of diseased plants under the leaf sheath of a healthy plant. It is unlikely that the amount of this liquid transported by the mites from one plant to another is sufficient to cause an infection in the field. The spread of the two mite species was not studied but it is of interest that *S.spinki* was described from specimens collected on the plant hopper *Sogata orizicola* Muir (Smiley, 1967).

No indications were obtained, nor do the properties of the virus suggest, that hemipterous insects transmit RYMV.

Because several beetles, some of which were present abundantly in the field, were able to transmit the virus, this group of insects is thought to be of prime importance in the spread of the virus in the field. The beetles which transmitted RYMV all belong to the family Chrysomelidae. Transmission of the virus was obtained with the following chrysomelids, classified according to subfamily:

- Criocerinae: *Oulema dunbrodiensis* Jac. f. *nigripennis* Hze.
- Cryptocephalinae: *Cryptocephalus* sp. ? *chalybeipennis* Suffr. and *Cryptocephalus* sp. ? W – *nigrum* Suffr.
- Galerucinae: insects of a genus near *Apophylia*, *Monolepta flaveola* Gerst., *M. haematura* Fairm. and *Sesselia pusilla* Gerst.
- Halticinae: *Chaetocnema abyssinica* Jac., *C.kenyensis* Bryant (?), *C.pulla* Chapuis, and a mixture of *Chaetocnema* sp. with *C.pallidipes* Fairm., while *C.pusilla* Lab. might prove to be another vector
- Hispinae: *Dactylispa bayoni* Gest., *Dicladispa* (*Chrysispa*) *paucispina* (Weise), *D.* (*C.*) *viridicyanea* (Kraatz) and *Trichispa sericea* (Guérin).

Of these beetles *Chaetocnema* spp. and *S.pusilla* were collected in high numbers in the disease stricken area. It was also proved that *C.pulla* carried the virus in the field and therefore this insect as well as the other *Chaetocnema* spp. are thought to be the main vectors of the virus in the rice fields of the Ahero Pilot Scheme and probably in other rice growing areas as well.

A low percentage of transmission was also obtained with the long-horned grasshopper *Conocephalus merumontanus* Sjöstedt after being allowed a few bites on the test plants only. The importance of long-horned grasshoppers in transmission of the virus in the field has not been ascertained, but they are thought to be of only secondary importance because of their feeding behaviour and the type of feeding damage caused.

The relations between virus, vector and host plant were determined with field collected insects. However for this type of experiment insects of known age and kept in constant laboratory conditions are to be preferred.

As far as could be ascertained, the most efficient vectors of RYMV from and to 'Sindano' rice are insects of the genus near *Apophylia* and *S.pusilla*, while the *Chaetocnema* spp., *T.sericea* and the other Hispinae generally caused lower percentages of infection.

S.pusilla, *C.pulla* and *T.sericea* were able to acquire the virus when allowed for 15 min on diseased rice 'Sindano'. Although these insects were also able to infect a rice seedling in 15 min, in general the beetles seemed to acquire the virus faster than they were able to infect a plant. The willingness of the insects to feed from the plants was of prime importance in these experiments. Therefore it seems justified to conclude, that these beetles are able to acquire the virus and to infect a rice plant in short periods.

S.pusilla and *C.pulla* were able to retain the virus for several days, often causing infection of the test plants on several consecutive days, while *T.sericea* retained the virus for one day only in these experiments.

These findings substantiate the conclusion that beetles which occur abundantly in the field like *C.pulla* and other *Chaetocnema* spp. and *S.pusilla* cause a fast spread of the virus in a rice field with a variety highly susceptible to RYMV.

6 Physical, chemical and serological characterization

Determination of the properties of RYMV in crude sap provided useful information for the selection of the procedures needed for further characterization of the virus. Intrinsic properties of RYMV determined with purified virus or its extracted nucleic acid were: ultraviolet absorption spectra of the virus and its nucleic acid; behaviour of the virus in the ultracentrifuge; type of carbohydrate, base composition and molecular weight of the nucleic acid; percentage of nucleic acid in virus particles, and morphology of the virus. Electron microscopy of infected rice leaves was, however, primarily aimed at obtaining information about the virus in situ.

In the serological tests mainly the ability of RYMV to react with antisera prepared against other similar shaped viruses was studied.

6.1 Persistence of infectivity

Whole 'Sindano' plants, ratooned 4 weeks after mechanical inoculation and harvested two weeks later, were dried at room temperature (about 20°C). The young leaves, which originally showed clear symptoms remained greenish. Inoculum prepared from these leaves still proved infective 155 days after the harvest.

Inoculum prepared from young rice leaves with clear symptoms, which were cut in small pieces and stored above $CaCl_2$ at 4°C, according to the methods described by Bos (1969), was still infective when tested a year later.

The following studies were performed according to the methods described by Bos et al. (1960) when expressed sap was used.

Dilution end-point Sap from young 'Sindano' leaves with clear symptoms, 2 – 3 weeks after inoculation was still infective at a dilution of 10^{-10} when tested on 'Sindano', whereas with sap from plants inoculated 4 – 5 weeks earlier a dilution of 10^{-6} was the highest infective dilution on this variety (Bakker, 1970).

At Wageningen, series of diluted inocula were compared by inoculating 5 plants of 'Sindano', 'Basmati 217' and 'IR 22', and the grass *Dinebra retroflexa* in a single experiment. The inocula were prepared from

- young 'Sindano' leaves, harvested 14 days after inoculation,
- young 'Sindano' leaves, harvested 7 days after inoculation and
- from purified virus suspensions with a concentration of 1 mg virus/ml.

The results of these experiments are given in Table 23.

Table 23. Highest dilution of sap still resulting in infection of 'Sindano', 'Basmati 217', and 'IR 22', and *Dinebra retroflexa* (Vahl) Panz.

Inoculum prepared from	'Sindano'	'Basmati 217'	'IR 22'	*D. retroflexa*
Young 'Sindano' leaves, 14 days after inoculation	10^{-9}	10^{-6}	10^{-6}	10^{-6}
Young 'Sindano' leaves, 7 days after inoculation	10^{-6}	10^{-4}	10^{-5}	10^{-3}
Purified virus at a concentration of 1 mg/ml	10^{-5}	10^{-3}	10^{-5}	10^{-3}

Thermal inactivation point To obtain a sufficient amount of sap, 1 ml 0.01 M phosphate buffer pH 7.0 was used per gram of leaves during squeezing of the leaves. Most infectivity was lost by heating sap at 65°C for 10 min. Occasionally sap tested up to 80°C still caused infection in a low number of inoculated seedlings.

Ageing in vitro Diluted sap as prepared for the standard inoculum was divided in two parts. The part stored at room temperature (about 20°C) remained infective for 99 days but not for 120 days; the sap stored at 4°C still was infective after 260 days.

Freeze-dried virus, used for the determination of virus concentrations (Section 3.5) and stored at room temperature was suspended in 1 ml bidistilled water and inoculated onto 'Sindano'. Virus, freeze-dried 1 month earlier, still proved to be infective.

Influence of organic solvents Sap diluted with 0.01 M phosphate buffer pH 7.0 and shaken with equal amounts of chloroform, chloroform and butanol, and carbon tetrachloride, resulted in complete infection of the 'Sindano' plants (Bakker, 1970).

6.2 Physical properties of RYMV

The final purified virus suspensions (S_5, Table 4) were strongly opalescent and proved to be highly infectious when inoculated onto 'Sindano'. No impurities were seen when studied in the electron microscope.

6.2.1. UV absorption spectrum

The ultraviolet absorption spectrum of purified RYMV gave a spectrum typical for a nucleoprotein (Fig. 20). Maximum UV absorption was at 260 nm and minimum at 242 nm, with $E_{max}/E_{min} = 1.34 \pm 0.01$ and $E_{280}/E_{260} = 0.65 \pm 0.005$.

By plotting the absorbancy between 310 - 1000 nm on log/log graph paper (Noordam, 1973) no satisfactory line was obtained and therefore no correction for light scattering could be made. The light brown impurities noticed in the high-speed pellet (P_4) and the high virus concentration in the measured samples are thought to be responsible for this irregularity.

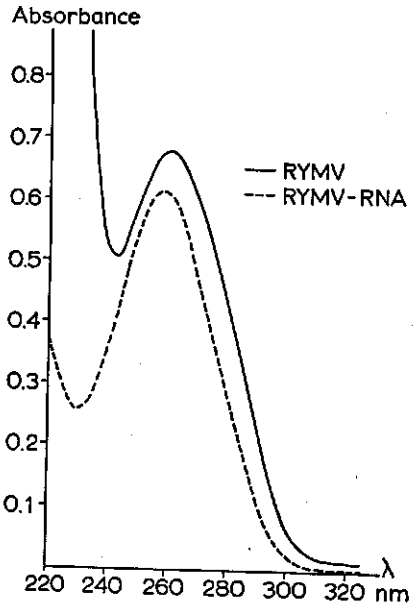


Fig. 20. Ultraviolet absorption spectra of RYMV and its RNA in 0.01 M phosphate buffer pH 7.0, uncorrected for light scattering.

6.2.2 *Determination of virus concentrations*

For the determination of virus concentrations, as described in Section 3.5, the amount of buffer constituents was deducted from the dried virus portions. E^{1cm}_{260nm} and $E^{1cm}_{260-290\ nm}$ were plotted against the virus concentrations as given in Fig. 21. From the slope of the line the following specific extinction coefficients, uncorrected for light scattering, were obtained: $E^{0.1\%}_{1\ cm,260\ nm} = 6.5$ and $E^{0.1\%}_{1\ cm,260-290\ nm} = 4.2$.

The followed purification method yielded about 1 mg virus/g leaves.

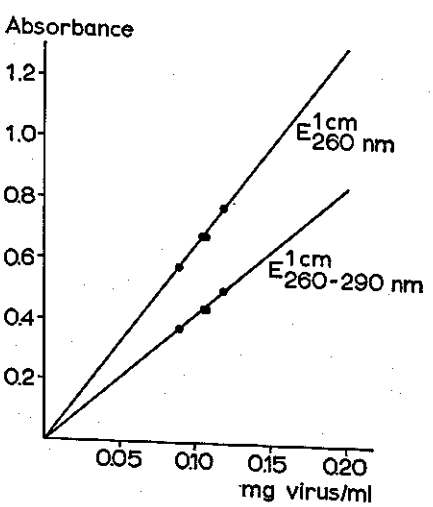


Fig. 21. Relationships between E^{1cm}_{260nm} and $E^{1cm}_{260-290\ nm}$, and the concentration of a RYMV suspension.

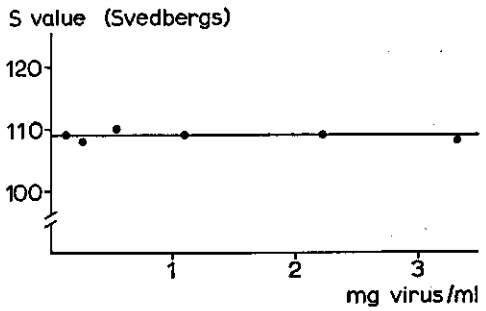


Fig. 22. Concentration-dependence of the sedimentation coefficient of RYMV.

6.2.3 Behaviour of RYMV in the ultracentrifuge

Sedimentation pattern When virus solutions containing 1 – 2 mg virus/ml in 0.01 M phosphate buffer pH 7.0 were run in the analytical ultracentrifuge with schlieren optics at 31410 rpm, the schlieren pattern revealed only one component.

Sedimentation coefficient The sedimentation coefficient was measured as described under Section 3.6. After plotting the S values of the different virus concentrations s^0_{20} was determined by extrapolation to zero concentration (Fig. 22). At the virus concentrations used, the sedimentation coefficient proved independent of the concentration. For RYMV $s^0_{20} = 109$ (Svedbergs).

Analysis of the effect of freezing of RYMV suspensions To study the effect of freezing, virus suspended in 0.01 M phosphate buffer pH 7.0 was divided in two equal amounts and centrifuged for 100 min at 78500 *g* (Spinco L50, rotor R30). One pellet was suspended in bidistilled water, the other again in 0.01 M phosphate buffer pH 7.0. The virus concentration of each suspension obtained was about 3 mg/ml. Of each suspension half was frozen in small conical glass centrifuge tubes at −25°C and kept there for 40 hours, while the other half was kept at 4°C. Thawing was done at room temperature. Virus in water was adjusted to 0.01 M with 0.1 M phosphate buffer pH 7.0. The suspensions were subsequently diluted with 0.01 M phosphate buffer pH 7.0 to concentrations of about 1.5 mg virus/ml.

After thawing both frozen solutions showed more opalescence and tended to foam upon agitation, than those stored at 4°C. When run in an analytical ultracentrifuge, the solutions kept at 4°C showed only a single component, the structurally unaffected RYMV. The solutions frozen in bidistilled water and in 0.01 M phosphate buffer pH 7.0 both showed two components however. One component was presumed to be unaffected RYMV; the other faster sedimenting component (147S) was possibly due to aggregation of virus particles (Fig. 23A). For the results of the electron microscopy of virus suspensions frozen in water and unfrozen see Section 6.5.1.

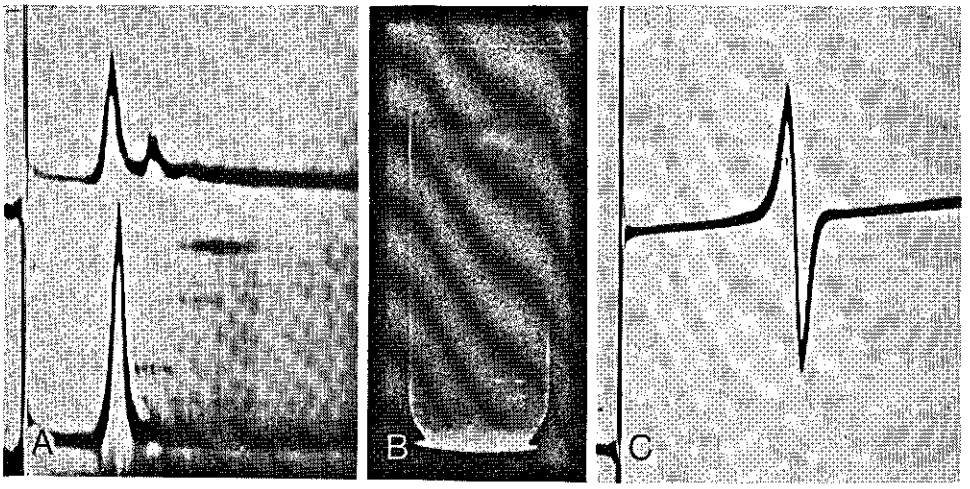


Fig. 23. Centrifugal analysis of purified RYMV.
A. Analytical ultracentrifuge pattern of in bidistilled water frozen and unfrozen solutions of RYMV which were adjusted to 0.01 M with 0.1 M phosphate buffer pH 7.0 before centrifuging. Concentrations: 1.5 mg virus/ml. Temperature of run 20.5°C. Picture taken at 70° schlieren angle about 5 min after the centrifuge reached a speed of 31410 rpm. Upper pattern, RYMV kept at —25°C for 40 h; lower pattern, RYMV kept at 4°C for 40 h.
B. Results of centrifuging of 0.02 ml RYMV suspension in 0.01 M phosphate buffer pH 7.0 (16.3 mg virus/ml) on top of 5 ml CsCl (ρ = about 1.40 g/cm^3) in a Spinco L50 centrifuge, SW 39 rotor for 26 h at 34000 rpm at 5°C.
C. Schlieren diagram of 0.01 ml RYMV suspension in 0.01 M phosphate buffer pH 7.0 (16.3 mg virus/ml) in 1.6 ml CsCl (ρ = about 1.40 g/cm^3) centrifuged to equilibrium in the analytical ultracentrifuge for 22 h at 44770 rpm at 20°C. Schlieren angle: 65°.

Density-gradient centrifugation Density-gradient centrifugation was done on sucrose and cesium chloride gradients. The sucrose gradients were prepared by mixing 14 ml of 0% and 14 ml of 40% sucrose in 0.01 M phosphate buffer pH 7.0 in a mixing chamber. The cesium chloride solution was made by adding to 5.90 g CsCl, 0.01 M phosphate buffer pH 7.0 to a total weight of 15.0 g. The refractive index of this solution was measured with an Atago refractometer. The density was about 1.40 g/cm^3 at 20°C when calculated with the formula (Stols, 1964):

$$\rho^{20} = 10.250\, n_D^{20} - 12.679 \qquad 1.25 < \rho < 1.50$$

in which ρ is the density at 20°C and n_D^{20} the refractive index at 20°C at the wavelength of the Na-D line.

Sugar gradient; 0.4 ml of a virus suspension (10 mg virus/ml) was layered on the sugar gradient and spun for 100 min in the Spinco L50, SW 25.1 rotor, at 23000 rpm at 5°C. Only one band containing infectious virus was visible at 1.5 – 1.9 cm under the meniscus.

CsCl gradient; 0.02 ml of a virus suspension (16.3 mg virus/ml) was layered on top of 5 ml of the CsCl solution in the tubes of the Spinco L50,SW 39 rotor. The tubes were spun for 26 h at 34000 rpm at 5°C. Only one band was visible between 2.1 and

2.3 cm under the meniscus (Fig. 23B). After pinning the bottom of the tubes with a MSE tube piercer the contents were pumped by a LKB Varioperpex pump through a LKB Uvikord (type 4701A), scanned at 254 nm and 281 nm and registered on a Servogor recorder. Only one peak was recorded at these wavelengths. The fractions which caused the absorbancy were collected and dialysed against 0.01 M phosphate buffer pH 7.0. When inoculated onto 'Sindano', these suspensions proved infectious.

Equilibrium centrifugation For equilibrium centrifugation 0.01 ml of a virus suspension (16.3 mg virus/ml) was mixed with 1.6 ml CsCl solution and spun for 22 hours at 44770 rpm at a temperature kept at 20°C. The schlieren pattern revealed here one component too (Fig. 23C).

6.3 Physical and chemical properties of RYMV nucleic acid

In the determination of the properties of RYMV nucleic acid, purified virus was used in the orcinol and diphenylamine reactions, in the determination of the base composition and in the determination of the phosphorus content. The ultraviolet absorption spectrum and the molecular weight of the extracted nucleic acid were determined. The effect of RNase and DNase on the infectivity of the extracted nucleic acid was also studied.

The nucleic acid extracted from the virus by the methods as described under Section 3.7 formed a flocculent precipitate on standing after ethanol had been added to the aqueous solution and could not be wound on a glass rod. Mechanical inoculation of the final water clear supernatant onto 'Sindano' resulted in infection of the seedlings.

6.3.1 UV absorption spectrum

The ultraviolet absorption spectrum of the nucleic acid showed maximum absorption at 258 – 259 nm and minimum absorption at 229 – 230 nm (Fig. 20). The value for E_{max}/E_{min} was about 2.39 and that for E_{280}/E_{260} about 0.46.

6.3.2 Type of carbohydrate

Orcinol reaction For the Mejbaum orcinol method (Putman, 1957) purified virus, containing 0.05 – 0.07 mg virus/ml and blanks of 0.01 M phosphate buffer pH 7.0 were used. To 3 ml of the samples 3 ml of a 1% orcinol solution in 12 N HCl containing 0.1% $FeCl_3$ was added. After thorough mixing, the tubes were heated for 40 min in a boiling waterbath and then cooled. The virus samples then showed a green colour – a typical reaction for pentose – while the blanks remained yellowish.

Diphenylamine reaction This reaction was performed according to the method described by Burton (1956) with calf-thymus DNA as control. The reagent which was prepared by dissolving 1.5 g diphenylamine in 100 ml glacial acetic acid and adding

1.5 ml concentrated sulphuric acid, was stored in the dark; 20 ml of this solution was mixed with 0.1 ml aqueous acetaldehyde (16 mg/ml) just before use.

Samples of calf-thymus DNA and RYMV were made in 0.005 N NaOH, giving concentrations of 0.2 mg DNA/ml and 1 – 2 mg virus/ml. Blanks consisted of 0.005 N NaOH. The samples were mixed with an equal amount of 0.5 N $HClO_4$ and heated at 70 °C for 15 min. After cooling to 30°C, 1 ml portions of the solutions were mixed with 2 ml of the diphenylamine reagent containing acetaldehyde and kept in the dark at 30°C for 18 hours.

On standing the treated DNA samples developed a blue colour. Their absorption curve in the 400 – 700 nm range was similar to the curve obtained by Peters & Dieleman (1963). The treated virus samples in which a slight precipitate was noticed, and the blanks had not changed colour, indicating the absence of deoxyribose.

Influence of RNase and DNase on infectivity The infectivity of nucleic acid preparations in 0.01 M phosphate buffer pH 7.0, was abolished by treatment at 0°C for 100 min with pancreatic RNase-A (Sigma, 5× cryst., 1 mg/l). Treatment with DNase I (Sigma, RNase-free, 5 mg/l) in the presence of 2 mM Mg^{2+} had no effect. RYMV preparations (about 1 mg virus/ml) treated with RNase-A at 5 mg/l still proved to be infective.

