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Rice yellow mottle, a mechanically transmissible virus disease of rice in Kenya [1]

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Abstract

In Kenya around Lake Victoria rice is affected by a hitherto undescribed virus for which the name Rice Yellow Mottle Virus (RYMV) is proposed. The virus was easily transmitted mechanically to rice and to *Oryza barthii* and *Oryza punctata*, but not to *Oryza eichingeri*, barley, bulrush millet, durum wheat, finger millet, maize, oats, rye, sorghum, wheat, sugarcane, 20 other monocotyledonous and 9 dicotyledonous plant species.

The disease is characterised by stunting and reduced tillering of the rice plant, crinkling, mottling and yellowish streaking of the leaves, malformation and partial emergence of the panicles, and sterility. In severe cases the plant may die. RYMV is stable and highly infective.

The vector is the beetle *Sesselia pusilla* Gerstaeker (fam. Chrysomelidae, Galerucinae). The beetle has been identified by Mr John A. Wilcox, New York State Museum and Science Service, New York. *S. pusilla* transmitted the virus for at least five successive days after feeding on an infective plant.

Purified virus preparations consisted of polyhedral particles about 32 mμ in diameter. The sedimentation coefficient was 116S.

Introduction

In Kenya rice, mainly the indica variety 'Sindano', is grown by peasant farmers along the coast of the Indian Ocean and along the shores of Lake Victoria, but also by tenants of the more inland Mwea Irrigation Settlement. The crop is of increasing importance because it has also been successfully planted at the Kano Pilot Scheme, which it is intended shall be developed into an extensive irrigation scheme.

At Otonglo near Kisumu along the shore of Lake Victoria in November 1966 a hitherto unknown disease was observed. It attacked a high percentage of rice plants and was tentatively called rice yellow mottle. In the field the diseased plants were first noticeable 3–4 weeks after transplanting by their striking yellowish appearance. The youngest leaves showed mottling or a mild yellow-green streaking (Fig. 1). The plants were stunted, showed reduced tillering and the flowers were sterile (Fig. 2).

The disease was soon found to be caused by a mechanically transmissible virus not resembling any known rice virus. The present paper describes this apparently new disease and its causal agent rice yellow mottle virus (RYMV), and suggests possible ways of control, since potentially the disease is of great importance.

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Fig. 1. Symptoms of RYMV on 'Sindano' rice leaves.

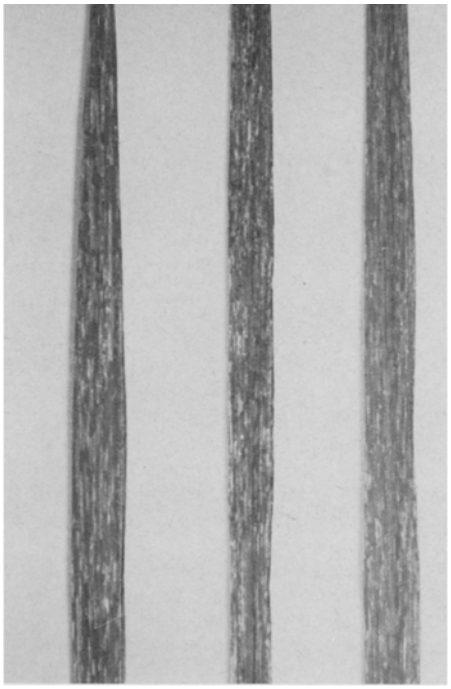

Fig. 1. Symptomen van RYMV op 'Sindano' rijstbladeren.

Fig. 2. Rice ,'Sindano', 7 months after sowing, just before harvest. Left, manually inoculated with RYMV 3 months after sowing. Right, healthy control.

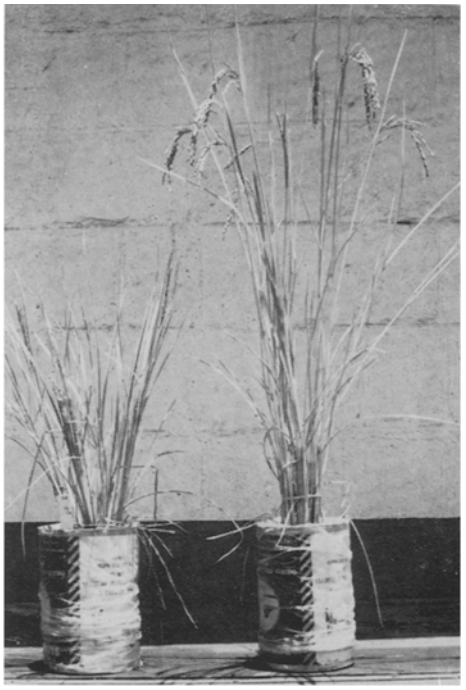

Fig. 2. Rijst, 'Sindano', 7 maanden na het zaaien, juist voor de oogst. Links, mechanisch geïnoculeerd met RYMV 3 maanden na het zaaien. Rechts, gezonde controle.