The presence of ribose as carbohydrate in the nucleic acid (positive orcinol reaction), rather than deoxyribose (negative diphenylamine reaction), while infectivity of the nucleic acid was not lost by DNase, shows that the nucleic acid of RYMV is RNA.

6.3.3 Base composition

Purified virus (13 – 24 mg) was used for determination of the base composition according to the methods as described by Knight (1963). The nucleic acid was hydrolyzed in 1 N HCl by heating the virus in a closed ampulla at 100°C for one hour. The hydrolysate was chromatographed on Whatman No. 1 paper in a solvent consisting of *t*-butanol, 6 N HCl and H_2O (70:13:17) at 30°C for 20 hours. When the chromatography paper was dry, four well separated spots were visible in ultraviolet light. The spots and also non-spotted pieces of the same size and of the same height, were marked, cut out and eluted from the paper with 0.1 N HCl. Identification and determination of the amounts of bases and nucleotides was done spectrophotometrically using the blanks as standard. The absorption curves were identical to

Table 24. Molar base ratio of RYMV. The numbers are the averages of the analyses made on four different virus preparations. Values stated as moles (%).

Guanine	Adenine	Cytosine	Uracil	Number of analyses
29.2 ± 2.0	21.2 ± 1.0	25.1 ± 1.5	24.5 ± 1.5	12

those of guanine, adenine, cytidylic acid and uridylic acid. The molar base ratio is given in Table 24.

The nucleic acid of RYMV appears to be relatively rich in guanine. Guanine and adenine together account for about 50% of the bases, while the G/C ratio is 1.16.

6.3.4 Percentage nucleic acid in virus particles

From the E_{280}/E_{260} ratio of 0.65 for purified RYMV, as given in Section 6.2.1, a nucleic acid content of about 20% is estimated (Paul, 1959).

The nucleic acid content of RYMV was calculated from the phosphorus content which therefore had to be determined. In the commonly applied virus purification procedure as given in Section 3.5, use was made of phosphate buffers, but for this determination virus suspensions without additional phosphate were preferred. Therefore the purification procedure had to be changed.

At first 0.1 M sodium citrate/HCl pH 5.0 + 0.2% 2-mercaptoethanol was used instead of 0.1 M phosphate buffer pH 5.0 + 0.2% 2-mercaptoethanol in the first purification steps. The high speed pellet (P_4) was suspended in bidistilled water, which was used in the following steps too. This procedure was not satisfactory. During dialysis the virus suspension turned milky white as much of the virus precipitated. The method was therefore discarded and the following procedure was chosen for the purification of RYMV used for the phosphorus determination.

In the first steps 0.1 M phosphate buffer pH 5.0 + 0.2% mercaptoethanol was used also, but the high speed pellet (P_4) was suspended in 0.01 M imidazol/HCl buffer pH 7.0 and dialysis in this buffer was extended to 3 days, with 3 changes of the buffer.

Phosphorus content was determined according to the micromethod of Morrison (1964). Glassware was kept in bichromate-sulphuric acid overnight and rinsed with tap water and bidistilled water before use. A standard phosphorus solution was made of $NaH_2PO_4.H_2O$ in bidistilled water in a concentration of 0.89406 g/l.So this solution contained 200 μg P/ml. By making a dilution series a standard curve of optical density against μg P was obtained.

An amount of 0.125 – 0.150 mg of purified virus was dried in 'Pyrex' test tubes – calibrated at 5 ml level – at 100°C. After cooling 0.3 ml sulphuric acid (98%) was added and the tube gently heated over a gas flame till when charring was completed, no further discolouration of the solution was noted. With a Pasteur pipette one drop of hydrogen peroxide (30% w/v) was added and the tube well shaken. The tube was gently boiled for 1 min above a gas flame and allowed to cool. Then first 3.4 ml bidistilled water was added, using the water to wash the walls of the tube and subsequently 0.1 ml sodium sulphite (Na_2SO_3) 16.5% (w/v) after which the tube was gently shaken. 1.0 ml ammonium paramolybdate $(NH_4)_6Mo_7O_{24}.4H_2O$ 2% (w/v) was added directly to the acid followed by 0.1 ml freshly prepared ascorbic acid 10% (w/v) and the solution mixed. The tubes were heated at 100°C for 10 min in a water-bath and cooled. The volume of solution was adjusted to 5.0 ml with bidistilled water, the tubes stoppered, shaken and the optical density measured at 822 nm against blanks

containing the reagents only. Each determination was performed in triplicate and included also standard phosphorus in amounts of 2 and 4 μg P.

From the base ratio, given in Section 6.3.3, the phosphorus content of the nucleic acid was derived by means of the formula $\%P = \Sigma p_i w_i$ in which p_i is the phosphorus content of the base i, and w_i is the relative proportion in weight of this base. So the calculated phosphorus content of the viral nucleic acid was found to be 9.109%. Phosphorus analysis of four different virus preparations gave a mean value of 2.15 ± 0.05% phosphorus by weight. From these data the calculated nucleic acid content of RYMV was 23.6% ± 0.5%.

6.3.5 Molecular weight

The molecular weight of RYMV-RNA was determined by gel electrophoresis by Dr L. Reijnders (Laboratory of Molecular Biology, Agricultural University, Wageningen), applying the methods as described by Reijnders et al. (1973).

Electrophoresis of the RNA was done on 2.2% polyacrylamide gels in a buffer containing 36 mM Tris-HCl, 30 mM NaH_2PO_4, 1 mM Na_2-EDTA (pH 7.5) at 10°C for 3½ h at 7.5 mA/gel. Electrophoresis of the RNA was also performed in 8 M urea at 60°C in 20 mM Tris-HCl, 2 mM EDTA (pH 7.5) for 5 h at 5 mA/gel using 2.1% polyacrylamide gels. Under these conditions RYMV-RNA was completely denatured.

By both methods only one species of RNA was detected. The molecular weight for RYMV-RNA was calculated from its electrophoretic mobility, relative to those of the two ribosomal RNAs of *Escheria coli*, present as internal references (mol. weights 1.07×10^6 D and 0.55×10^6 D). Under the first mentioned conditions the molecular weight was calculated to be 1.36×10^6 D, while under complete denaturing conditions a value of 1.41×10^6 D was found.

6.4 Serology

The antiserum against RYMV prepared in the Netherlands, still reacted at a dilution of 1/4096 against sap at 1/128 dilution. The clearest lines of precipitation were observed at 1/32 dilution of sap and 1/512 – 1/1024 dilution of the antiserum. No reactions were noticed in tests with sap from diseased and healthy leaves against normal serum, nor in tests with sap from healthy leaves against antiserum.

Tests were done to detect possible serological relationships between RYMV and a number of other isometric viruses. In these tests the antisera were used in dilutions of 1/1, 1/4, 1/16 and 1/64 against sap obtained from young leaves of RYMV infected 'Sindano' rice by extracting 10 g leaves with 5 ml 0.85% NaCl. Undiluted antisera were used against sap of healthy leaves, prepared in the same way as sap from diseased leaves. The antisera were kindly provided by Dr D. Noordam, Dr D. Peters, Ir C. P. de Jager and Ir B. J. M. Verduin (Laboratory of Virology, Agricultural University, Wageningen); Mr D. Z. Maat (Institute of Phytopathological Research, Wageningen); Prof. Dr T. T. Hebert (Department of Plant Pathology, North Carolina State Univers-

ity, Raleigh, USA) and Dr A. J. H. Carr (Welsh Plant Breeding Station, near Aberystwyth, Wales).

The viruses whose antisera were tested are listed below. They are grouped according to the system proposed by Harrison & Murant (1973). The names of persons in parentheses refer to those who donated the respective antisera.

- Bromoviruses: broad bean mottle virus (Peters); brome mosaic virus, cowpea chlorotic mottle virus (Verduin).
- Comoviruses: cowpea mosaic virus – Vs isolate, cowpea mosaic virus – Sb isolate (de Jager); Echtes Ackerbohnenmosaik virus (Peters).
- Nepoviruses: *Arabis* mosaic virus, tobacco ringspot virus (Maat).
- Tombusviruses: carnation ringspot virus (Maat).
- Tymoviruses: Andean potato latent virus, *Belladonna* mottle virus, *Dulcamara* mottle virus, eggplant mosaic virus, *Physalis* mosaic virus (Peters).
- Others: carnation mottle virus (Noordam); cocksfoot mottle virus, *Phleum* mottle virus (Carr); pea enation mosaic virus (Peters); sowbane mosaic virus (Maat); Peru corn virus (Hebert).

In addition purified turnip yellow mosaic virus provided by Dr D. Peters, was tested at a concentration of 2 and 1 mg virus/ml against RYMV antiserum at dilutions of 1/1, 1/4, 1/16, 1/64, 1/128 and 1/256, and against undiluted normal serum.

None of these antisera reacted against RYMV, nor did turnip yellow mosaic virus react with RYMV antiserum.

6.5 Electron microscopy

6.5.1 Negatively stained purified virus preparations

Samples of purified virus suspensions contained many isometric particles. Often these particles had an angular outline, usually hexagonal and showed no subunits. A part of the particles had been partially or fully penetrated by the PTA, but the majority seemed intact and had not been penetrated. In the preparations stained with uranyl acetate, particles occurred with an electron-dense central area (Fig. 24).

Virus particles in which no PTA had penetrated and which were arranged in honeycomb structures (Fig. 24A), measured 25 nm between the nearest sides and 26 – 30 nm between the extreme points. When compared with the width of tobacco mosaic virus particles (16 nm), these distances were for the particles not penetrated by PTA 23 nm and 25 nm, and for the stain penetrated particles 22 and 24 nm, respectively.

In bidistilled water, frozen and unfrozen virus suspensions which were subsequently adjusted to 0.01 M with phosphate buffer pH 7.0, as described in Section 6.2.3, were stained with PTA and examined in the electron microscope. Compared with the unfrozen suspension, the frozen suspension contained particles which appeared less angular, but also many broken particles, while more particles were fully penetrated with stain (Fig. 25). No aggregation of the particles into honeycomb structures were noticed in the frozen suspension either.

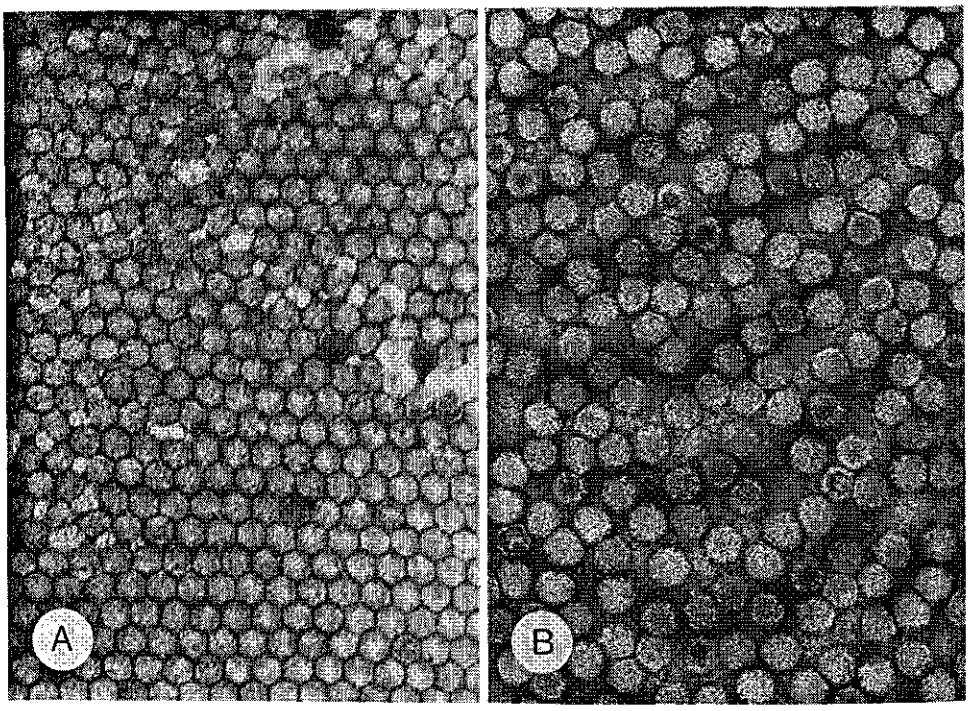


Fig. 24. Purified preparations of RYMV.
A. Stained with 1% sodium phosphotungstate pH 4.5, 160000 ×.
B. Stained with 1% uranyl acetate pH 4.3, 160000 ×.

6.5.2 RYMV in leaf tissue

In this study observations were confined primarily to the epidermis and the mesophyll of the leaves in transverse sections only. Although most attention was paid to visualization of RYMV in the rice leaves mainly, some regularly observed anomalies in the diseased leaves are also reported.

Differentiation between individual virus particles and the ribosomes was difficult. However identification of the virus particles was possible because the virus particles were seen in large numbers, often in accumulations, giving structures not observed in healthy leaf tissue (Fig. 26 and 27).

Virus particles were observed in the cells of the epidermis, the stomata, the mesophyll and also in the mestome sheath. In the fibres between epidermis and vascular bundle, virus-like particles were also noticed. No particles were seen in the nuclei and the chloroplasts, while the vascular bundle was studied insufficiently to draw conclusions.

In the youngest leaf cut (Stage A), the epidermis cells possessed large vacuoles already and although virus particles were present, the mesophyll cells showed higher concentrations of the virus (Fig. 27). At this stage of leaf and symptom development,

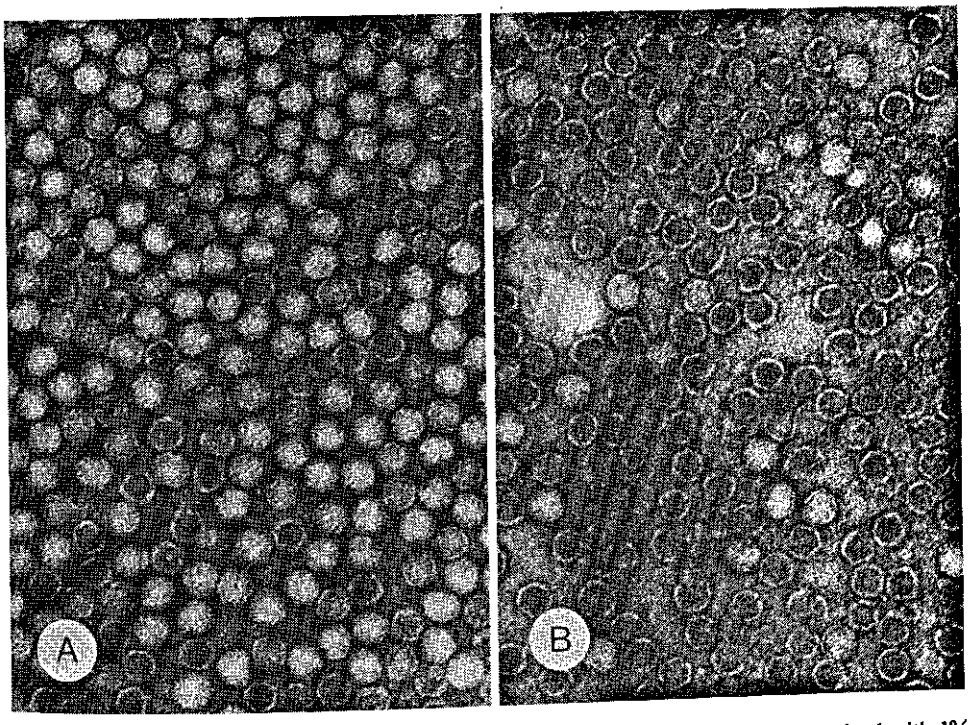


Fig. 25. Influence of freezing on RYMV suspended in bidistilled water. Preparations stained with 1% sodium phosphotungstate pH 4.5.
A. RYMV kept at 4°C for 40 h and adjusted to 0.01 M with phosphate buffer pH 7.0, 160000 ×.
B. RYMV kept at −25°C for 40 h and adjusted to 0.01 M with phosphate buffer pH 7.0 after thawing, 160000 ×.

accumulations of virus particles were noticed in the mesophyll. In the incomplete developed leaf which showed mottling (Stage B), aggregations of particles were more common (Fig. 28). In the mature leaf with mottling and chlorotic areas (Stage C), the mesophyll cells possessed a large vacuole and densily accumulated particles were seen here less often.

Crystals of virus-like particles, surrounded by a membrane were occasionally noticed (Fig. 29C). The diameter of these particles was about 22 nm. Fibres totally filled with crystals of particles resembling RYMV were also occasionally observed in the chlorotic regions of the mature leaf (Stage C) (Fig. 29A). The diameter of these individual particles was about 21 nm.

The regular occurrence of fibrils, often grouped together, in the virus-infected cells was striking (Fig. 30). Often two other types of structures, which also could not be identified, were seen in the virus-infected leaves only. The first type was of tubular nature and the other an inclusion being enveloped by a membrane, showing an electron-dense centre surrounded by an electron less dense area. The long flexuous tubules were only noticed in the mature leaf (Stage C) (Fig. 31 and 32). They were

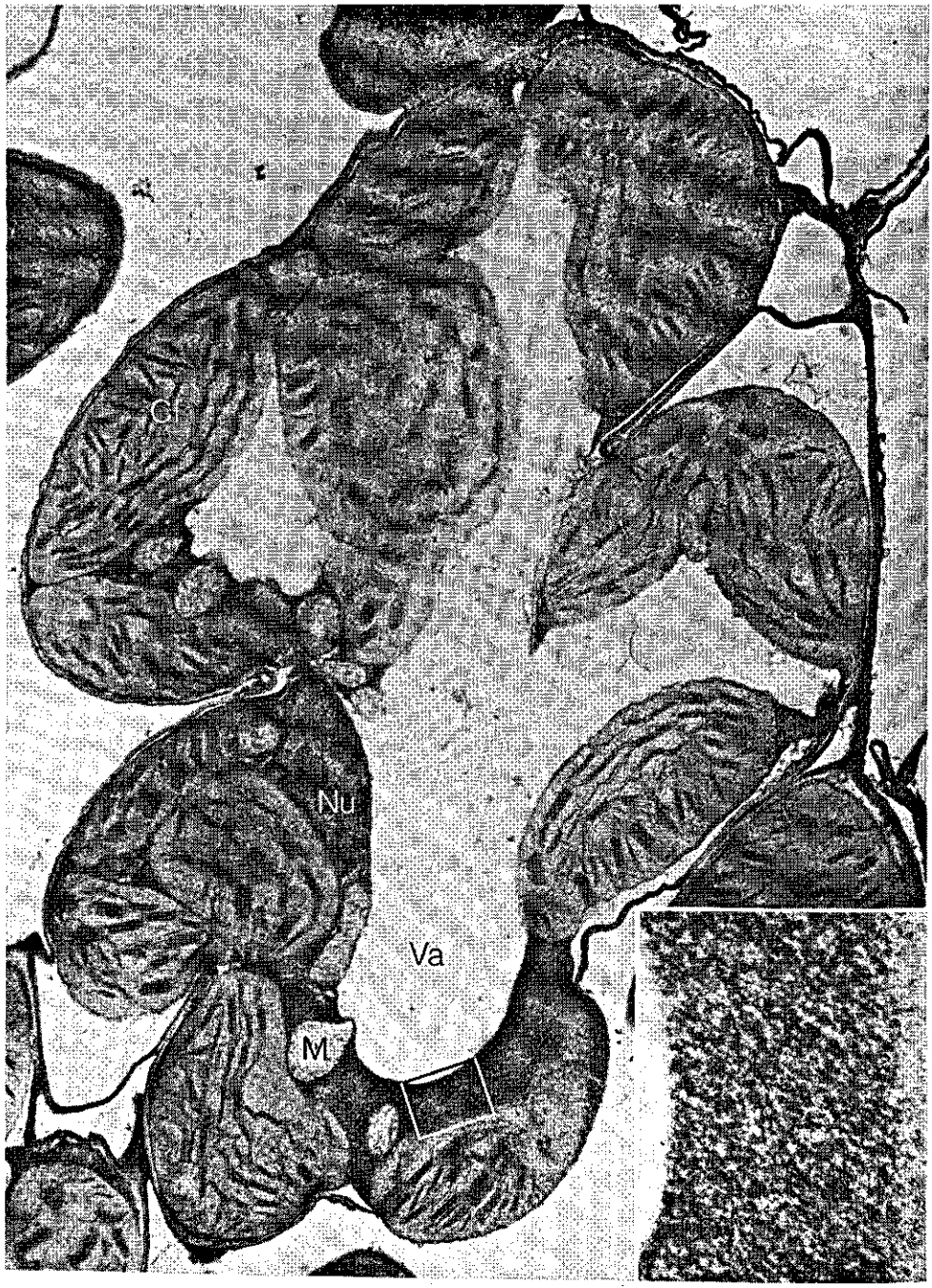


Fig. 26. Mesophyll cell of young leaf of healthy 'Sindano' rice (stage A), 9000 ×. Inset: blocked portion of cytoplasm with ribosomes at higher magnification, 48000×.
Cl: chloroplast, M: mitochondrion, Nu: nucleus, Va: vacuole.

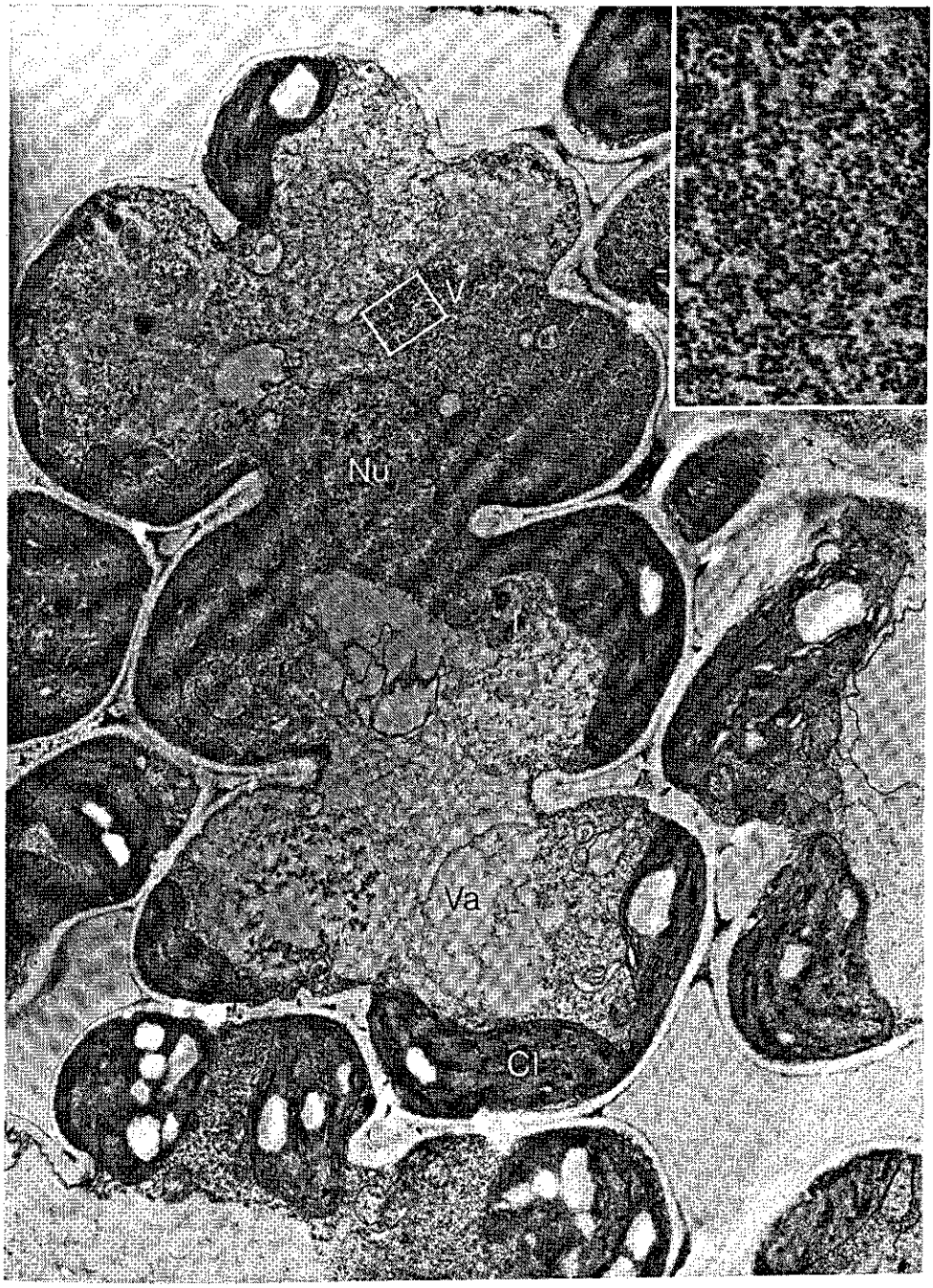


Fig. 27. Mesophyll cell of young dotted leaf of RYMV-infected 'Sindano' rice (Stage A), 6200×. Inset: blocked portion of densely packed virus particles at higher magnification, 48000×.
Cl: chloroplast, I: inclusion with electron-dense centre, Nu: nucleus, V: cluster of RYMV particles, Va: vacuole.

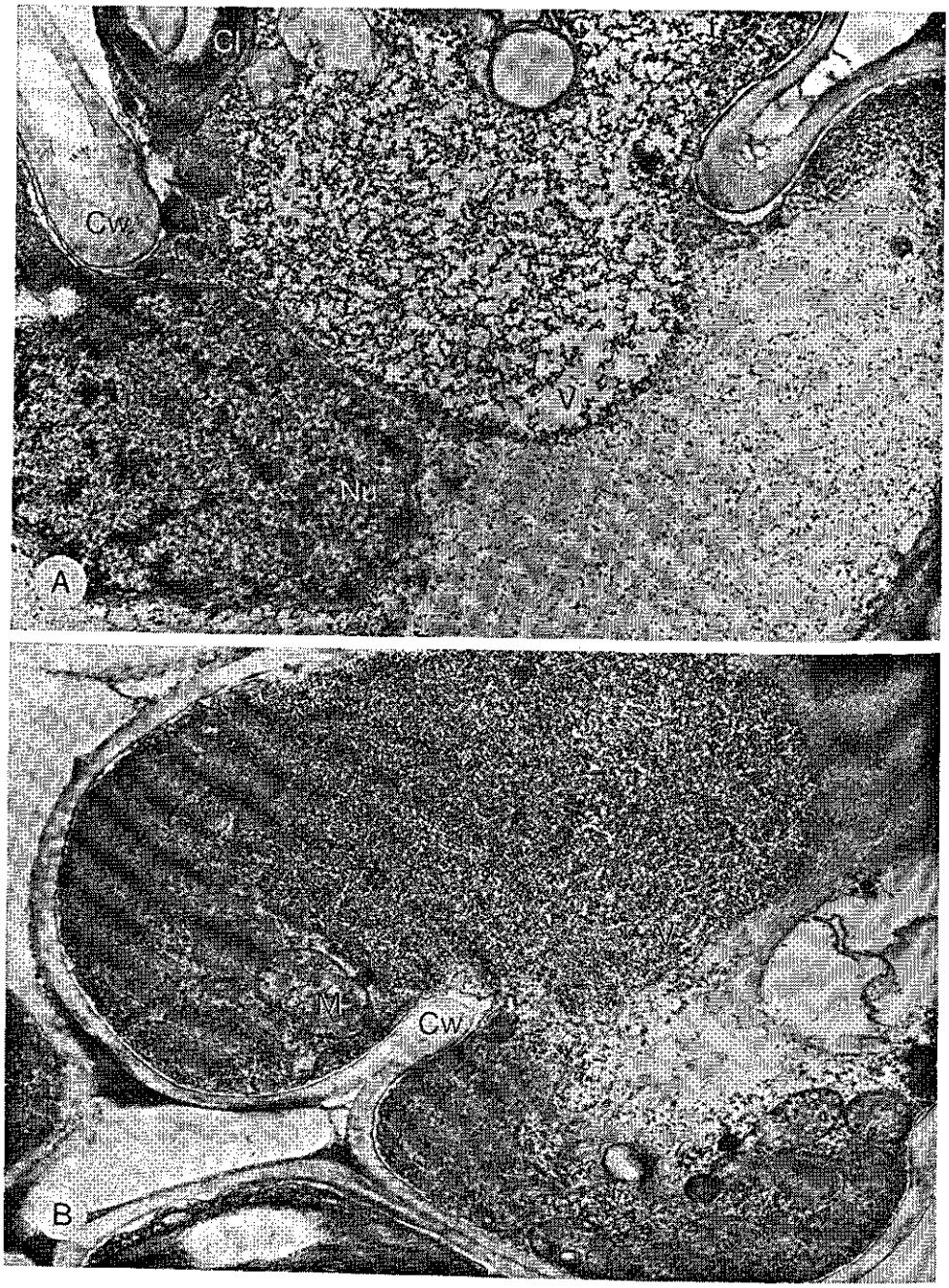


Fig. 28. Accumulation of RYMV particles in mesophyll cells of an incompletely developed 'Sindano' rice leaf with clear mottling (Stage B), 18000×.
Cl: chloroplast, Cw: cell wall, M: mitochondrion, Nu: nucleus, V: rice yellow mottle virus.
A. Loosely packed virus particles.
B. Densely packed virus particles.

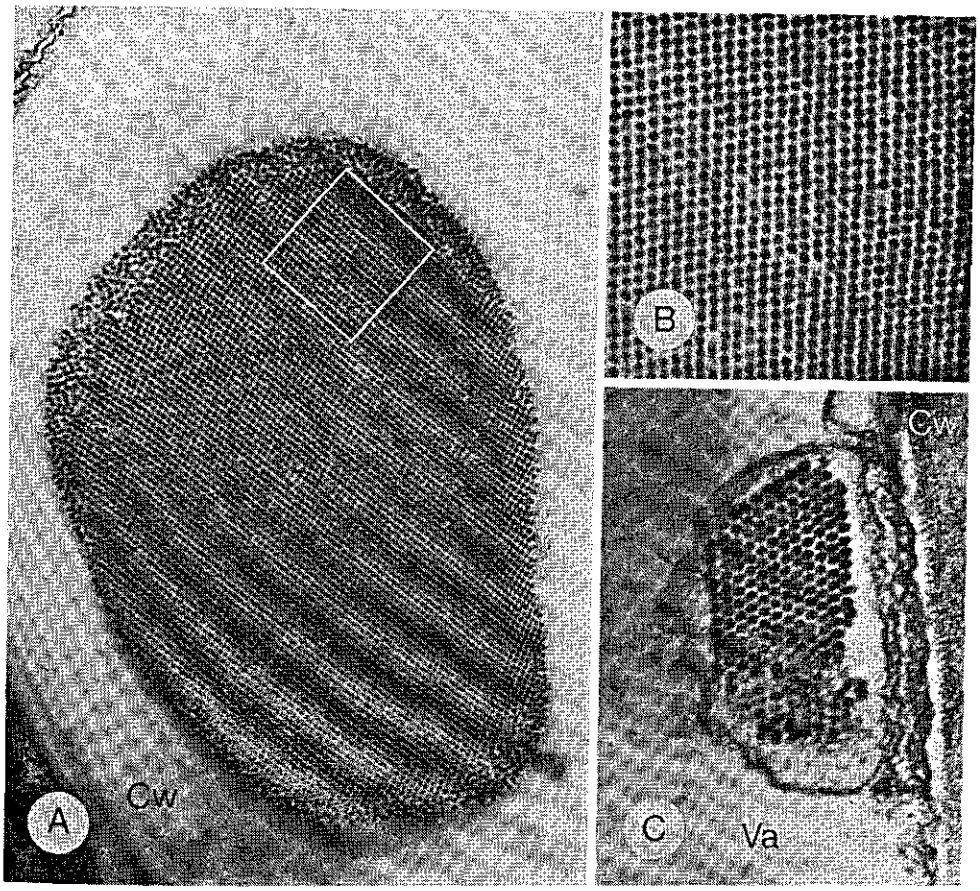


Fig. 29. Crystals in mature RYMV-infected 'Sindano' rice leaf (Stage C).
Cw: cell wall, Va: vacuole.
A. Fibre filled with crystal; in chlorotic part of leaf, 40000×.
B. Higher magnification of blocked portion of Fig. A, 80000×.
C. Virus-like crystal in mestome cell; in mottled part of leaf, 80000×.

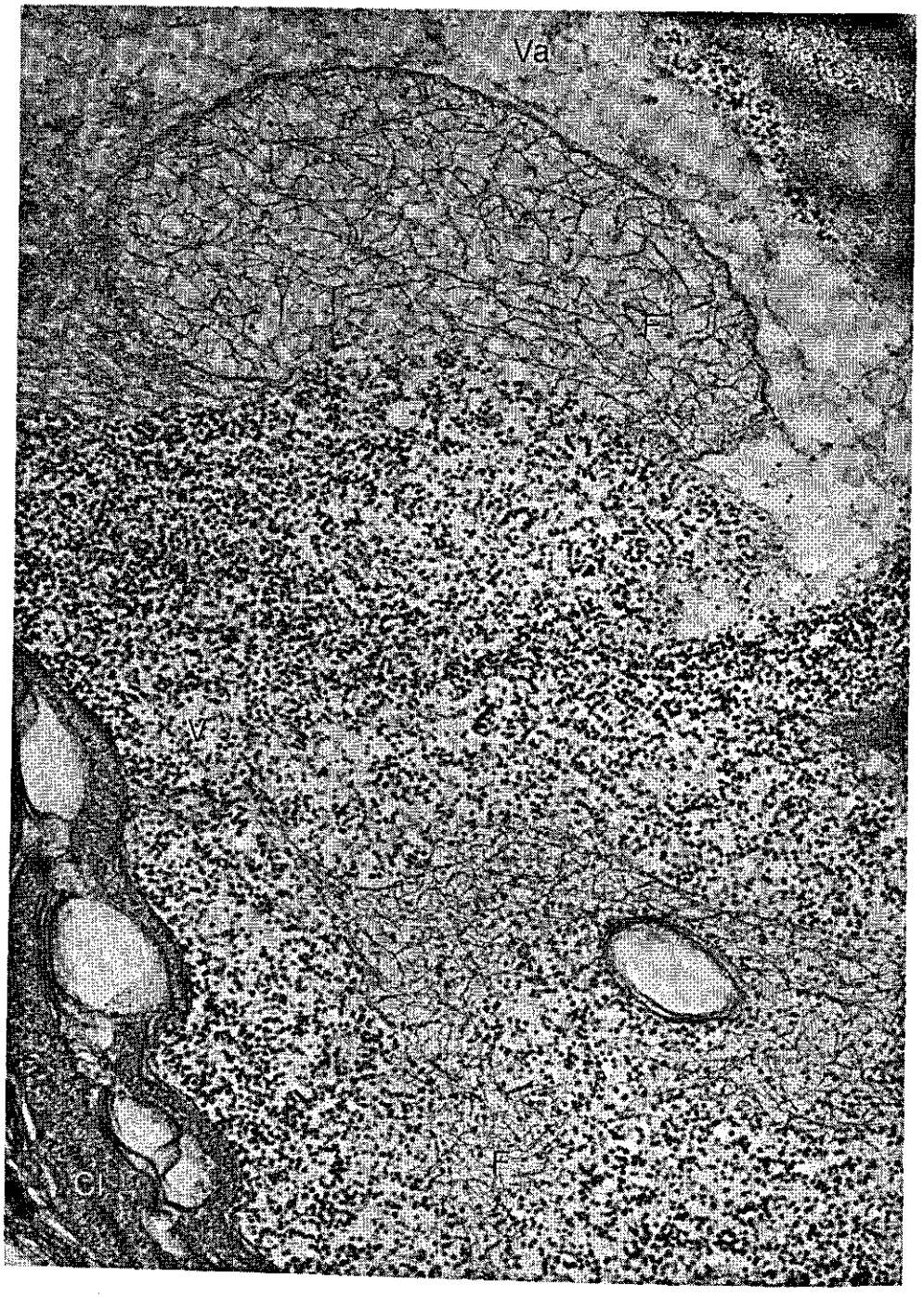


Fig. 30. Aggregations of fibrils in mesophyll cell of young RYMV-infected dotted leaf of 'Sindano' rice (Stage A), 40000×.
Cl: chloroplast, F: fibrils, V: rice yellow mottle virus, Va: vacuole.

Fig. 31. Flexuous tubules (→) in mesophyll cell of mature RYMV-infected 'Sindano' rice leaf (stage C), 18000×.
Cl: chloroplast, M: mitochondrion, Nu: nucleus, T: flexuous tubules.

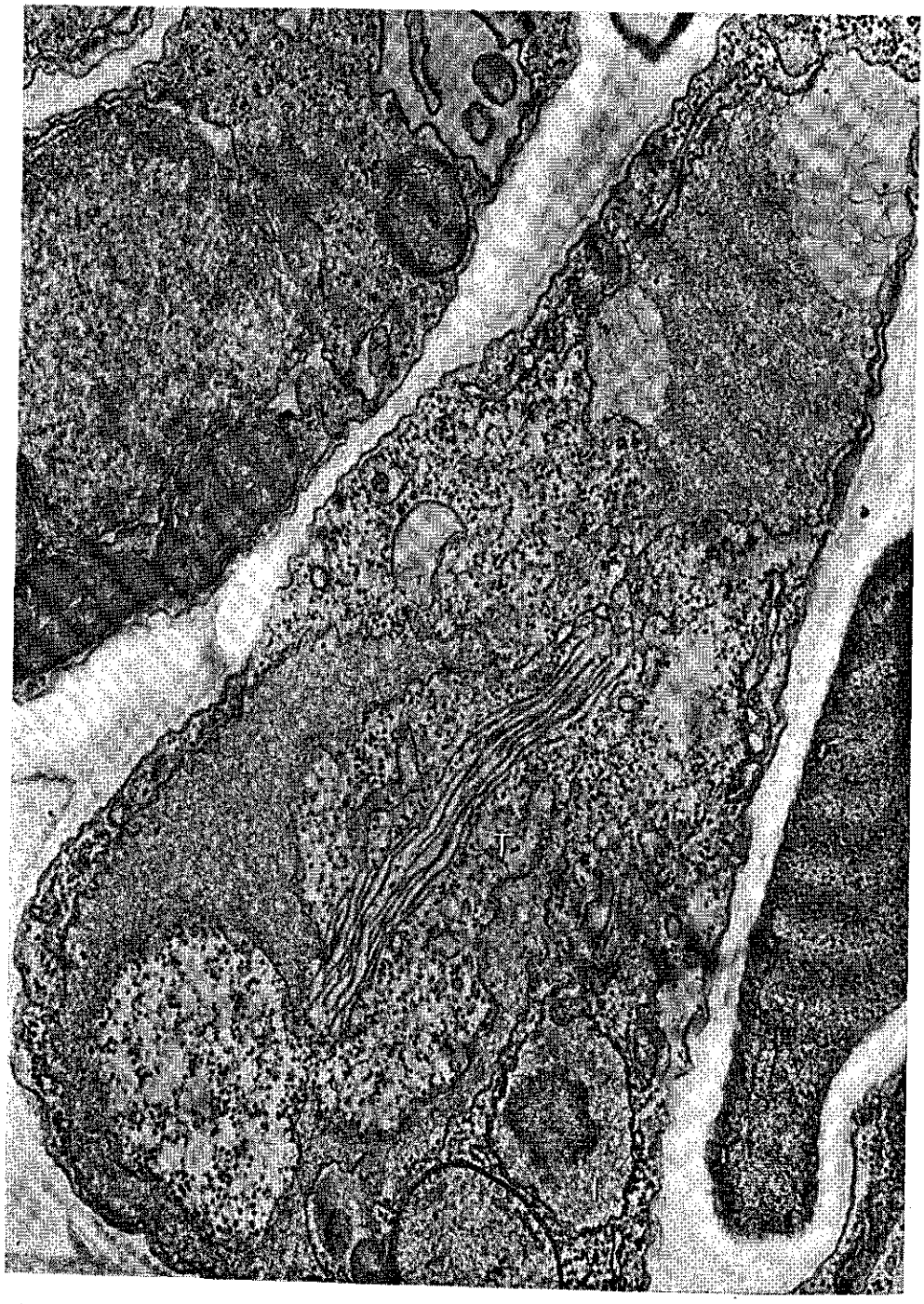


Fig. 32. Flexuous tubules in subsidary cell of stoma in chlorotic area of a mature RYMV-infected 'Sindano' rice leaf (stage C), 36000×.
I: inclusion with electron-dense centre, T: flexuous tubules.