Materials and methods

The experiments were performed with an isolate obtained from a young rice plant from Otonglo. Seedlings of 'Sindano' rice, used for the experiments were grown from seed in sterilised compost in 8 cm pots – 5 seeds per pot – in an insect-proof glasshouse. The temperature in the glasshouse was 20°–32 °C. Due to insufficient lighting most plants were etiolated. Other test plants belonging to the Gramineae were also grown from seeds – 2–5 seeds per pot – or from stools collected in the field. *Chenopodium amaranticolor* and *C. quinoa* were grown under artificial light for 16 h per day. Extreme care was exercised with the watering of the rice plants to prevent contamination. Details on various techniques are given in the sections concerned.

Host range and symptoms

The range of hosts was determined by mechanically inoculating test plants belonging to the Gramineae at the 3–6 leaf stage and the other test plants at the commonly used

stage. The inoculum was prepared by cutting young leaf blades from young rice with clear symptoms with a razor blade in some 0.01 M phosphate buffer pH 7.0. After cutting, the leaf material was ground in a mortar together with some more buffer and squeezed through muslin cloth. In total 1 ml of buffer per gram of leaf was used. Back inoculations to rice were performed 3–4 weeks after the inoculation.

The following species and varieties were found to be susceptible and showed symptoms: *Oryza sativa* (rice), 'Basmati', 'Basmati 217', 'Faya Sl', 'Gamti', 'Kialangawa', 'Kibawa chekundu', 'Kibawa cheupe', 'Madevu', 'Mbuyu', 'Mkarafuu', 'Portugues', 'Shingo la Majani', 'Sindano', 'Uchuki', and 'Zira', *Oryza barthii* (wild rice), and *Oryza punctata*.

Rice showed the first symptoms about 7 days after inoculation. With 'Sindano' seedlings sap-inoculated at a young stage (3–6 leaves), the first newly formed leaves were mottled, streaked and spirally twisted as well, as if they met difficulties in emerging (Fig. 3). Leaves formed later were mostly normal in shape. Inoculation at a later stage does not give this malformation. It can occasionally be seen in the field. In older plants (8–10 leaves) inoculated manually, the first symptoms consisted of a few yellow-green spots on the youngest leaves. These spots enlarged along the veins to give the characteristic streaking. Such leaves sometimes turned yellow and later became necrotic. In severe cases the plant died. Mottling of the leaf sheath also occurred. The inoculated plants were stunted, formed fewer tillers, and the production of viable seeds was greatly reduced or totally absent, depending on the age of the plant at the moment of inoculation. Many panicles did not emerge properly from the flag-leaf

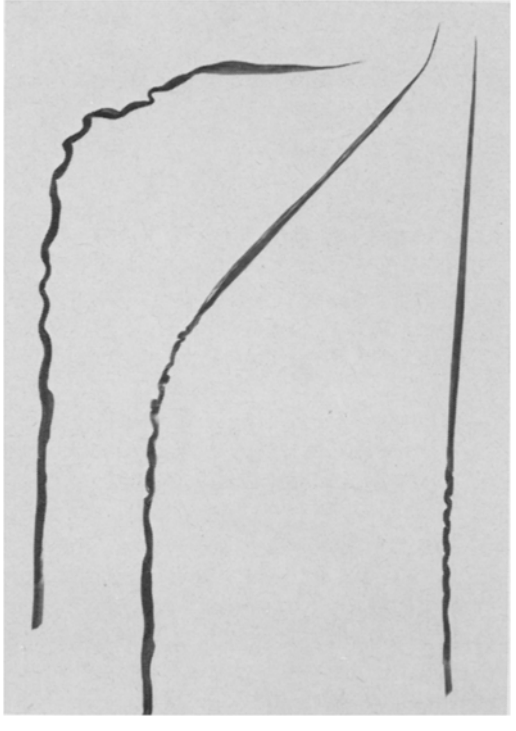

Fig. 3. Crinkling of 'Sindano' rice leaves infected with RYMV.

Fig. 3. Krinkeling van 'Sindano' rijstbladeren geïnfecteerd met RYMV.

Fig. 4. Panicles from 'Sindano' rice plants infected with RYMV. Left panicle from healthy control.

Fig. 4. Pluimen van 'Sindano' rijst geïnfecteerd met RYMV. Linker pluim is van de gezonde controle.

Fig. 5. Leaf symptoms of Oryza punctata infected with RYMV.