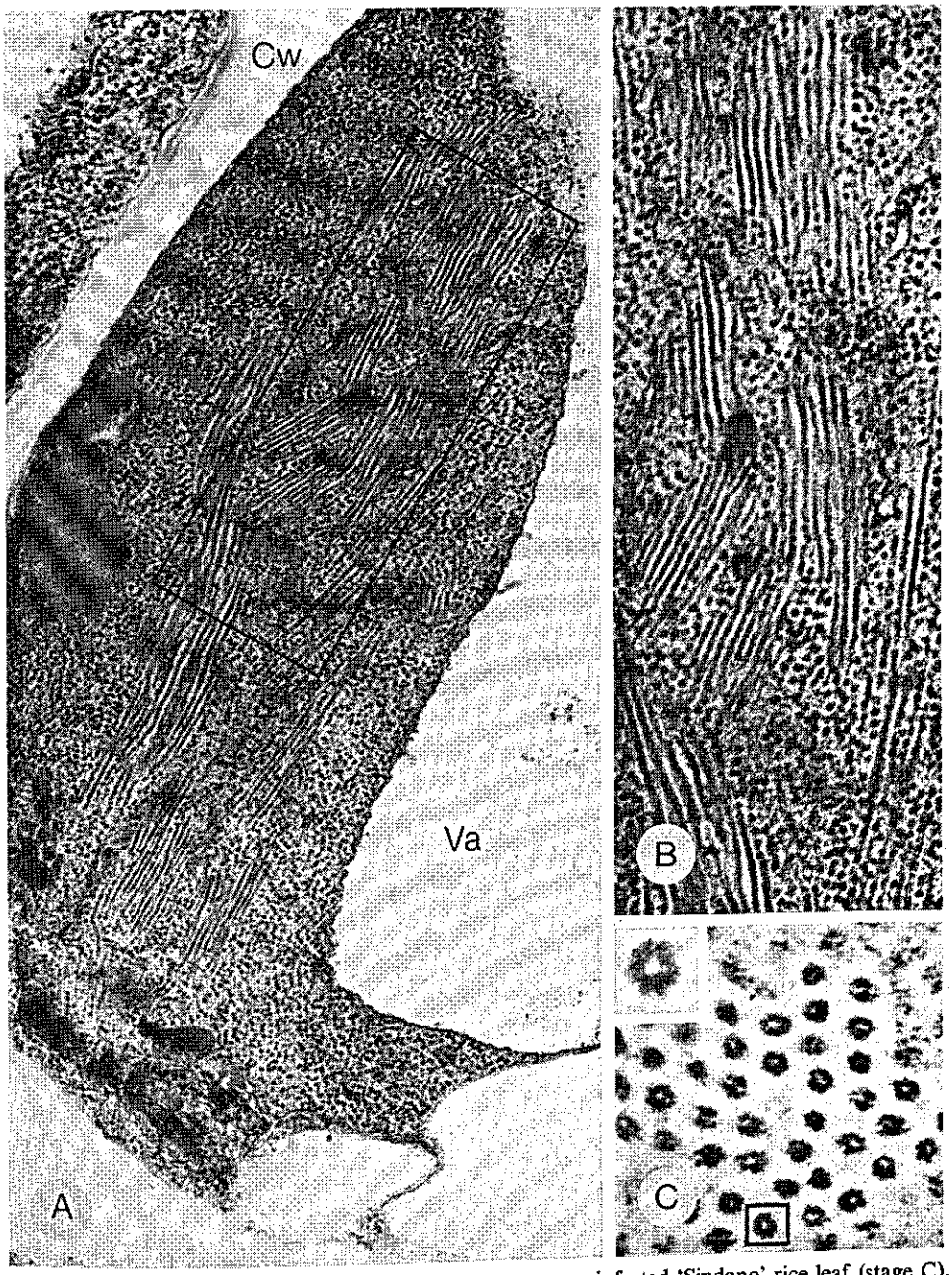


Fig. 33. Flexuous tubules in epidermis cells of mature RYMV-infected 'Sindano' rice leaf (stage C). Cw: cell wall, Va: vacuole.

A. In chlorotic area of leaf. Note the alignment of particles with about the same diameter as the tubules along and in line with the tubules, 36000×.

B. Higher magnification of blocked portion of Fig. A, 64000×.

C. Cross-section of tubules in mottled part of leaf, 216000×. Inset: higher magnification of blocked portion, 510000 X.

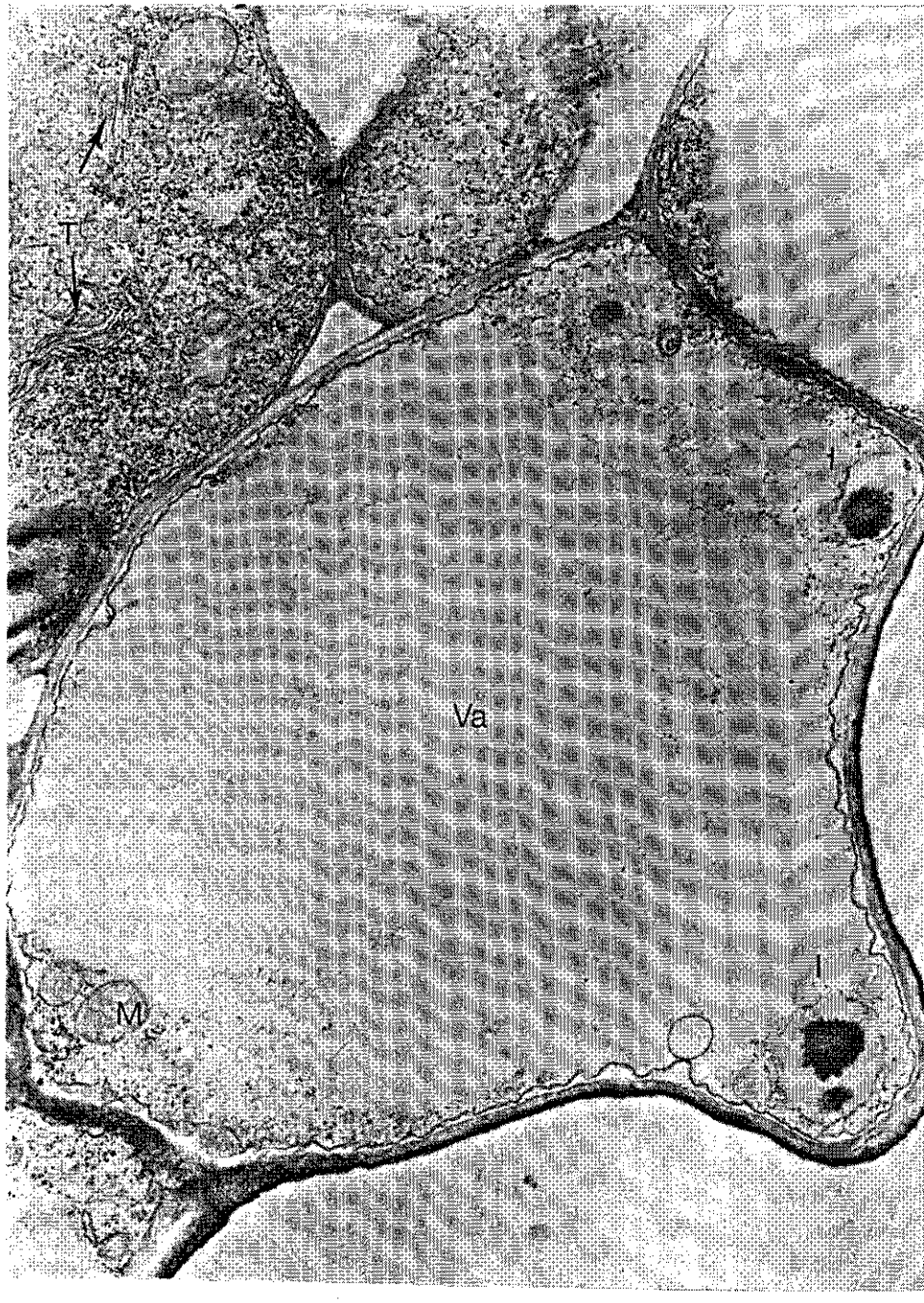


Fig. 34. Inclusions with electron-dense centre in epidermis cell of mature RYMV-infected 'Sindano' rice leaf in mottled area (Stage C). Flexuous tubules are present in adjoining mesophyll cell (→), 17000×.

I: inclusion with electron-dense centre, M: mitochondrion, T: flexuous tubules, Va: vacuole.

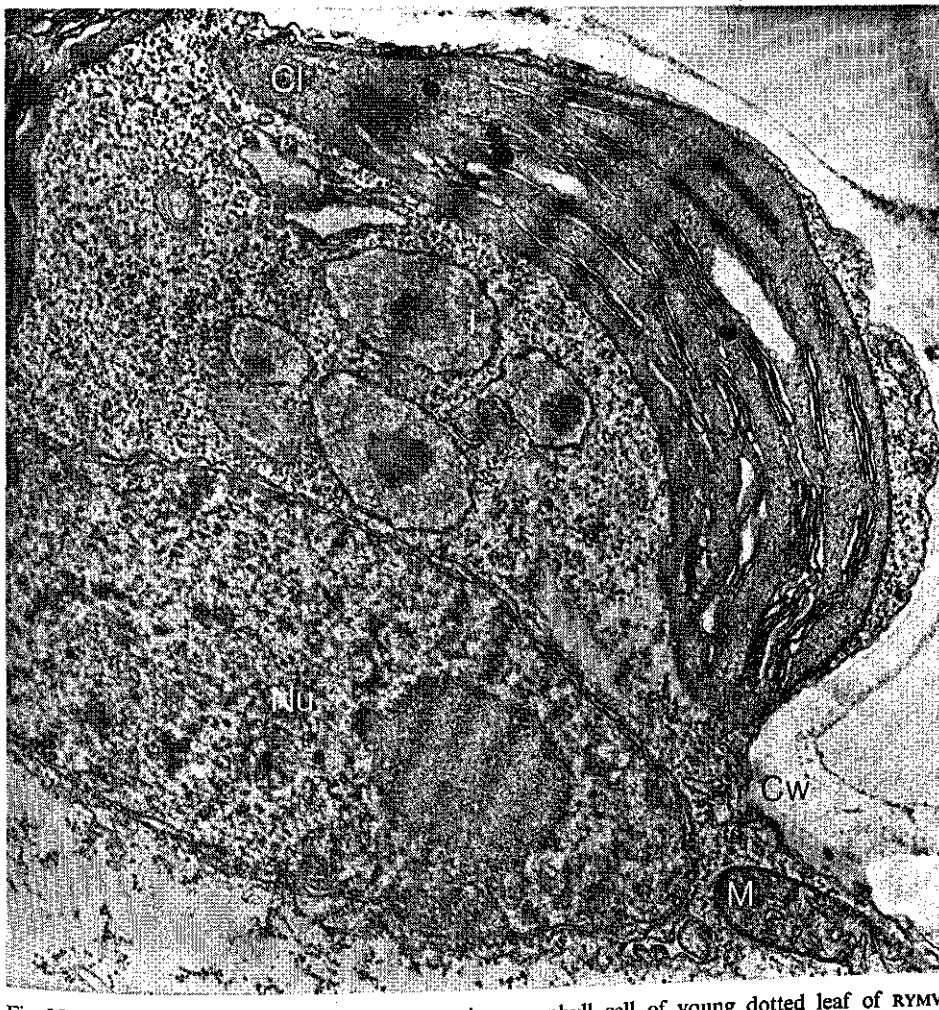


Fig. 35. Inclusions with electron-dense centre in mesophyll cell of young dotted leaf of RYMV-infected 'Sindano' rice (stage A), 27000 X.
Cl: chloroplast, Cw: cell wall, I: inclusion with electron-dense centre, M: mitochondrion, Nu: nucleus.

grouped together in bundles in the cytoplasm. The hollow nature of the tubules was demonstrated in transverse sections, and subunits could be differentiated in the ring (Fig. 33C). The diameter of these tubules was 10 – 15 nm, with a central hole of about 4 nm in diameter. Virus-like particles with about the same diameter as the tubules were often noticed aligned along these tubules, or in direct line with them (Fig. 33A).

The inclusions with the electron-dense centre (Fig. 34 and 35), often occurred in groups of two or three together. They varied in size and were of the same order of size as the mitochondria.

6.6 Conclusions

RYMV is a stable virus which in highly diluted suspensions still proved to be infectious in 'Sindano' and 'IR 22'. Higher levels of virus concentrations were, however, necessary to infect 'Basmati 217' and the grass species *Dinebra retroflexa*.

With the purification procedure applied good yields of purified virus were obtained, even from low amounts of leaves. This implies a high virus concentration in the leaves. The diameter of RYMV particles in 0.01 M phosphate buffer pH 7.0 and stained with 1% sodium phosphotungstate is about 25 nm.

RYMV obtained from fresh or deep-frozen leaves sediments as a single component with a s^0_{20} value of 109S. Virus, suspended in bidistilled water or in 0.01 M phosphate buffer pH 7.0, which was frozen and subsequently thawed sediments, however, as two components. One component was presumed to be the unaffected RYMV; the other faster sedimenting component (147S) was possibly due to aggregation of virus particles. Many damaged, or in severe cases totally ruptured particles were seen in the electron microscope then. Freezing and thawing of turnip yellow mosaic virus, however, results apart from a barely discernible amount of intact virus in two predominating, slower sedimenting components (its RNA and protein component) as reported by Kaper & Alting Siberg (1969). RYMV suspended in 0.01 M phosphate buffer pH 7.0 and needed for further analysis should therefore not be frozen, as it appears to precipitate.

The nucleic acid of RYMV is RNA. From the base composition and the abolishment of infectivity of the nucleic acid by RNase it is concluded that the RNA is single stranded. Only one species of RNA with a molecular weight of 1.4×10^6 D was revealed by polyacrylamide gel electrophoresis. The base composition is similar, but not identical, to that of cocksfoot mottle virus (Paul & Huth, 1970).

Chemically the RNA content was determined as $23.6 \pm 0.5\%$, while from the E_{280}/E_{260} ratio of the virus suspension, an RNA content of about 20% was estimated. The RNA content of RYMV was therefore assessed at 23%.

Purified RYMV proved to be highly antigenic. No serological relationship was established between RYMV and a number of small isometric plant viruses including cocksfoot mottle virus and *Phleum* mottle virus.

In young 'Sindano' leaves a high virus concentration was present in the tissue between the veins, especially in the mesophyll, as observed in the electron microscope. This is the part of the leaves specifically eaten by the *Chaetocnema* spp. and the Hispinae used in the transmission experiments. In the initial period of the process of enlargement of the mesophyll cells, densily packed virus aggregations occurred in the cytoplasm. In later stages of cell development, at increasing size of the vacuole, the virus particles were again distributed evenly over the cytoplasm. At this stage of leaf development, crystalline structures, presumably composed of virus particles, appeared surrounded by a membrane.

The presence of the inclusions with an electron-dense centre in diseased tissue only, provides presumptive evidence that the inclusions are a result of the virus infection.

Conclusions about the tubules and fibrils are made with more reservations. The tubules resemble microtubules. Microtubules have been generally defined as straight cylinders, 24 $\pm$ 2 nm in diameter with a hollow 15 nm core, although diameters of the tubules of 18 – 34 nm have also been reported (Olmsted & Borisy, 1973). They have been postulated to be functionally important in a variety of eukaryotic cellular phenomena. In plant cells for instance, the presence of microtubules has been correlated with defining the plane of cell cleavage, formation of the cell plate, development of cell walls and maintenance of specific cell shapes. I tend to believe that the presence of the tubules in cells of the rice leaves are merely a consequence of virus infection, rather than of cell enlargment. The tubules appear more flexuous than straight, with a diameter smaller than those given for microtubules. The wall of the tubule is proportionally thicker and apparently consists of less subunits than noticed in microtubules. Presence of virus particles in the tubules themselves would not be possible because of the diameter of the virus particles and the inner diameter of the tubules. The presence of particles which appear smaller than the RYMV particles and are aligned along or in line with the tubules is intriguing, but can as yet not be explained.

I also think that the fibrils noticed in the RYMV-infected cells are nucleic acid fibrils and that they are closely connected with the presence of virus, but no attempt has been made to substantiate these hypotheses.

7 Ecological aspects

Understanding of the ecology of RYMV is necessary for the development of methods for the control of the virus in the affected area and for prevention of further spread of RYMV.

Matthews (1970) stated that as with most other obligate parasites, the dominant ecological factors to be considered are usually the way viruses spread from plant to plant and how other factors influence this spread. Knowledge of the biological and physical factors as well as the survival of the virus through the seasonal cycle are of prime importance.

The exact date of introduction of rice near Lake Victoria is not known, but compared with traditional rice growing centres in the world, it is relatively recent. Initially rice was cultivated here in isolated spots on small scale, but gradually as near Mwanza in Tanzania, larger areas were taken into production. The establishment of the Ahero Pilot Scheme (APS), where mainly one variety of rice is grown twice a year under supervision was a further step to more intensive rice cultivation in this region. The high costs involved in establishing and running these schemes necessitate good yields, and the influence of any factor that depresses yield is soon felt by the farmer.

Recently some insight in the virus diseases of rice has been obtained, but still little is known of the viruses affecting rice in Africa. As RYM only occurs in a relatively small area (as far as information has been obtained), it may be concluded that the virus has not been introduced into this area, but was present near Lake Victoria.

To understand the ecology of RYMV, information had to be obtained about:
- the natural sources of RYMV and its spread from the wild host(s) to rice;
- the spread of RYMV in the rice crop;
- the chances of further spread of the virus over large distances, this involves the question why the virus had not spread to other areas where rice cultivation had started earlier like at the Mwea Irrigation Settlement.

The basic properties of RYMV and the characteristics of the disease it provokes have been given in the preceding chapters. Other facts contributing to the understanding of the ecology of RYMV are dealt with first.

7.1 Occurrence of RYM

The presence of RYM in rice has been confirmed in the neighbourhood of Kisumu only, for example at Otonglo, Paponditi and Ahero (Fig. 4). At Bunyala and Mwea the disease has not been observed, while in the few plots visited in the Coast Province

of Kenya, no RYM was noticed, nor has the condition been reported from this area. Neither was the disease reported from the important rice growing area near Mwanza (Tanzania), an area which I did not visit.

7.2 Influence of rice cultivation on the natural vegetation

Before the APS existed, this area of the Kano Plains was used by smallholders mainly for grazing cattle and growing of crops like maize and sorghum. Water control was insufficient, causing flooding of some areas in the rainy seasons. In the dry seasons the land dried and then water was available only in the rivers and in some ponds. The combined effects of grazing cattle and in the dry season little or no rain influenced especially the grass vegetation. Large areas in the Kano Plains can be regarded as 'cultivation steppe', i.e. land which has been heavily cultivated and grazed for many years (Allnutt, 1942).

Growing rice under irrigation necessitates the presence of water, which is spread over a considerable area. With two crops of rice a year, the water used for irrigation, drained off water and that lost through leakages, enhances the nearby vegetation, especially that of grasses, which is abundant. The influence of the natural break between seasons was therefore less sharply noticeable in the vegetation near the rice fields. An increase of *Dinebra retroflexa* and sedges was easily noticed at the APS. In general a situation was created favouring the growth of grasses and thereby likely to attract insects which feed or find shelter on them.

7.3 Presence of rice during the year

The general practice of the smallholders near Kisumu outside the irrigation scheme, was to grow the main crop of rice in the short rainy season. If the water was easily available, the period of rice growing could be extended while a second crop could be planted too. At Paponditi, Ahero and Otonglo the rice was planted in the immediate neighbourhood of swamps. At Otonglo additional water was available from the prison, while at Ahero after the start of the pilot scheme, water drained off from this scheme could be used. At Paponditi the rice was mainly rain fed. In all these areas it was customary to graze cattle on the rice fields after harvest. The main points of interest for the areas during the time of observation are summarized:

- Paponditi: one crop a year in the short rainy season; rice fields dried after harvest; little rice present outside the growing season.
- Otonglo: main crop in the short rainy season; two crops a year; part of the rice fields could be wet after the harvest, resulting in abundance of ratoon rice; rice present throughout the year.
- Ahero: Before the APS: one crop a year in the short rainy season; rice fields dried after harvest; little rice present outside the growing season. After the APS: the area under rice was expanded; one crop a year in short rainy season; most fields dried after harvest; some rice present outside the growing season mainly near the swamp.

– APS: two crops of rice a year; rice present throughout the year.

For the areas under cultivation by smallholders outside the APS there was seasonal fluctuation in the acreage of rice.

7.4 Spread of RYM in rice fields

One out of a hundred seedlings collected from nurseries at Otonglo proved to be infected with RYMV when transplanted in the glasshouse. In 'Sindano' seedlings collected from the Irrigation Research Station Ahero, this was the case for 3 out of 113 plants. At transplanting no symptoms of RYMV had been observed. These figures indicate a low percentage of infection of the rice in the nursey stage.

In most rice varieties, the first diseased plants were easily seen in the field about 2 – 3 weeks after transplanting. In the small trial fields at the Irrigation Research Station Ahero and at Otonglo, the diseased plants were spread at random over the whole field, while in the larger fields of the APS most diseased plants were noticed along the edges of the field at this stage. In the variety 'Sindano' the disease spread fast to neighbouring plants, while also new centres of infection were noticed. This process proceeded fast, and about 4 – 8 weeks after transplanting (Fig. 36), small fields could be totally diseased. In the larger fields, more gradually large parts or even whole fields became affected.

In the APS, incidence of RYM was not the same for all sections when 'Sindano' was still the main variety. Sections in which a high incidence of RYM did occur in one season, could show a much lower incidence of diseased plants in the next season. Then other sections could be seriously affected.

In 1972 at Otonglo, when rice was grown here again on a small scale after several seasons of no rice growing, only a few plants along the edges of the field were found affected by RYM late in the season. At Paponditi and Ahero, incidence of RYM never reached similar proportions to those seen initially at Otonglo, or later in the APS with 'Sindano'. The incidence of the disease was, however, higher at Ahero than at Paponditi.

At the Irrigation Research Station Ahero, RYM was observed and occasionally confirmed by mechanical inoculation of sap to 'Sindano' test plants and by serological tests in the rice varieties given in Table 25.

By the host range studies described in Section 4.1.1, a number of non-rice hosts for RYMV have been assigned. Only Gramineae and in this family only species in the tribes Bromeae, Eragrostideae, Paniceae, Phalarideae and Oryzeae belonged to this group. East Africa has however many species of Gramineae, in Kenya alone more than 450 are represented (Bogdan, 1958). Only some of these species have been tested by mechanical inoculation with the virus.

The Bromeae and Phalarideae are mainly found in the highlands in East Africa. They are represented here by relatively few genera and species (Clayton, 1970). Therefore it is not likely that the source of RYMV is present in species belonging to these tribes. In addition none of the assigned hosts is indigenous in East Africa. The

Fig. 36. Spots of RYM affected plants in a field with 'Sindano' rice.

Oryzeae are also represented by few genera with a limited number of species, but several genera with many species belong to the Eragrostideae (Napper, 1965). These two tribes are represented in the area around Lake Victoria. Natural sources of RYMV are therefore likely to be found in grasses belonging to these tribes, of which several species have been assigned as systemic host for the virus. The distribution and habitat of potential natural sources of RYMV are given in Table 26.

Several times plants of species other than rice with and without suspected symptoms were collected in the disease stricken area, and sap of these plants was mechanically inoculated onto 'Sindano' test plants. Plants so tested are given in Table 27. *Dinebra retroflexa* was collected at the Irrigation Research Station Ahero and the APS in the immediate neighbourhood of diseased 'Sindano' rice. Apart from some occasional

Table 25. Rice varieties affected by RYM at the Irrigation Research Station Ahero.

Variety	Incidence of RYM[1]		Variety	Incidence of RYM[1]	
Afaa Kilombero 0/906	A	++	SML 242	B	×
Afaa Kilombero 1/196	A	++	SML Apura	B	O
Afaa Kilombero 2/214	B	×	SML Temerin	B	O
Afaa Mwanza 0/746	A	+	Taichung Native 1	A	++
Afaa Mwanza 1/133	A	++	Uchuki	A	+
Afaa Mwanza 1/159	A	++	Yonechiro	B	×
Basmati 217	A	++	YRL I	A	+
Demarara Creole	A	++	Zira	A	++
Faya S.L.	A	+++			
Fujiminori	B	O	IR 5	A	++
Gamti	A	++	IR 8	A	+++
Introduction 1/324	A	+++	IR 12-178-2-3	A	+++
Kialangawa	B	O	IR 20	A	++
Lindi Safari	A	++	IR 22	A	++
Madevu	A	+	IR 24	B	×
Milfor 6 (2)	A	+	IR 52-18-2	A	++
Portuguese	A	+	IR 154-61-1-1	A	++
Radin Goi	A	++	IR 480-5-9-3-3	B	×
S.C. 70	A	++	IR 520-1-26-3-3	A	++
Shimokita	B	×	IR 532E537	B	×
Shingo la Majani	A	++	IR 532-1-33	B	×
Sialkot 16	A	++	IR 579-48-1-2	B	×
Sindano	A	+++	IR 589-66-2-1	B	×
Sindano Nsemevu	A	++	IR 661-1-127-3-1	B	O
SML 128/4	B	O	IR 790-28-6	B	×
SML 140/5	B	O	IR 822-347	B	×
SML 140/10/4	B	O			

1. As observed in the most seriously affected trial fields at heading of the rice.
A: Observations made during several seasons,
+ : only very few plants affected,
++ : up to 50% of the plants affected,
+++: more than 50% of the plants affected.
B: Observations made during 1 – 2 seasons and limited to a very small area,
O: no RYM observed,
× : RYM present.

light spots, not resembling RYM symptoms, no other symptoms were seen and therefore most plants were taken at random. *Eragrostis ciliaris* was found as weed in a maize patch on higher grounds not near rice at Paponditi. Sugarcane showing mottling and mosaic of the leaves was regularly found in the Kano Plains. After inoculation of sap of these leaves onto 'Sindano', a mild mosaic was incited in the test plants. The symptoms did not resemble those incited by RYMV and serologically no relation-

Table 26. Occurrence and habitat of systemic RYMV host plants, indigenous in East Africa.

Systemic host	In Kenya collected by myself at	Occurrence in Africa[1]	Habitat[1]
Dinebra retroflexa (Vahl) Panz.	Ahero, Bunyala, Mwea	throughout tropical Africa	annual; common in black heavy seasonally waterlogged soil, in grassland, on road sides and as weed of arable land
Diplachne caudata K.Schum.	Mwea	Kenya, Uganda, eastern Zaire	perennial; banks of streams, lake shores, swamps
Eragrostis aethiopica Chiov.	Otonglo, Paponditi	throughout eastern tropical Africa	annual; grasslands, occurs frequently as weeds, especially in poor and in semi desert areas
Eragrostis ciliaris (L.) R.Br.	Paponditi	throughout tropical Africa	annual; in grasslands, rocky grounds, roadsides, old cultivations, weed of arable land
Eragrostis namaquensis Nees var. *namaquensis*	Mwea	throughout tropical Africa	annual; pools, riverbanks and seasonally innundated places in dry grasslands
Eragrostis tenella (L.) Roem. et Schult.	Kisumu	throughout tropical Africa	annual; old cultivations, weed in arable land and in waste ground
Oryza barthii A.Chev.	—	Tanzania, Zambia, West Africa from Mauritania to the Sudan Republic	annual; in shallow water and as weed in rice fields
Oryza punctata Steud.	—	throughout tropical Africa and Madagascar	annual; swampy soils by banks of streams and edges of ponds, usually in the open

1. Information derived from Bogdan (1958), Clayton (1970), Harker & Napper (1961) and Napper (1965).

Table 27. Field collected non-rice plants, tested for presence of RYMV by mechanical inoculation onto 'Sindano' rice.

Plants species	Symptoms	Number of plants tested	Number of tests	Symptoms induced
Commelina diffusa Burm. f.	mosaic	3	3	none
Crotalaria pallida Air. var. *obavata* (G.Don) Polhill	mosaic	2	2	none
Cynodon dactylon (L.) Pers.	yellows	10	3	none
Dinebra retroflexa (Vahl) Panz.	mild spotting	84	25	none
Echinochloa colona (L.) Link.	mild spotting	13	4	none
Eragrostis ciliaris (L.) R.Br.	mottling (physiological ?)	56	14	none
Eriochloa nubica (Steud.) Thell.	mild spotting	1	1	none
Leersia hexandra Sw.	mild spotting	15	4	none
Rottboellia exaltata L. f.	mosaic	1	1	none
Saccharum officinarum L.	mosaic (sugarcane mosaic ?)	5	5	[1]

1. Mosaic not resembling RYMV incited symptoms.

ship to RYMV was established. Sugarcane mosaic virus is thought to be responsible for the affection of the sugarcane. The other plants were mostly collected on or near the bunds of the rice fields at Ahero. RYMV was not recovered in any of the cases.

7.5 Distribution and behaviour of beetle vectors

The insects tested for ability to transmit RYMV represented only a small part of the species found in and around the rice fields. Evidence was, however, obtained that the insects able to transmit RYMV from rice to rice are principally to be found in several subfamilies of the Chrysomelidae (Coleoptera) as described in Section 5.3.2 More detailed information about relations between virus, vector and host plant were obtained with *Chaetocnema pulla, Sesselia pusilla* and *Trichispa sericea*, representing the subfamilies Halticinae, Galerucinae and Hispinae. These insects were able to acquire the virus and to infect 'Sindano' rice plants in short feeding periods, while *C.pulla* and *S.pusilla* retained the virus for several days. Also it was proved, that *C.pulla* carried the virus in the field, while this beetle was also able to transmit RYMV from rice to *Dinebra retroflexa* and conversely.

In Table 28 information about the distribution of the main insects able or strongly suspected to transmit RYMV is given. Although the information is still incomplete, it indicates that the insects are wide spread over Africa and that vectors of RYMV are present in the main Kenya rice growing areas.

The behaviour of the vectors is also important for the ecology of the virus. Observations revealed the following points of interest.

Table 28. Distribution of the main beetle vectors of RYMV in Kenya and other African countries.

Insect	In Kenya collected by myself at	Incidence[1]	African countries from which insects have been reported
Sesselia pusilla Gerst.	Ahero	moderate/low	East Africa (Weise, 1924), Mozambique (Laboissière, 1931)
	Kaloka	moderate	
	Mwea	low	
	Otonglo	very high	
	Paponditi	moderate/low	
Chaetocnema abyssinica Jac.	Ahero	moderate/low	Sudan, Ethiopia, Congo, Rwanda, Tchaad (Scherer, 1972), Erithrea (Scherer, 1962a)
	Bunyala	low	
	Otonglo	low	
Chaetocnema kenyensis Bryant (?)	Bunyala	low	Erithrea (Scherer, 1962b)
	Mwea	low	
	Otonglo	low	
Chaetocnema pallidipes Fairm.[2]	Ahero	moderate	Somalia, South Africa (Cape Province, Transvaal), Tanzania, Ethiopia (Scherer, 1963)
	Otonglo	low	
Chaetocnema pulla Chapuis	Ahero	high	Sierra Leone Guinea, Ivory Coast, Congo, Rwanda, Burundi, Ethiopia (Scherer, 1972)
	Bunyala	moderate	
	Mwea	very high	
	Otonglo	moderate	
	Paponditi	moderate	
Chaetocnema pusilla Lab.[2]	Ahero	low	Congo (Scherer, 1962a)
	Mwea	low	
	Nairobi	low	
	Otonglo	low	
	Ruiru	low	
Dactylispa bayoni Gest.	Ahero	occasionally high	Uganda, East Africa, Congo, Rwanda, Burundi, South Africa (Uhmann, 1958)
	Otonglo	low	
Dicladispa (*Chrysispa*) *paucispina* (Weise)	Nairobi	low	Central Africa, Fr.Guinea, Nigeria, Ivory Coast, Togo, Cameroon, Congo, Rwanda, Ethiopia (Uhmann, 1958), Angola (Uhmann, 1964)
	Otonglo	low	
Dicladispa (*Chrysispa*) *viridicyanea* (Kraatz)	Otonglo	low	Gaboon, Congo, Cameroon (Uhmann, 1958)
Trichispa sericea (Guérin)	Ahero	low	Tanzania (Mwanza (*Hispa sericea* Guérin), Nye, 1960), Madagascar, Zanzibar, Br.East Africa, Uganda, Congo, Ethiopia, Sudan, Natal, Swaziland, South Africa, Angola, Fr.Guinea (Uhmann, 1958)
	Mwea	at times very high	

1. As observed in collections mostly made with a net during day-time; very high: regularly more than 100 insects collected in a single catch, high: regularly 50 – 100 insects collected in a single catch, moderate: regularly up to about 50 insects collected in a single catch, low: seldom or regularly only in low numbers collected.
2. Suspected vector.

S.pusilla drops when in danger. On the ground the beetle will climb a plant, mostly a grass or a sedge and move upwards. From this higher position they may fly off. The insects do not stay for long periods on the same plant, although on the large blooming flowers of *Cyperus* spp. considerable longer than on a small grass flower. Here they eat and move around for some time and fly then to another plant. Most *S.pusilla* were collected on plants near water, e.g. in slightly wet ratoon rice fields or on blooming *Cyperus* spp. The insects proved to be phototrophic. In the laboratory when placed on rice seedlings, the beetles flew off to another plant after short feeding periods. Insects starved for a long period (24 hours or longer) or which had fed on blooming *Cyperus articulatus* flowers for a considerable time, were attracted by the fluid arising from damaged rice plants. Treated insects placed on a wet filter paper seemed to take up the water here too.

Being a halticid, *C.pulla* often leaps over short distances. Large numbers of this insect were collected on young grasses, especially *Echinochloa colona* and ratoon rice, both found on humid places. This beetle also proved to be phototrophic and in the laboratory the insects stayed only for short periods on the same plant. In the laboratory it was also noted, that this insect was considerably more active in the warmer than in the cooler period of the year.

In the field very high numbers of *T.sericea* were found on rice, both on young and older leaves, which because of severe feeding damage were turning yellow and dry. In the laboratory, after having found a rice plant to feed upon, this beetle stayed for considerable periods on the same leaf and than moved to another leaf of the same plant or went to another plant.

7.6 Conclusions

In the preceding chapters it was concluded that RYMV is a stable virus, both inside and outside the plant. In certain rice varieties like 'Sindano', the virus reaches a very high concentration and spreads rapidly through the plant. For other hosts, like *Dinebra retroflexa* as well as for some other rice varieties like. 'Basmati 217', higher minimum levels of inoculum are needed for an infection, or spread in the plant is less rapid as for 'Sindano'. In the field most rice plants survive an infection by the pathogen.

7.6.1 Sources of RYMV and its spread to rice

Under natural conditions the virus was only found in rice. The results of the host range studies must therefore be used to indicate the most likely sources of infection. By assuming, that (1) the virus was present around Lake Victoria before rice cultivation was introduced here and (2) the systemic host is to be found in the family Gramineae, the source of RYMV is likely to be one or more species belonging to the tribes Oryzeae and Eragrostideae. Because of the apparently narrow host range of the virus and the breaks in growing seasons, a perennial is more likely than an annual.

The host must also be able to can withstand a rise and fall in water level for the areas where the disease is endemic. Of the grasses reported around Lake Victoria in the floras (Bogdan, 1958; Clayton, 1970; Harker & Napper, 1961; Napper, 1965), there are some which meet these conditions, such as *Diplachne caudata* (Fig. 37). This grass proved to be a systemic host for the virus. An annual host like *Oryza punctata*, if it is able to survive the dry period like rice can, however, not be excluded totally. Grasses like these could well have been the prime source of RYMV.

RYMV may have spread from one of these possible hosts to rice direct or via other

Fig. 37. *Diplachne caudata* K. Schum. ($\frac{1}{5}\times$).

hosts, which do not necessarily have to be perennials or confined to swampy areas. These plants could be *Dinebra retroflexa* (Fig. 38), *Eragrostis aethiopica*, *E.ciliaris* and *E.namaquensis* var. *namaquensis* (Fig. 39).

When the pathogen was introduced in a very susceptible rice variety like 'Sindano', a basic condition for further spread in this crop (a high inoculum potential) was established.

The ways in which the virus spreads from the wild host(s) to rice, direct or via other wild hosts, are still more difficult to explain. Viruses transmissible by beetles are mostly transmitted by a number of different beetle species e.g. cowpea mosaic virus by several galerucids (Jansen & Staples, 1971), but also by other insects with biting mouth parts as for certain turnip viruses (Martini, 1958). Both facts proved to be the case for RYMV too. In addition there is a considerable lack of knowledge of the insect fauna of the grasses near Lake Victoria. From the efficiency in transmitting RYMV, primarily beetles are thought to be responsible for the spread of the virus from the wild host(s) to rice. During this spread different beetle species may act as transmitting agents of the virus from one plant species to another plant species.

Beetles able to transmit RYMV and regularly collected on the wild vegetation included several *Chaetocnema* spp., *Sesselia pusilla*, several Hispinae and *Monolepta* spp., while others like the insects of a genus near *Apophylia*, *Oulema dunbrodiensis* f. *nigripennis* and *Cryptocephalus* spp. were less regularly collected. All of them and possibly many others may play a role in the spread of the virus from the wild host(s) to rice, although the insects which were present in high numbers are likely to be of more importance than insects which were less abundantly present. *C.pulla* has been collected in substantial numbers at several places in the Kano Plains, while *S.pusilla* was mostly confined to areas near the shores of Lake Victoria. Only a few *T.sericea* were caught here, but Nye (1960) noted damage caused by this insect to rice near Mwanza in Tanzania.

Little is known about the food plants of these insects. From the hosts on which the insects were collected, is clear that the *Chaetocnema* spp. may feed on several grasses. *C.pulla* was observed feeding on *Dinebra retroflexa*, while in captivity this insect was able to transmit RYMV to and from this plant to rice. Although *S.pusilla* was frequently found on the blooming flowers of *Cyperus* spp. and grasses, the gready eating of the leaves of rice and other Gramineae in the laboratory, justifies the assumption that this will happen in the field too. *T.sericea* is known to feed preferably on rice, but in Swaziland this insect feeds also on *Eragrostis aethiopica* (Ministry of Overseas Development, 1970) (a systemic host of RYMV). *Monolepta* spp. appeared to feed mainly from pollen of *Cyperus* spp. In captivity they caused less damage to rice than *S.pusilla*.

7.6.2 The spread of RYMV in the rice crop

Facts of prime importance for this subject are:

- the incidence of RYM in rice was considerably higher and earlier noted in areas

Fig. 38. *Dinebra retroflexa* (Vahl) Panz. ($\frac{3}{5}\times$).

Fig. 39. *Eragrostis namaquensis* Nees var. *namaquensis* ($\frac{1}{4}\times$).

where rice was present throughout the year, than in areas where no rice had been present for some time;
– no non-rice hosts of the virus were found to be naturally infected by the virus, even when growing next to diseased 'Sindano' plants, like *Dinebra retroflexa*.

These facts provide strong evidence that in the rice areas, rice itself must be the main source of infection for this crop.

When rice is transplanted, the seedlings infected in the nursery of which it seems there are only a few, are spread over the whole field. The fact that the disease initially spreads faster near the edges of a large field than in the centre indicates that:
– the virus sources are also present outside the field; rice on the bunds, in the irrigation ditches and in nearby ratoon or earlier planted fields may act as such;
– the vectors do come from the surroundings of the field.

As described in Chapter 5, throughout the year *Chaetocnema* spp., especially *C.pulla* were collected in substantial numbers at the Ahero Pilot Scheme. Here *S.pusilla* was caught in low numbers and at times not at all on the plants on the bunds and in the ditches around the rice fields. At Otonglo *S.pusilla* was always present while *Chaetocnema* spp. were collected too close to the rice fields. At times members of the Hispinae were present here and there in substantial numbers in the Ahero schemes, but large numbers of this subfamily were never collected at Otonglo. *Chaetocnema* spp. are therefore thought to be of main importance in the spread of RYMV from rice to rice. In addition, *C.pulla* proved to carry the virus in the field. *C.pulla* does not stay for long periods on the same plant and normally moves over short distances. This may explain the spread of the disease from the edges of the field and the initial patchy occurrence of the diseased plants.

The role of *S.pusilla* in the spread of RYMV in the rice fields cannot yet be explained. It is less likely that this insect will be of the same importance as *C.pulla*, because of its habit of feed upon blooming flowers of *Cyperus* spp. and grasses. The fact, however, that *S.pusilla* appeared to take water after prolonged feeding on *Cyperus* flowers, indicates that this insect probably does not only feed on these flowers.

The Hispinae are not regarded as having played a major role in the spread of the virus in the rice field as yet.

Apart from transmission of the virus by insects, during certain stages of the rice cultivation, the possibility of mechanical infection exists. This could occur at transplanting, especially when the leaves and roots of the seedlings are trimmed, during weeding and at harvest, then causing an increase of infected ratoon plants. Because of the low percentage of infected plants, while the virus concentration has not yet reached a high level, it is not likely that this will happen in the field on a large scale at transplanting. The danger of infection during weeding is also not considered to be high, because of the distances in the planting system and the dilution of the virus by water. A certain amount of transmission of the virus by contact, caused by wind or otherwise, is possible. In the laboratory no evidence was obtained of infection by the water in which the plants grew. The practice to allow a slight streaming of water in the fields, also keeps the chances of infection by the irrigation water low.

7.6.3 Chances of spread of RYM to other rice growing areas

From the Tables 26 and 28 it is seen that both at Bunyala and at Mwea possible natural hosts and vectors are present. The situation at Mwea with high numbers of *C.pulla* and *T.sericea*, a number of systemic hosts (perennial as well as annual) and 'Sindano' as main rice variety, gives cause for concern. If the virus is introduced in this area, it will spread quickly, and it will not be possible to eliminate the disease.

To explain why RYM has not yet spread to these areas, it may be assumed that the area where the disease occurs is ecologically isolated. The Kano Plains and the Lake Victoria shores in Kenya are enclosed inland by areas of higher altitude (Fig. 3). Furthermore to reach Mwea, the Rift Valley (a steep and wide depression) and the Aberdares, a mountain range, must be crossed. Before the Ahero Pilot Scheme was initiated, rice cultivation was limited to a small acreage, spread over several areas. In the area between the Kano Plains and Mwea, vectors and possible natural hosts in the Eragrostidae, Bromeae and Phalaridae are likely to be present. With the enlargement of the area under rice, the chances of spread over large distances will increase. For Bunyala, which is much closer to Ahero and more readily accessible than Mwea, an infection by natural spread of the virus is therefore earlier to be expected.

A more imminent danger is the introduction of diseased plant material into these schemes, or the spread being quickened by smallholders using seedlings from infected areas. Transfer of seedlings from one rice area to another is very likely to accur within short distances initially, but by increased contacts between the Ahero Pilot Scheme and Mwea, it is higly possible that the disease with be introduced here too.

From the still limited knowledge of the ecological aspects of RYM, in general it can be stated that in other rice growing African and overseas countries, vectors and likely non-rice hosts of the virus are to be found.

8 Considerations for the control of RYM

To control RYM the disease should be controlled in the affected area and its spread over large distances should be prevented. An understanding of the disease and its pathogen and co-ordinated actions between the parties involved in the development of rice production is absolutely necessary.

8.1 Ways to control the disease in the affected area

Direct measures against a virus in an affected plant does not exist, therefore indirect measures must be applied. These methods involve ways directed towards prevention of infection by eliminating or decreasing the number of infection sources, limiting the spread of the virus and so reducing the effects of an infection on yield.

Indications have been obtained that RYMV may be present in grasses belonging to the tribes Eragostideae and Oryzeae. Even when it is known exactly which plants are the sources of RYM, elimination of these plants will hardly be feasible. In the areas themselves, infected ratoon rice and plants grown from dropped seeds which later were infected, are a main source of infection. Methods must therefore be devised to eradicate the stubble and sanitation must be improved, together with a reduction in the time lapse between planting the first and the last fields. The aim is to have a closed season for rice of maximum duration. Therefore when two crops of rice are grown annually, selection of a rice variety with short vegetative periods is to be preferred. By changing over to one crop of rice a year, more time will be available to destroy the stubble, while the closed season will be extended. If, however, the fields only are rotated when preparing for a new crop, as done at Mwea, this will be of little use for control of the disease.

The use of insecticides for control of the vectors is generally the first method suggested by those dealing with the practice of rice growing. In the case of RYM it should be realised, that:

- apart from *T.sericea*, in large numbers, the vectors themselves do not cause serious damage to the rice plants;
- apart from those Hispinae that prefer rice as food plant, the vectors move regularly between the rice in the field and the surroundings;
- too little is known of the biology of most vectors of RYMV. Very likely certain stages in the development of these insects are passed in the ground;
- transmission of the virus can be effected in short feeding periods of the vectors.

Together with the disadvantages of insecticides viz. development of resistance, its

influence on the environment and higher chances of outbreaks of secondary pests caused by the destruction of their natural enemies, its effects on the control of RYM may be limited. Some of the dangers of insecticides against the vectors of RYMV in the area near Lake Victoria are:

- For reasons of economy, DDT – notorious for its persistence – is likely to be chosen, as is the case already for control of *T.sericea* at Mwea.
- To be effective in limiting the spread of RYM in the rice field, repeated applications must be given, especially in the first stages of development of the rice plants. Spraying should not be limited to the rice fields only, but should also include the bunds and verges of the roads. When aerial application is selected, the risk for man and cattle in the schemes is even greater.
- The irrigation water is also used for human and animal consumption, while fish are caught in the irrigation ditches.
- After having been used for irrigation of the rice fields, excess water streams into Lake Victoria. This lake is the main drinkwater supply for a large population, provides water for the River Nile, and fish are caught here.
- By applying DDT natural enemies of the mites may be destroyed as noted in the control of Hispinae in Madagascar in the case of *Steneotarsonemus madecassus* (Gutierrez, 1967). At Otonglo *Steneotarsonemus spinki* was collected on rice.

It is not easy to create circumstances which do not favour the presence of the vectors nearby the rice fields. This would mean no plant growth, especially no grasses on the bunds. However the size of the bunds at the APS, even when allowing for shrinkage, are unnecessarily large. Apart from high costs during construction, this situation also creates an unnecessary amount of vegetation, resulting in extra work in keeping the vegetation low. Ways of reducing the vegetation on the bunds for existing and future rice schemes should be studied.

No evidene has been obtained, that a change in the time of planting would result in less RYM affected plants. In addition it is practically impossible to change the time of planting because two crops of rice are grown a year in the irrigation scheme, and the rice has to ripen at times when there is little likelihood of rain. Even if only one crop of rice is grown annually, the time difference between the first and last plantings of the rice should be limited.

Generally one of the most efficient ways to reduce the effects of a virus disease in a plant, is breeding for resistance or immunity. This however takes considerable time. Therefore refined techniques for screening rice varieties by laboratory methods and by field trials have to be developed first.

Up to now all rice varieties mechanically inoculated with slightly diluted sap proved to be susceptible. However two properties of the rice varieties with regard to exposure to RYMV seem to be of importance in indicating differences in resistance. First the time when the symptoms are visible after inoculation; this property, however, also depends on the growth habitat of the rice variety. Secondly the differences in the dilution end-point of the virus in the varieties. If inoculation, by a paint spray apparatus for example, would be possible, it should not be difficult to devise a standardized

method. When these two properties are compared with those obtained with standard varieties like 'Sindano' and 'Basmati 217', together with the results of the field trials, a relationship may be established.

Observations on varietal resistance in rice to RYMV are made at the Irrigation Research Station Ahero, where incidence of RYM is high. Mostly the information is obtained from experiments not set up for this purpose. In addition insecticides are applied, the use of which is not advisable in this type of experiments. Furthermore the creation of a source of RYM in the centre of the APS should not be allowed if a serious attempt to control RYM in this scheme is to be made. It would therefore be better to screen rice varieties for RYM resistance at another place where the disease occurs, for example at Otonglo.

On an experimental scale a low incidence of RYM was noted in some rice varieties such as 'Madevu', 'Milfor 6 (2)' and 'Portuguese'. Occasionally, however, in these varieties a plant was attacked at a young stage, and the low incidence of the disease may reflect a preference of the vectors for other rice varieties. When these varieties are planted on large scale, the vectors have no choice, and possibly a seasonal increase in the amount of affected plants occurs.

Breeding for resistance to RYM or to the vectors of the virus is a long-term project. In addition blast and borers have to be considered too. The chance is small that in the screened varieties a variety will be found showing resistance to the diseases and pests, while being suitable for the area. In the host range studies of the virus, some *Oryza* spp. have been assigned which only became locally infected. Crossings of *Oryza* spp. generally give problems. But the value of this work has been demonstrated in crossings of an accession of *O.nivara* and rice 'IR 661–1–140' for grassy stunt resistance by breeders of the International Rice Research Institute (Anononymus, 1970). A recently named variety bred at this institute 'IR 26', has been claimed to be resistant or moderately resistant to most major insects and diseases of rice in tropical Asia (Anonymus, 1973b). The actual and potential acreage of rice in East Africa make it sufficiently worthwhile to pay attention to this aspect of rice development in this part of the world.

8.2 Prevention of spread of RYM over large distances

Before the first rice was planted in the APS, directions to prevent accidential spread of the disease to other not yet affected rice areas, were issued. The advised measures included no transport of rice seeds and seedlings from the affected area to other rice growing areas. The danger of transport of seedlings from one area to another area have been discussed. When new and better rice varieties are introduced, this danger is imminent. Although no evidence of seed transmission of the virus has been demonstrated, the fact that the virus survives for long periods in dry green plant material, makes it quite likely, that the virus is present in bags with unwinnowed seed. Another danger is the spread of the white-tip nematode. Therefore it is better to carry out these directions. Seeds, preferably certified, of new rice varieties should be made

available to smallholders on easy terms. These seeds should come from areas where the named diseases are not present, like Mwea. Due to difficulties in milling rice of the variety 'Basmati 217' at Kisumu, seeds from the APS have been brought to the mill at Mwea. This situation also means increased communication between the two rice schemes, which therefore raises the chance of earlier introduction of the virus at Mwea. Burning of the debris of the milled seeds from Ahero is recommended. Milling locally, however, is to be preferred.

9 General discussion

9.1 Characterization and identification of RYMV

Viruses affecting Gramineae often induce symptoms which are quite similar in the same host (Carr, 1968; Huth, 1972; Slykhuis, 1962). Comparison of RYMV with these and other viruses affecting non-Gramineae, to detect an affinity, must therefore be based on the biological and intrinsic properties of the viruses.

A summary of the most important properties of RYMV shows, that it is a RNA-containing virus with isometric particles about 25 nm in diameter. It is readily transmissible by inoculation with sap. Insects with biting mouth parts, especially many species of chrysomelid beetles, are able to transmit the virus. Only a limited number of Gramineae, mainly in the tribes Oryzeae and Eragrostideae have been recorded as host plants for RYMV. A systemic host in which symptoms are induced is also *Phleum arenarium*, while grasses in which infection remains localized include *Bromus hordeaceus* and *Setaria viridis*. In rice the virus provokes a discolouration of the leaves, stunting and reduced tillering of the plant and sterility of the flowers.

Other important properties of RYMV are its stability, its high concentration in sap, its sedimentation behaviour and it contains single-stranded RNA. High concentrations of the virus occur in mesophyll cells of young affected 'Sindano' leaves. Serologically no relationship was established between RYMV and many other isometric viruses.

These properties allow to consider RYMV as a stable virus and to classify it as a small RNA virus; i.e. a virus with a particle diameter of 30 nm or less and consisting of protein and single-stranded RNA (Brown & Hull, 1973).

The cryptogram (Gibbs, 1968) for RYMV is: R/1:1.4/23:S/S:S/Cl.

The influence of ionic strength, pH and temperature on the composition and structure of RYMV were not studied in detail, although minor changes in these conditions may markedly affect the stabilizing interactions in viruses (Brown & Hull, 1973; Kaper, 1972). RYMV was normally suspended in 0.01 M phosphate buffer pH 7.0, in which the virus remained infective for a long time.

Viruses transmitted by beetles have many properties in common. Usually they are relatively stable, develop a high titre in infected plants, can be readily transmitted by mechanical inoculation, have spherical or polyhedral particles from 25 to 30 nm in diameter and are highly antigenic. Host ranges and symptoms are usually not correlated with any specific features of particles or with their vectors (Walters, 1969). These properties apply for RYMV as well.

The data obtained for the RYMV particles and their RNA, in comparison to other

small RNA viruses, belonging to groups (Harrison et al., 1971; Brown & Hull, 1973) in which beetle-transmitted viruses occur, or to other beetle-transmitted viruses not yet grouped, are shown in Table 29.

Based on size, shape, sedimentation properties, nucleic acid content and base composition, some similarity between RYMV and cocksfoot mottle virus (CFMV) can be deduced. However, the absence of a serological relationship and differences in host range (Serjeant, 1967) provides evidence that RYMV and CFMV are two distinct viruses. Further investigations into the degree of affinity between the two viruses is necessary.

Phleum mottle virus (PMV), the properties of whose particles and their RNA is still not completely known, differs in host range from RYMV, while no serological relationship could be established. For PMV, which is also not related serologically to CFMV (Serjeant, 1967), several hosts in the tribe Agrostideae of the family Gramineae have been recorded (Catherall, 1970), while barley can also be infected (Benigno & A'Brook, 1972). *Phleum arenarium* is a host for PMV and RYMV, but *Apera spica-venti*, *Phleum bertolonii*, *P.pratense* and barley are hosts for PMV only.

Other mechanically transmissible viruses of similar shape and comparable size are, as far as information is available, also readily distinguishable by their host ranges, absence of serological relationships, properties of the particles and their RNA or by the groups of vectors which transmit the virus. This applies, among others, for brome mosaic virus – claimed to be nematode transmitted (Bancroft, 1970), but also for cocksfoot mild mosaic virus – transmitted inefficiently by the aphid *Myzus persicae* (Huth & Paul, 1972); *Lolium* mottle virus (A'Brook, 1972); strains of tomato black ring virus (Carr, 1968) and Peru corn virus (Hebert, pers. commun., 1973). Too little information about ryegrass spherical virus, which is seed transmitted in Italian ryegrass 'S.22' (Plumb, 1973), is available to permit conclusions.

RYMV is therefore considered to be distinct from all other known viruses and can not be classified in the present groups of plant viruses in which affinity between members exists.

9.2 Fine structure of RYMV-infected mesophyll cells

The effects of a RYMV infection on the fine structure of mesophyll cells of a rice leaf show resemblance to the patterns observed with other small isometric viruses in their hosts. Clear differentiation between the ribosomes and virus particles is possible only if the virus particles have crystallized. RYMV was often observed in densily packed accumulations, resembling structures as observed by Milne (1967) with sowbane mosaic virus in *Chenopodium amaranticolor* leaves. A treatment which promotes crystallization of the virus particles, like wilting (Milne, 1967) has not been studied, but may be of use in further studies. RYMV was not observed in the nuclei and chloroplasts, nor was a vesiculation noticed as (among others) with pea enation mosaic virus (de Zoeten et al., 1972) or with turnip yellow mosaic virus (Hatta et al., 1973).

Nucleic acid-like fibrils were often observed in RYMV-infected mesophyll cells. Fibrils in the cytoplasm were also noticed by Milne (1967) and in vesicles induced

Table 29. Properties of virus particles and RNAs of isometric viruses or groups of such viruses with beetle vectors.

	Virus particles				RNA						
	number of sedimenting components	S values	diameter (nm)	RNA (%)	number of species	mol.weight $\times 10^{-6}$ (D)	base composition[1] G	A	C	U	reference[4]
Group											
turnip yellow mosaic virus	2	116,53	28	34,0	1	2.0	17	22	38	22	a
cowpea mosaic virus	3	115, 95, 58	28	32, 23, 0	2	2.6, 1.5	21	28	19	32B	a
							23	29	17	31M	
brome mosaic virus	1	87 (79)[2]	26	22	4	1.09, 0.99, 0.75, 0.28	28	27	21	24	a
tomato bushy stunt virus	1	132	30	17	1	1.6	29	26	21	26	a
Virus											
southern bean mosaic virus	1	115	28.5	21	1	1.4	27	23	23	27	a
turnip rosette virus[3]	1	112	28	—	—	—	25	26	22	27	b
cocksfoot mottle virus	1	118	30	25	—	—	27	23	26	24	c
Phleum mottle virus	1	112	30	—	—	—	—	—	—	—	c
rice yellow mottle virus	1	109	25	23	1	1.4	29	21	25	24	d

1. B: bottom component, M: middle component.
2. Members of the bromo group sediment more slowly above pH 7.
3. Probably transmitted by biting insects.
4. a: Brown & Hull (1973), b: Hollings & Stone (1973), c: Paul & Huth (1970), d: this study.

by cowpea mosaic virus (Assink, 1973), where RNA replication of the virus is associated with the vesicular membranes.

The inclusions with an electron-dense centre which were observed in diseased tissue only, showed some resemblance, although they were larger in size, to the electron-opaque bodies in the mitochondria in bean leaves infected with bean-pod mottle virus (Kim & Fulton, 1972).

Cell-wall projections into the cytoplasm have been noticed in cells of plants infected with a number of isometric plant viruses (Jones et al., 1973). The tubules noticed in RYMV-infected leaves were situated in the cytoplasm and appeared not to be associated directly with the cell wall. The diameter of the tubules appeared too small to contain virus particles, but often particles whose nature was not discovered, were noticed along or in line with these tubules. Further studies are required to provide more detailed information of the subcellular effects of a RYMV infection.

9.3 Transmission of RYMV by beetles

Most beetles that transmit viruses have been found in the family Chrystomelidae of the superfamily Chrysomeloidea and only a few in the superfamily Curculionoidea. The subject has been reviewed by Selman (1973) recently.

Virus transmission by insects with biting mouth parts immediately after acquisition feeding, is commonly explained by an effective dose of virus on contaminated mouth parts or in faeces entering partly damaged cells (Selman, 1973; Tinsley, 1973; Walters, 1969). Viruses retained by beetle vectors for prolonged periods of time have long been thought to be transmitted through regurgitation of fluids 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 haemolymph of chrysomelid beetles, so acting as a virus reservoir from which the virus can be transmitted at intervals as quoted by Selman (1973). This author stated that the differences between the infectivity of different species of chrysomelids feeding in the same host indicates that the determining factors in the mode of transmission remain to be discovered.

Because the beetles used in the RYMV transmission experiments could not be bred under controlled conditions, they were collected in the field, so that the relationship between virus and vector could not be explained precisely. The differences in the capability of some beetle species to infect rice are marked however. Although the experiments were not performed under strictly the same conditions, and large differences in the indivual experiments were noted, the few insects of a genus near *Apophylia* showed 100% transmission at 1 insect/test plant. For *Sesselia pusilla* this figure was about 50%, for *Trichispa sericea* and *Chaetocnema pulla* about 25%.

In virus transmission experiments, often the viruses were transmitted better with

small numbers of beetles feeding for short periods, than with large numbers of beetles feeding for long periods which seriously damage the plant (Campbell & Colt, 1967 (in Selman, 1973)). In the RYMV experiments those beetles, about whose relationship to the virus more information was obtained, generally acquired the virus faster, than they were able to infect a rice seedling. The amount of infected rice plants appeared to depend partly upon the appreciation of the beetles to accept rice as a food plant in general and possibly upon other not yet known factors, but also on feeding behaviour and type of feeding damage. In feeding behaviour there was a clear difference between feeding for prolonged periods on one area of a single leaf, as done by *T.sericea*, or for short duration on different spots on the leaves or on other parts of the plant, as performed by *S.pusilla*, the insects of a genus near *Apophylia* and *C.pulla*. The feeding damage by *C.pulla* and *T.sericea* consisted of clean-cut incisions, while *S.pusilla* and also the insects of a genus near *Apophylia*, caused frayed damage. *S.pusilla* was a fast feeder, *C.pulla* and *T.sericea* fed more slowly.

Insects, which after having damaged many cells partly in a short feeding period move to another plant, are therefore considered to be the most efficient vectors of RYMV. Thus *S.pusilla*, when feeding on rice in the field, could cause a vast spread of the virus. *C.pulla* appears to be a less efficient vector. The large numbers of this insect may favour a vast spread as has been seen in the variety 'Sindano' at the Ahero Pilot Scheme. In addition other *Chaetocnema* spp. which are suspected to be able to transmit RYMV in the field too, may play an important role.

S.pusilla and *C.pulla* retained the virus for several days, while the retention period of *T.sericea* appeared to be limited to a shorter period. It remains uncertain whether this difference has to be explained by assuming that *T.sericea* loses the virus faster than the other species, or that other basic differences in the mode of transmission are involved.

In general it can be stated that with the large numbers of several species of chrysomelid beetles that are able to transmit RYMV in and around the rice fields, a vast spread of the virus in a susceptible variety is to be expected when a virus source is present.

9.4 Control

The results of this study provide basic knowledge about RYMV, a virus which can seriously affect rice of many varieties. The ecological aspects teach us that intensive cultivation of rice favours a quick spread of the virus if virus sources are present. Because of the trend towards continuous cropping of rice, RYMV has to be taken very seriously.

Differences in susceptibility between the rice varieties 'Sindano' and 'Basmati 217' and the grass species *Dinebra retroflexa* has been demonstrated by the more diluted inocula which still caused infection in 'Sindano' but not in 'Basmati 217' and *D. retroflexa*. In the virus transmission experiments with *S.pusilla*, *C.pulla* and *T.sericea*, much lower numbers of infected seedlings were recorded in 'Basmati 217' than in 'Sindano'. Although these experiments should be considered as preliminary, they

indicate that differences in susceptibility to RYMV, and not differences in resistance of the varieties to the beetles are involved. The fact that higher levels of inoculum are needed to infect *D.retroflexa*, possibly combined with a lower appreciation of the beetles to accept this grass species as food plant, may explain why this species was not found to be naturally infected with RYMV.

In several *Oryza* spp. the infection with the virus proved to be restricted to the inoculated leaves only. These species may be of value in future breeding programmes. An early start with a screening and breeding programme to produce resistant varieties has to be undertaken in East Africa, for which co-operation with the International Rice Research Institute in the Philippines seems appropriate.

Summary

(1) Rice yellow mottle virus (RYMV) is the causal pathogen of yellow mottle of rice (RYM) and occurs in the area around Kisumu near Lake Victoria in Kenya. The characteristic symptoms of RYM are a yellow or orange discolouration of the leaves, stunting and reduced tillering of the plant, and sterility of the flowers.

(2) This study was aimed to characterize RYMV and to obtain information about its ecology to provide a basis for the development of appropriate methods for control.

(3) RYMV is readily mechanically transmissible to rice. Other hosts were found in the family Gramineae only, mainly in the tribes Oryzeae and Eragrostideae. Systemic hosts in which symptoms are induced are: *Phleum arenarium* L. (Phalarideae); *Dinebra retroflexa* (Vahl) Panz., *Diplachne caudata* K.Schum., *Eragrostis aethiopica* Chiov., *E.ciliaris* (L.) R.Br., *Eragrostis namaquensis* Nees var. *namaquensis* (Eragrostideae); *Oryza australiensis* Domin, *O.barthii* A.Chev., *O.brachyantha* A.Chev. et Roehr., *O.glaberrima* Steud., *O.nivara* Sharma et Shastry, *O.punctate* Steud., *O. ridleyi* Hook f., *O.rufipogon* Griff. and '*O.spontanea*' (Oryzeae).

A systemic host which develops no symptoms is *Eragrostis tenella* (L.) Roem. et Schult. (Eragrostideae).

Hosts from which the virus was regularly recovered from the inoculated leaves only are: *Bromus hordeaceus* L. (Bromeae); *Eragrostis chapelieri* (Kunth) Nees, *E.ciliaenensis* (All.) Lut., *E.macilenta* (A.Rich.) Steud., *E.tef* (Zucc.) Trotter (Eragrostideae); *Oryza alta* Swallen, *O.eichingeri* Peter, *O.grandiglumis* (Doell) Prodh., *O. latifolia* Desv., *O.minuta* C.B.Presl, *O.officinalis* Watt (Oryzeae); *Setaria viridis* (L.) P.Beauv. (Paniceae).

Irregular recovery of RYMV from the inoculated leaves only occurred with: *Dactyloctenium aegyptium* (L.) P.Beauv., *Eleusine coracana* (L.) Gaertn., *Eragrostis aspera* (Jacq.) Nees, *E.pilosa* (L.) P.Beauv. (Eragrostideae).

(4) Apart from sap of infected rice plants, the virus was also recovered from their guttation fluid and from standing irrigation water in a field with ratoon rice.

(5) Although all rice varieties tested were severely affected when inoculated with RYMV at a young stage of development, differences in susceptibility between the rice varieties exists. This was most pronounced when inoculation took place at increasing age of the plants. 'Sindano' rice proved highly susceptible, 'IR 22' perhaps slightly less than 'Sindano', while 'Basmati 217' at this stage was much more resistant and being infected showed fewer severe symptoms and less reduction in seed yield.

(6) Insects with biting mouth parts, mainly chrysomelid beetles commonly encountered in and around the rice fields, transmitted the virus. The following insects

were able to transmit RYMV from infected 'Sindano' to 'Sindano':
- Coleoptera, Chrysomelidae: *Oulema dunbrodiensis* Jac. f. *nigripennis* Hze. (Criocerinae); *Cryptocephalus* sp. ? *chalybeipennis* Suffr., *Cryptocephalus* sp. ? W – *nigrum* Suffr. (Cryptocephalinae); insects of a genus near *Apophylia*, *Monolepta flaveola* Gerst., *M.haematura* Fairm., *Sesselia pusilla* Gerst. (Galerucinae); *Chaetocnema abyssinica* Jac., *C.kenyensis* Bryant (?), *C.pulla* Chapuis, mixture of *Chaetocnema* sp. with *C.pallidipes* Fairm. (Halticinae); *Dactylispa bayoni* Gest., *Dicladispa* (*Chrysispa*) *paucispina* (Weise), *D.* (*C.*) *viridicyanea* (Kraatz) and *Trichispa sericea* (Guérin) (Hispinae).
- Orthoptera, Tettigonidae: *Conocephalus merumontanus* Sjöstedt.

(7) An insect strongly suspected to be able to transmit RYMV is: Coleoptera: Chrysomelidae: *Chaetocnema pusilla* Lab. (Halticinae).

(8) *C.pulla* proved to carry RYMV in the field, while in experiments this insect was able to transmit RYMV from rice to *Dinebra retroflexa* and conversely.

(9) Insects of a genus near *Apophylia* and *S.pusilla* were the most efficient vectors of RYMV, while the *Chaetocnema* spp., *T.sericea* and the other Hispinae generally caused lower percentages of infection.

(10) *S.pusilla*, *C.pulla* and *T.sericea* were able to acquire the virus when left for 15 min on diseased rice 'Sindano'. Although the insects were also able to infect a 'Sindano' seedling in 15 min, in general the beetles appeared to acquire the virus faster than they were able to infect a plant. *S.pusilla* and *C.pulla* were able to retain the virus for 8 and 5 days respectively, often causing infection of the rice plants on several consecutive days, while *T.sericea* retained the virus for one day only.

(11) After acquisition feeding on RYMV-infected 'Sindano', *S.pusilla*, *C.pulla* and *T.sericea* infected more 'Sindano' and 'IR 22' seedlings than 'Basmati 217' seedlings.

(12) Transmission of RYMV by seed was not found.

(13) RYMV is a stable virus. When tested on 'Sindano' and depending on the source of inoculum, sap from RYMV-infected plants has a dilution end-point of $10^{-6} - 10^{-9}$. Most infectivity was lost by heating sap at 65°C for 10 min. Sap diluted with 0.01 M phosphate buffer pH 7.0 and stored at room temperature was still infectious after 99 days, but not after 120 days. When stored at 4°C, this sap was still infectious after 260 days. Infected 'Sindano' leaves, cut in small pieces and stored above $CaCl_2$ at 4°C, still proved to be infectious after one year, the longest time tested. Inoculum prepared from young leaves from plants which were dried at room temperature still proved to be infective 155 days after harvest.

(14) RYMV is easy to purify from infected 'Sindano' leaves by clarification of strongly diluted sap with chloroform and precipitation of the virus with ammonium sulphate followed by dialysis and differential centrifugation. The yield was about 1 mg virus/g of leaves.

(15) Maximum UV absorption of purified RYMV in 0.01 M phosphate buffer pH 7.0, uncorrected for light scattering, is at 260 nm and minimum UV absorption at 242 nm. $E_{max}/E_{min} = 1.34 \pm 0.01$ and $E_{280}/E_{260} = 0.65 \pm 0.005$. $E^{0.1\%}_{1\,cm,260\,nm} = 6.5$ and $E^{0.1\%}_{1\,cm,260-290\,nm} = 4.2$ both values uncorrected for light scattering.

(16) RYMV is an isometric virus with a particle diameter of about 25 nm. In 0.01 M phosphate buffer pH 7.0, the virus sediments as a single component, $s^0_{20} = 109S$. When frozen in bidistilled water or in the above buffer, the virus sediments as 2 components. One was believed to be the structurally unaffected virus; the other faster sedimenting component (147S) was possibly due to aggregation of virus particles.

(17) RYMV contains one species of single stranded RNA with a molecular weight of about 1.4×10^6 D. The RNA constitutes about 23% of particle weight. The base composition of the RNA is: G29; A21; C25; U24. The base composition is similar but not identical to that of cocksfoot mottle virus.

(18) RYMV proved highly antigenic. No serological relationship was established between RYMV and any of several other isometric viruses tested, including cocksfoot mottle virus and *Phleum* mottle virus.

(19) When ultra-thin sections of RYMV-infected 'Sindano' leaf tissue were examined by electron microscopy, RYMV was found free in the cytoplasm of epidermis and mesophyll cells. Densely packed accumulations of RYMV were commonly observed in mesophyll cells of young leaves.

(20) Based on the intrinsic and biological properties of RYMV, it was not possible to place the virus in an established group of viruses.

(21) From the results of this study the following cryptogram for RYMV was concluded: R/1:1.4/23:S/S:S/Cl.

(22) In the field, rice was the only host found naturally infected with RYMV. Nearby diseased rice (ratoon rice, plants grown from dropped seeds, and earlier planted fields) is considered to be the main source of infection of newly planted fields.

(23) In the nursery a low percentage of seedlings proved to be infected with RYMV. After transplanting the number of infected plants increased fast, most rapidly in small fields, in large fields at a slower rate.

(24) The most important insects which contributed to the spread of the virus in the rice fields, are thought to be *Chaetocnema* spp., while in areas closer to Lake Victoria, *S.pusilla* is strongly suspected to be of importance too.

(25) Based on the results of the host range studies, other natural hosts of RYMV are likely to be species belonging to the tribes Eragrostideae and Oryzeae of the family Gramineae.

(26) By growing rice under irrigation, conditions are created which favour the growth of grasses and the presence of insects, including host plants and vectors of RYMV.

(27) In Kenya rice areas where RYMV is not present, host plants and vectors of RYMV have been found. Prevention of spread of the virus to these areas is of prime importance. Care has to be taken that RYMV is not spread by importing rice seedlings from the affected areas.

(28) The occurrence of RYMV in a relative small area only, may be explained by the fact that rice was only recently introduced and till shortly grown on a small scale in this area which is ecologically isolated.

(29) Control of RYMV must be sought in improvement of the sanitary conditions

(prevention of growth of ratoon and volunteer rice), in growing of less susceptible and sensitive rice varieties, in the creation of a closed season for rice and, especially when two crops of rice are grown annually, by selection of a variety with a short vegetative period. Control of vectors by a carefully selected insecticide is only of value when performed at an early stage of development of the rice. Then optimum results are to be expected only when the sanitation requirements have been fullfilled. A more rewarding and lasting answer to RYM and other diseases and pests affecting rice in the area is a screening and breeding programme to produce resistant rice varieties.

(30) From the still limited knowledge of the ecological aspects of RYMV, it can be stated that in other African and overseas rice growing countries, potential vectors and probable non-rice hosts of the virus are present.

Samenvatting

In november 1966 werd aan het hoofd van de planteziektenkundige afdeling van het Kenyaanse Ministerie van Landbouw een niet bekende ziekte van rijst gemeld. Deze ziekte kwam voor in de rijstvelden van klein-landbouwers nabij Kisumu aan het Victoriameer. De aanleg van een proef-irrigatieproject in de omgeving van Kisumu (Ahero Pilot Scheme) met rijst met twee gewassen per jaar als belangrijkste teelt, maakte een spoedige bestudering er van gewenst. Een mogelijke verspreiding van de ziekte naar het centraler gelegen Kenyaanse rijstgebied (Mwea Irrigation Settlement) benadrukte bovendien het belang van een onderzoek.

De ziekte veroorzaakt een gelige verkleuring van de rijstbladeren (of zoals later bleek in bepaalde rassen ook wel een oranje verkleuring), een achterblijven in de groei en verminderde uitstoeling van de plant. De zaadopbrengst wordt sterk verlaagd door het optreden van steriliteit. De resultaten van de eerste onderzoekingen werden reeds gepubliceerd (Bakker, 1970, 1971).

De ziekte, rice yellow mottle (RYM) genoemd, bleek te worden veroorzaakt door een niet eerder beschreven klein isometrisch virus, het rice yellow mottle virus (RYMV). Het virus kon gemakkelijk mechanisch worden overgebracht. Bovendien bleek een aantal kevertjes behorende tot de familie Chrysomelidae in staat het virus over te brengen.

In dit verslag worden de biologische en intrinsieke eigenschappen van RYMV beschreven. Tevens zijn gegevens opgenomen over het voorkomen van het virus in situ en wordt aandacht besteed aan ecologische aspecten van RYMV. Een zo volledig mogelijke karakterisering en kennis van de ecologie van RYMV zijn van het grootste belang voor het geven van richtlijnen ter bestrijding van het virus. Zij vormen het doel van deze studie.

In de inleiding wordt in het kort ingegaan op de thans bekende virus- en mycoplasma-ziekten van rijst. Tevens worden de te velde waargenomen symptomen bij door RYMV aangetaste rijstplanten beschreven.

Het tweede hoofdstuk behandelt aspecten van de rijstteelt in Oost-Afrika, zoals de produktiegebieden, de voornaamste ziekten en plagen, en de wijze van rijstverbouw zoals die door klein-landbouwers nabij Kisumu en in de grote irrigatieprojecten in Kenya wordt bedreven.

De veelvuldig in het onderzoek toegepaste methoden en het gebruikte materiaal zijn beschreven in hoofdstuk 3. 'Sindano', een lokaal rijstras dat in het veld sterk door het virus wordt aangetast, is als toetsplant gebruikt.

In hoofdstuk 4 is de mechanische overdracht van het virus en de gevolgen van een

infectie voor rijst en andere waardplanten beschreven. Afhankelijk van de groeiomstandigheden en de leeftijd van de met sap geïnoculeerde rijst, verschijnen bij 'Sindano' 5 – 7 dagen na de inoculatie de eerste symptomen, die uit kleine gele vlekjes op de jongste bladeren bestaan. Deze vlekjes verlengen zich evenwijdig aan de nerven en geven het blad een geel-groene vlekkerigheid. Later worden de bladeren geel en necrotisch. De groei van de plant blijft sterk achter. Jong geïnfecteerde planten sterven veelal af. De pluimen komen onvoldoende uit de bladschede, zijn misvormd en dragen veelal kleine en misvormde bloempakjes, die meestal loos zijn. Alleen in de familie Gramineae is een beperkt aantal waardplanten van het RYMV gevonden, voornamelijk in de stammen Oryzeae en Eragrostideae, terwijl *Phleum arenarium* L. (Phalarideae) eveneens een waardplant met systemische symptomen is. Lokale infectie treedt ook op in *Bromus hordeaceus* L. (Bromeae) en *Setaria viridis* (L.) P. Beauv. (Paniceae). Een volledige lijst van de gevonden waardplanten is gegeven in paragraaf 4.1.1 (tabel 5). De symptomen in de waardplanten die systemisch geïnfecteerd werden, bestaan voornamelijk uit vlekkerigheid.

Alle getoetste rijstrassen (tabel 6), vertonen symptomen van de ziekte en zijn erg vatbaar wanneer ze in een jong stadium geïnfecteerd worden. Wanneer inoculatie in een later stadium van de planten plaats vindt, is er een duidelijker verschil in gevoeligheid tussen de rijstrassen op te merken. Het ras 'Basmati 217' blijft dan minder in groei achter en de zaadopbrengst wordt minder beïnvloed dan bij 'Sindano'.

Geen overdracht van RYMV vond plaats door zaad. Wel bleek de guttatievloeistof van geïnfecteerde rijst en stilstaand irrigatiewater in een stoppelveld van rijst het virus te bevatten.

De overdracht van het RYMV door ongewervelde dieren wordt in hoofdstuk 5 behandeld. Insekten met bijtende monddelen zijn in staat het virus over te brengen. Deze insekten, veelal gevangen in of rondom de rijstvelden, zijn kevers behorende tot de onderfamilies Cryptocephalinae, Criocerinae, Galerucinae, Halticinae en Hispinae van de familie Chrysomelidae, terwijl de sprinkhaan *Conocephalus merumontanus* Sjöstedt (fam. Tettigoniidae) ook in staat blijkt het virus met een geringe efficiëntie over te brengen indien hij slechts weinig vraatschade aan de rijstplant veroorzaakt. Een lijst van insekten die het RYMV in de proeven konden overbrengen, is opgenomen in paragraaf 5.4. *Chaetocnema pulla* Chapuis (Halticinae) bleek met het virus in het veld besmet te zijn, terwijl dit insekt het virus ook kan overbrengen van rijst naar de waardplant *Dinebra retroflexa* (Vahl) Panz. (Eragrostideae). Het virus kon na een korte opname- en afgifteperiode (15 min) door enkele van de kevers overgebracht worden, hoewel een afgifteperiode van langere duur het aantal geïnfecteerde rijstzaailingen sterk verhoogde. *Sesselia pusilla* Gerst. (Galerucinae) kon 8 dagen na opname van het virus nog rijstplanten infecteren; voor *C.pulla* was dit 5 dagen en voor *Trichispa sericea* (Guérin) (Hispinae) slechts 1 dag. De vraag of er tussen de wijze van overdracht van het RYMV door deze kevers fundamentele verschillen zijn, kon niet beantwoord worden. *C.pulla*, *S.pusilla* en *T.sericea* infecteerden ongeveer tweemaal zoveel 'Sindano' – als 'Basmati 217' – planten.

In hoofdstuk 6 wordt de fysische, chemische en serologische karakterisering van

RYMV behandeld. RYMV is een stabiel virus. Sap verdund met 0,01 M fosfaatbuffer pH 7,0 was nog infectieus na 99 dagen, maar niet na 120 dagen wanneer het bij kamertemperatuur werd bewaard. Bij bewaring bij 4°C bleek het sap nog na 260 dagen infectieus te zijn. Jong geïnfecteerd blad bij 4°C boven $CaCl_2$ bewaard, bevatte na minstens 1 jaar nog infectieus virus. Inoculum bereid van bij kamertemperatuur gedroogde planten was nog infectieus na 155 dagen. Bij toetsing op 'Sindano' werd, afhankelijk van de virusbron, een verdunningseindpunt van sap gevonden van 10^{-6} – 10^{-9}. Voor het veroorzaken van een infectie waren bij 'Basmati 217' hogere virusconcentraties nodig dan bij 'Sindano'. De infectiositeit van sap ging grotendeels verloren bij verwarming bij 65°C gedurende 10 min.

Door sterk verdund sap van zieke rijstbladeren uit te schudden met chloroform en het virus te precipiteren met ammoniumsulfaat gevolgd door dialyse en differentiële centrifugering, is RYMV gemakkelijk te zuiveren. De opbrengst bedraagt ongeveer 1 mg virus/g blad. Het UV-absorptiespectrum vertoont een maximum bij 260 nm en een minimum bij 242 nm. $E_{max}/E_{min} = 1{,}34 \pm 0{,}01$; $E_{280}/E_{260} = 0{,}65 \pm 0{,}005$ en $E^{0,1\%}_{1\ cm,260\ nm} = 6{,}5$. Deze waarden zijn niet gecorrigeerd voor lichtverstrooing.

In 0,01 M fosfaatbuffer pH 7,0 sedimenteert het virus als een enkele component met een s^0_{20}-waarde van 109S. Na bevriezen en ontdooien in gedestilleerd water of in de genoemde buffer werden 2 componenten waargenomen. Eén component stelde waarschijnlijk het structureel niet veranderde virus voor, de tweede sneller sedimenterende component (147S) was mogelijk ontstaan door aggregatie van virusdeeltjes. Elektronenmicroscopisch werden vele kapotte virusdeeltjes waargenomen.

RYMV bevat ongeveer 23 gewichtsprocenten enkelstrengig RNA, dat uit 1 type bestaat, met een molekuulgewicht van ongeveer $1{,}4 \times 10^6$ D. De basensamenstelling is: guanine 29%, adenine 21%, cytosine 25% en uracil 24%. Deze basensamenstelling vertoont overeenkomst met, maar is niet identiek aan die van het cocksfoot mottle virus.

Tegen RYMV werd een antiserum (titer 1/4096) bereid. Er kon geen serologische verwantschap aangetoond worden tussen enerzijds het RYMV en anderzijds cocksfoot mottle virus, *Phleum* mottle virus en vele andere isometrische plantevirussen van ongeveer dezelfde diameter.

De diameter van de RYMV-deeltjes is ongeveer 25 nm. Hoge concentraties van het virus werden waargenomen in mesofyl-cellen van jonge systemisch geïnfecteerde bladeren van 'Sindano'. Vaak kwamen de virusdeeltjes in dichte opeenhopingen voor, terwijl in ouder weefsel een enkele keer kristallen gezien werden die mogelijk waren opgebouwd uit virusdeeltjes. Nucleïnezuurachtige fibrillen, insluitsels met een elektronendicht centrum en lange in bundels voorkomende flexibele buisjes werden regelmatig tijdens het elektronenmicroscopisch onderzoek in de epidermis- maar vooral in mesofyl-cellen waargenomen.

De resultaten van het onderzoek geven het volgende cryptogram voor RYMV: R/1:1,4/23:S/S:S/Cl. Plaatsing van RYMV in één van de huidige groepen van virussen waarbij tussen de leden overeenkomst bestaat, is nog niet mogelijk. Een eventuele overeenkomst tussen RYMV, cocksfoot mottle virus en mogelijk ook het *Phleum* mottle

virus dient echter nader onderzocht te worden.

Op de ecologische aspecten van RYMV wordt in hoofdstuk 7 ingegaan. De enige in het veld gevonden plantesoort, die was geïnfecteerd met RYMV, was rijst. De aanwezigheid van eerder geïnfecteerde rijst, zoals stoppelrijst, opslag, of eerder geplante rijst, wordt als de belangrijkste infectiebron voor de nieuw beplante velden beschouwd. Door het verbouwen van rijst onder irrigatie wordt de groei van grassen in de nabijheid van de velden, en daardoor ook de aanwezigheid van vectoren van RYMV bevorderd. In de kweekbedden was het percentage geïnfecteerde planten laag. Na het overplanten waren de eerste zieke planten na 2 – 3 weken duidelijk zichtbaar. De ziekte breidde zich toen snel uit, eerst voornamelijk langs de kanten van de velden, zodat kleine velden reeds spoedig geheel ziek waren. In grote velden liep het proces langzamer, maar ook hier konden volledig zieke velden worden waargenomen. De insekten, die het meest bijdragen tot de verspreiding van het virus zijn waarschijnlijk *Chaetocnema* spp., terwijl langs het Victoriameer *S.pusilla* mogelijk ook een rol zou kunnen spelen. In de overige Kenyaanse rijstgebieden werd de ziekte nog niet waargenomen, maar de aanwezigheid van waardplanten en vectoren van RYMV benadrukt het belang om introductie van het virus in deze gebieden te voorkomen. Een mogelijke verklaring van het feit dat het RYMV zich nog niet over grote afstanden verspreid heeft, is een combinatie van het tot voor kort slechts beperkte rijst areaal en de aanwezigheid van natuurlijke 'grenzen'. Het gebied waar de ziekte voorkomt, wordt omgeven door het Victoriameer en hoger gelegen gebieden, zodat het als ecologisch geïsoleerd beschouwd kan worden.

Hoofdstuk 8 behandelt de mogelijkheden voor een samenstel van maatregelen ter beheersing van de ziekte. Mogelijke wijzen waarop de ziekte in nog niet besmette gebieden geïntroduceerd zou kunnen worden en maatregelen ter voorkoming hiervan, worden hierbij ook behandeld.

Bestrijding van de ziekte moet allereerst gericht zijn op het verwijderen van de infectiebronnen in en nabij de rijstvelden. Daartoe moet de sanitaire toestand verbeterd worden. Overlapping van de groeiseizoenen moet worden voorkomen. Gebruik van kortgroeiende rijstrassen is daartoe van belang. Een gerichte toetsing en een kweekprogramma om zo tot resistente en voor het gebied bruikbare rijstrassen te komen, moeten zo spoedig mogelijk een aanvang nemen.

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Appendix

Part I. Plant species collected at different places.

Angiospermae

MONOCOTYLEDONEAE

Gramineae

Andropogon pratensis Hack.: Molo
Aristida adoensis Hochst.: Nairobi
Bothriochloa pertusa (L.) A.Camus: Ahero
Brachiaria brizantha (A.Rich.) Stapf: Kisumu, Otonglo
Brachiaria eruciformis Griseb.: Ahero
Brachiaria radicans Napper: Otonglo
Chloris pycnothrix Trin.: Otonglo
Cymbopogon excavatus (Hochst.) Stapf: Kaloka
Cynodon dactylon (L.) Pers.: Ahero
Cynodon nlemfuensis Vanderyst var. *nlemfuensis*: Ahero
Dactyloctenium aegyptium (L.) P.Beauv.: Otonglo
Digitaria ciliaris (Retz.) Koel.: Otonglo
Digitaria melanochila Stapf: Miwani
Digitaria scalarum (Schweinf.) Chiov.: Ahero, Otonglo, Rabur
Digitaria ternata (A.Rich.) Stapf: Otonglo
Dinebra retroflexa (Vahl) Panz.: Ahero, Bunyala, Mwea
Diplachne caudata K.Schum.: Mwea
Echinochloa colona (L.) Link: Ahero, Mwea, Otonglo
Echinochloa pyramidalis (Lam.) Hitchc. et Chase: Otonglo
Eleusine coracana (L.) Gaertn.: Ahero
Eleusine indica (L.) Gaertn. subsp. *africana* (Kenn.-O'Byrne) Phillips: Ahero, Otonglo
Eleusine multiflora A.Rich.: Njoro
Enneapogon schimperanus (A.Rich.) Renv.: Elementeita
Eragrostis aethiopica Chiov.: Otonglo, Paponditi
Eragrostis aspera (Jacq.) Nees: Mwea, Paponditi
Eragrostis atrovirens (Desf.) Steud. var. *congesta* Robyns et Tournay: Bunyala
Eragrostis barbinodis Hack.: Nairobi (NAL)
Eragrostis braunii Schweinf.: Nairobi
Eragrostis cilianensis (All.) Lut.: Ahero, Mwea, Nairobi, Njoro, Paponditi

Eragrostis ciliaris (L.) R.Br.: Paponditi
Eragrostis exasperata Peter: Bunyala, Miwani, Nairobi (NAL), Otonglo
Eragrostis heteromera Stapf: Ahero, Nairobi
Eragrostis macilenta (A.Rich.) Steud.: Nairobi
Eragrostis minor Host: Otonglo
Eragrostis namaquensis Nees var. *namaquensis*: Mwea
Eragrostis paniciformis (A.Br.) Steud.: Kedowa, Mwea, Ruiru
Eragrostis pilosa (L.) P.Beauv.: Nairobi, Otonglo
Eragrostis schweinfurthii Chiov.: Keroka
Eragrostis superba Peyr.: Mtito Andei, Mwea, Paponditi
Eragrostis tenella (L.) Roem. et Schult.: Kisumu
Eragrostis tenuifolia (A.Rich.) Steud.: Ahero, Kisumu, Mwea, Nairobi, Otonglo, Rabur
Eriochloa meyeranum (Nees) Pilg.: Ahero, Paponditi
Eriochloa nubica (Steud.) Thell.: Ahero, Otonglo
Eriochloa procera (Retz.) C.E.Hubbard: Ahero
Harpachne schimperi A.Rich.: Koru
Hemarthria natans Stapf: Kisumu
Hyparrhenia rufa (Nees) Stapf: Koru, Otonglo
Imperata cylindrica (L.) P.Beauv. var. *africana* (Anderss.) C.E.Hubbard: Koru
Leersia hexandra Sw.: Ahero, Otonglo
Panicum infestum Anderss.: Otonglo
Panicum maximum Jacq.: Ahero, Nairobi
Panicum repens L.: Kaloka, Otonglo
Paspalum orbiculare Forst.: Ahero, Mwea, Otonglo
Pennisetum dowsonii Stapf et C.E.Hubbard: Mwea
Pennisetum mezianum Leeke: Ahero
Phragmites australis (Cav.) Steud.: Kaloka
Rhynchelytrum repens (Willd.) C.E.Hubbard: Kisumu
Rottboellia exaltata L. f.: Ahero, Otonglo
Setaria aurea A.Br.: Rabur
Setaria holstii Herrm.: Ahero
Setaria incrassata (Hochst.) Hack.: Kaloka
Setaria pallidifusca (Schumach.) Stapf et C.E.Hubbard: Kisumu
Setaria plicatilis (Hochst.) Hack.: Nairobi
Setaria sphacelata (Schumach.) Stapf et C.E.Hubbard: Otonglo
Setaria verticillata (L.) P.Beauv.: Nairobi (NAL)
Sorghum verticilliflorum (Steud.) Stapf: Miwani
Sporobolus africanus (Poir.) Robyns et Tournay: Kabete
Sporobolus agrostoides Chiov.: Nairobi
Sporobolus confines (Steud.) Chiov.: Sotik
Sporobolus consimilis Fres.: Paponditi
Sporobolus ioclados Nees: Ahero

Sporobolus pyramidalis P.Beauv.: Ahero, Kisumu, Otonglo
Themeda triandra Forsk.: Nairobi
Tragus berteronianus Schult.: Ahero

Cyperaceae
Cyperus alopecuroides Rottb.: Ahero
Cyperus articulatus L.: Ahero, Nanga, Otonglo
Cyperus difformis L.: Mwea, Otonglo
Cyperus digitatus Roxb. subsp. *auricomus* (Spreng.) Kük.: Ahero
Cyperus immensus C.B.Cl.: Otonglo
Cyperus laevigatus L.: Kusa
Cyperus latifolius Poir.: Otonglo
Cyperus longus L.: Ahero
Cyperus papyrus L.: Fort Hall
Cyperus teneriffae Poir.: Kaloka
Fimbristylis quinquangularis (Vahl) Kunth: Otonglo
Kyllinga bulbosa P.Beauv.: Otonglo
Lipocarpha chinensis (Osb.) Kern: Mwea
Pycreus flavescens (L.) Reichenb.: Otonglo
Pycreus polystachyos (Rottb.) P.Beauv.: Kaloka
Scirpus confusus N.E.Br.: Mwea, Otonglo

Typhaceae
Typha domingensis Pers.: Ahero

Araceae
Pistia stratiotes L.: Ahero

Commelinaceae
Commelina diffusa Burm. f.: Ahero
Commelina reptans Brenan: Nairobi

DICOTYLEDONEAE
Onograceae
Ludwigia stolonifera (Guill et Perr.) Raven: Paponditi

Polygonaceae
Polygonum salicifolium Willd.: Mwea

Pteridophyta
Azollaceae
Azolla nilotica Decne.: Ahero

Part II. Species of insects and mites collected at different places and on different plants.

Insects

Coleoptera

ALLECULIDAE

Alogista sp.: Otonglo; *Cyperus* sp., grasses, rice

BUPRESTIDAE

Sphenoptera (Haplistura) sp.: Otonglo; grasses

CARABIDAE

Amblystomus aeneolus (Chd.): Ahero; rice

CHRYSOMELIDAE

Cassidinae

Cassida sp.: Otonglo; rice
Conchyloctenia parummaculata Boh.: Ahero; grasses

Chrysomelinae

Mesoplatys ochroptera Stål: Otonglo; grasses
Phaedonia areata (F.): Ahero, Otonglo; grasses

Clytrinae

Gynandrophthalma sp. near *dorsalis* Lac.: Mwea, Otonglo; ditch vegetation
Herma juillielmi Weise: Kisumu; *Bracharia brizantha* (A. Rich.) Stapf flower
Melitonoma sp.: Otonglo; grasses
Melitonoma sp.: Otonglo; grasses

Criocerinae

Lema sp.: Otonglo; grasses
Lema sp.: Ahero, Mwea, Otonglo; ditch vegetation
Lema affinis Clark: Otonglo; grasses, *Commelina* sp.
Lema chalcoptera Lac.: Nairobi; rice (screenhouse)
Lema chalcoptera Lac. var. *sanguinicollis* Lac.: Nairobi; grasses
Lema diversicola Hze.: Ahero, Otonglo; *Commelina* sp., grasses, rice
Lema mulangensis Hze.: Mwea; rice
Lema rubricollis Klug: Otonglo; *Commelina* sp., grasses
Oulema dunbrodiensis Jac. f. *nigripennis* Hze.: Ahero; grasses

Cryptocephalinae

Cryptocephalus sp.: Ahero, Otonglo; *Cyperus* sp. flower, grasses, rice
Cryptocephalus sp. ?*chalybeipennis* Suffr.: Ahero, Otonglo; *Cyperus* sp. flower, grasses, rice
Cryptocephalus sp. ?W-*nigrum* Suffr.: Mwea; ditch vegetation, grasses, rice
Cryptocephalus callias Suffr.: Otonglo; *Cyperus* sp. flower
Cryptocephalus hildebrandti Har.: Otonglo; rice

Galerucinae

Genus near *Apophylia*: Ahero, Otonglo; grasses
Hallirhotius sp.: Otonglo; *Cyperus* sp., grasses
?*Lamprocopa* sp.: Otonglo; grasses

Genus near *Leptaulaca*: Ahero; grasses
Luperodes quaternus Fairm.: Mwea; grasses
Monolepta flaveola Gerst.: Mwea; *Cyperus* sp. flower, ditch vegetation, *Polygonum salicifolium* Willd.
Monolepta haematura Fairm.: Otonglo; *Cyperus* sp. flower
Monolepta intermedia Rits.: Ahero, Otonglo; rice
Monolepta irregularis Rits.: Otonglo; *Cyperus* sp. flower
Monolepta vincta Gerst.: Otonglo; *Cyperus* sp. flower, grasses
Sesselia pusilla Gerst.: Ahero, Kaloka, Mwea, Otonglo, Paponditi; *Cyperus* spp. and grass flowers, rice (see 5.3.2)

Halticinae

Altica sp.: Ahero; rice (no feeding)
Altica sp.: Mwea; ditch vegetation
Altica malvernensis Jac.: Ahero, Otonglo, Paponditi; *Ludwigia stolonifera* (Guill et Perr.) Raven, rice (no feeding)
Altica pyritosa Er.: Mwea; ditch vegetation
Apthona sp.: Ahero, Mwea, Otonglo; *Cyperus* sp. flower, grasses
Apthona bamakoensis Bech.: Ahero; grasses
Apthona friguiagbensis Bech.: Ahero, Mwea; grasses, rice
Chaetocnema sp.: Ahero, Mwea; grasses
Chaetocnema abyssinica Jac.: Ahero, Bunyala, Otonglo; grasses
Chaetocnema bamakoensis Bech.: Mwea; grasses
Chaetocnema bilunulata Demais: Ahero; grasses
Chaetocnema kenyensis Bryant (?): Bunyala, Mwea, Otonglo; grasses
Chaetocnema pallidipes Fairm.: Ahero, Otonglo; grasses
Chaetocnema pulla Chapuis; Ahero, Bunyala, Mwea, Otonglo, Paponditi; grasses, rice (see 5.3.2)
Chaetocnema pusilla Lab.: Ahero, Mwea, Nairobi, Otonglo, Ruiru; grasses, rice
Chaetocnema wollastoni Baly: Ahero; grasses
Longitarsus sp.: Ahero, Otonglo; grasses, rice
Podagrica nigriceps Bryant: Otonglo; grasses
Podagrica sp. ?*puncticollis* Weise: Ahero, Otonglo; grasses, okra
Torodera octomaculata Weise: Otonglo; rice

Hispinae

Callispa sp.: Otonglo; grasses
Dactylispa bayoni Gest.: Ahero, Otonglo; grasses, rice
Dactylispa chapuisii Gest.: Ahero; grasses
Dactylispa spinigera Gyll.: Otonglo; grasses
Dactylispa spinulosa Gyll.: Otonglo; grasses
Dactylispa tenella Péring.: Ruiru; grasses
Dicladispa quadrifida Gerst.: Ahero, Nairobi; grasses
*Dicladispa (Chrysispa) pauci*spina (Weise): Nairobi, Otonglo; grasses
Dicladispa (Chrysispa) viridicyanea (Kraatz): Otonglo; grasses
Dorcathispa alternata (Weise): Ahero; grasses
Leptispa clavareaui Weise ab. *ruficollis* Uh.: Otonglo; grasses
Trichispa sericea (Guérin): Ahero, Mwea; grasses, rice

COCCINELLIDAE

Brumoides nigrifrons Gerst.: Mwea, Otonglo; grasses
Cheilomenes sulphurea Oliv.: Otonglo; rice

Cheilomenes vicina Muls.: Otonglo; grasses
Epilachna sp.: Otonglo; rice
Epilachna multinotata Gerst. var. *punctipennis* Muls.: Otonglo; grasses, rice
Isora anceps Muls.: Ahero, Otonglo; rice

CURCULIONIDAE

Rhamnopoderus dumosus Gyll.: Otonglo; *Cyperus* sp., grasses

LAGRIIDAE

Chrysolagria sp.: Otonglo; grasses
Chrysolagria sp.: Mwea, Otonglo; grasses
Derolagria sp.: Mwea; grasses
Derolagria sp.: Otonglo; grasses
Lagria (Lagriella) sp. ?*quadrivittata* Fairm.: Otonglo; grasses

LAMPYRIDAE

Luciola sp.: Otonglo; grasses

MELYRIDAE

Apalochrus sp.: Ahero; grasses
Apalochrus sp.: Otonglo; grasses, rice
Apalochrus elgonensis Champ.: Njoro; wheat flower

PHALACRIDAE

Phalacrus sp.: Otonglo; grasses
Stilbus dollmani Champ.: Ahero; rice

SCARABAEIDAE

Cetoniinae

Leucocelis plebeja Klb.: Otonglo; *Cyperus latifolius* Poir.
Rhabdotis sobrina (G. et P.): Otonglo; *Cyperus latifolius* Poir.

Scarabaeinae

Sisyphus crispatus Gory: Otonglo; *Cyperus* sp., grasses

Diptera

DIOPSIDAE

Diopsis acanthophthalma Eggers: Otonglo; *Cyperus* sp., grasses, rice
Diopsis apicalis group (*D. apicalis* Dalm. and *D. tenuipes* Westw.): Ahero, Mwea; grasses, rice
Diopsis sp. ?*confusa* Wiedeman: Mwea; ditch vegetation
Diopsis thoracica Westw.: Ahero, Mwea; ditch vegetation, rice

Hemiptera

APHIDIDAE

Hysteroneura setariae Thos.: Nairobi; *Eragrostis caespitosa* Chiov., *Leersia hexandra* Sw., rice (screenhouse), *Cynodon dactylon* (L.) Pers.
Kugegania ageni Eastop: Nairobi; *Leersia hexandra* Sw. (screenhouse)
Metopolophium dirhodum Wlk.: Nairobi; oats
Rhopalosiphum maidis Fitch: Nairobi, Njoro; maize, wheat

Rhopalosiphum padi L.: Njoro; wheat
Rhopalosiphum rufiabdominalis Sasaki: Otonglo; rice
Schizaphis minuta v. d. Goot: Ahero, Otonglo; maize
Sitobion sp.: Nairobi; *Setaria* sp.
Sitobion chanikiwiti Eastop: Nairobi; *Brachiaria ruziziensis* Germain et Evrard
Sitobion graminis Tak.: Nairobi; *Setaria* sp.?
Tetraneura nigriabdominalis subsp. *bispina* H.R.L.: Nairobi; grasses

APHROPHORIDAE

Cordia sp.: Otonglo; rice
Poophilus sp.: Otonglo; grasses
Poophilus grisescens Schaum.: Otonglo; grasses, rice
Ptyelus grossus F.: Nairobi; cotton

CERCOPIDAE

Locris areata Wlk.: Nairobi; *Cyperus* sp., grasses
Locris auripennis Dist.: Otonglo; *Cyperus* sp., grasses, rice
Locris cardinalis Gerst.: Nairobi; grasses
Locris rhodesiana Dist.: Otonglo; *Cyperus* sp., grasses

CICADELLIDAE

Cicadella spectra Dist. complex: Ahero, Otonglo; rice
Nephotettix afer Ghauri: Ahero, Mwea, Otonglo; grasses, rice
Nephotettix modulatus Melichar: Ahero; rice
Signoretia sp.: Otonglo; rice

COREIDAE

Hydara tenuicornis (Westw.): Otonglo; grasses
Cletus sp. (pronus Bergr.?): Otonglo; grasses
Hydara tenuicornis (Westw.): Otonglo; grasses

CORIZIDAE

Agraphopus sjöstedti Schout.: Otonglo; grasses
Liorhyssus hyalinus F.: Mwea; grasses

FLATIDAE

Dalapax postica Spin.: Nairobi; grasses (screenhouse)

FULGORIDAE

Zanna flammea (L.): Ahero;

LYGAEIDAE

Diniella nitida Reut.: Otonglo; grasses
Ischnodemus grossus Slater: Otonglo; grasses
Ischnodemus parabasalis Slater or n.sp.: Otonglo; okra
Lasiosomus lasiosomoides (Berger.): Mwea; grasses
Opistholeptus elegans (Hesse): Otonglo; grasses
Oxycarenus albidipennis Stål: Otonglo; okra
Oxycarenus rufiventris (Germ.): Otonglo; *Cyperus* sp.
Pachybrachius dubius (Reut.): Ahero; grasses

Stigmatonotum capucinum (Stål): Mwea; grasses

MEENOPLIDAE

Nisia sp.: Mwea; grasses, rice

MEMBRACIDAE

Xiphopoeus sp.: Otonglo; *Cyperus* sp., grasses

MIRIDAE

Deraeocoris fülleborni Popp.: Mwea; grasses
Paramixia suturalis Reut.: Otonglo; okra
Polymerus longirostris (Reut.): Otonglo; grasses
Stenotus pulchellus Popp.: Otonglo; grasses
Stenotus transvaalensis (Dist.): Mwea; grasses

PENTATOMIDAE

Aeliomorpha griseoflava (Stål): Otonglo; grasses
Agonoscelis versicolor (F.): Otonglo; grasses
Aspavia armigera (F.): Otonglo; grasses
Aspavia pallidispina Stål: Mwea; ditch vegetation
Diploxys acanthura (Westw.): Ahero, Otonglo; grasses
Dorycoris pavoninus (Westw.): Mwea; ditch vegetation
Dorycoris pavoninus var. *miniatus* (Westw.): Otonglo; rice
Eysarcoris inconspicuus (Herr.-Sch.): Otonglo; grasses
Hermolaus gestroi Schout.: Otonglo; grasses
Menida transversa (Sign.): Otonglo; grasses
Nezara viridula (L.): Mwea; *Veronia lasiopus* O.Hoffm.
Sphaerocoris annulus (F.): Otonglo; *Cyperus* sp. flower
Sphaerocoris annulus var. *ocellatus* (Klug): Mwea; *Vernonia lasiopus* O.Hoffm.
Sphaerocoris testudogrisea (de Geer): Otonglo; *Cyperus* sp. flower
Steganocerus multipunctatus (Thunb.): Otonglo; *Cyperus* sp., grasses
Stenozygum decoratum Schout.: Otonglo; grasses

PLATASPIDAE

Coptosoma marginellum Stål: Otonglo; grasses

REDUVIIDAE

Coranus metallicus (Serv.): Otonglo; *Cyperus* sp., grasses
Coranus varipes Stål: Otonglo; *Cyperus* sp., grasses
Ectomocoris quadrimaculatus (Serv.): Nairobi;
Nagusta punctaticollis Stål: Otonglo; *Cyperus* sp.
Rhinocoris albopilosus (Sign.): Otonglo; *Cyperus* sp., grasses

Hymenoptera

BRACONIDAE

Centistes sp.: Otonglo; soil round *Cyperus articulatus* L.
Rhaconotus sp. near *menippus* Nixon: Otonglo; larva in *Cyperus articulatus* L.

Lepidoptera

NOTODONTIDAE

Rigema woerdeni Snellen: Otonglo; rice

PYRALIDAE

Scirpophage sp. ?*subumbrosa* Meyr.: Ahero; rice

TORTRICIDAE

Bactra sp. ?*fasciata* Diak.: Otonglo; out *Cyperus articulatus* L.

Orthoptera

ACRIDIDAE

Atractomorpha orientalis Kevan: Otonglo; rice

TETTIGONIIDAE

Conocephalus conocephalus L.: Ahero; grasses
Conocephalus merumontanus Sjöstedt: Nairobi; grasses

Dictyoptera

MANTIDAE

Pseudocreobotra ocellata Palis.: Otonglo; *Cyperus latifolius* Poir. flower

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Mites

ERIOPHYDIDAE

Aceria bakkeri K.: Otonglo; *Paspalum orbiculare* Forst., rice

PHYTOSEIIDA

Amblyseius sp.: Otonglo; rice

TARSONEMIDAE

Steneotarsonemus spinki Smiley: Otonglo; *Brachiaria radicans* Napper, rice

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