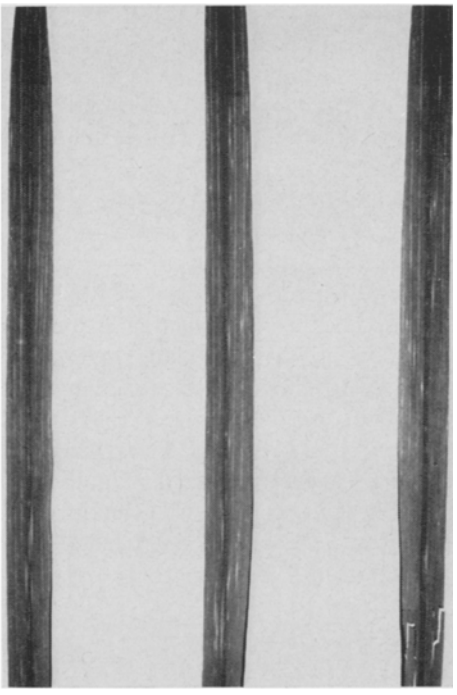

Fig. 5. Bladsymptomen van Oryza punctata geïnfecteerd met RYMV.

sheath and were malformed with small, usually empty spikelets (Fig. 4). 'Sindano' rice plants inoculated 3 weeks before heading still showed a clear reduction in seed yield.

With *O. barthii* and *O. punctata*, small yellow-green spots appeared on the youngest leaves 14 days after inoculation. These spots extended along the veins (Fig. 5). As the plants grew older a slightly darker patch could be seen in the centre of the yellow streaks.

Most plant species tested were found not to be susceptible: *Oryza eichingeri* (forest rice), *Avena sativa* (oats), 'M.F.C.15/67' and 'Lampton', *Eleusine coracana* (finger millet), *Hordeum vulgare* (barley), 'Proctor', *Pennisetum typhoideum* (bulrush millet), *Saccharum officinarum* (sugarcane), 'NCo 310' and 'Q 45', *Secale cereale* (rye), *Sorghum vulgare* (sorghum), 'H 726' and 'H 6060', *Triticum aestivum* (wheat), 'Kenya Kudu' and 'Wisconsin', *Triticum durum* (durum wheat), and *Zea mays* (maize), 'Hybrid 611B', 'Hybrid 612' and 'Hybrid 613B'. Twenty grasses belonging to the tribes Andropogoneae, Eragrosteae, Chlorideae, Festuceae, Hordeae, Paniceae and Sporoboleae were tested and also nine dicotyledonous plant species, among which were the most commonly used test plants, but none were found to be hosts.

Persistence of infectivity in expressed sap

The studies were carried out according to the methods described by Bos et al. (1960).

Dilution end point. Sap derived from young rice leaves with clear symptoms, 2–3 weeks after inoculation, was still infective at a dilution of 10^{-10}, whereas with sap obtained from plants inoculated 4–5 weeks earlier, a dilution of 10^{-6} was the highest infective dilution.

Thermal inactivation point. To obtain a sufficient amount of sap 1 ml 0.01 M phosphate buffer pH 7.0 was added per gram of leaves while grinding in a mortar. The thermal inactivation point was above 80 °C.

Ageing in vitro. 20 ml of diluted sap, obtained by grinding 70 g of young rice leaves with clear symptoms together with 25 ml 0.01 M phosphate buffer pH 7.0 ,was divided in two parts of 10 ml. In the part stored at room temperature (16°–25 °C) the virus remained infective for 33 days but not for 51 days; the sap stored in a refrigerator (9 °C), on the other hand, was still infective after 71 days.

Influence of organic solvents. Sap diluted with 0.01 M phosphate buffer pH 7.0 was shaken for 1 min with an equal amount of chloroform, chloroform and butanol, and carbon tetrachloride and ether. After centrifuging for 20 min at 3000 rpm the aqueous phase was inoculated on to 'Sindano' seedlings. In all three cases 100 % infection was obtained.

Insect transmission

Adult beetles (*Sesselia pusilla* Gerstaecker, fam. Chrysomelidae, Galerucinae; see Fig. 6) collected from rice fields at Otonglo were placed on young mechanically infected 'Sindano' seedlings and then transferred to healthy seedlings. Two types of experiment were performed:

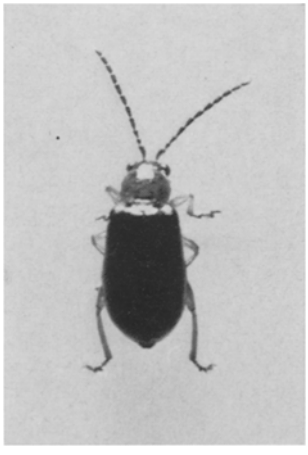

Fig. 6. Adult beetle, *Sesselia pusilla*, vector of RYMV.

Fig. 6. Volwassen kever, Sesselia pusilla, vector van RYMV.

a. Single beetles were transferred daily after an acquisition feeding period of 4 days.
b. Groups of five beetles were placed on a rice seedling for 3 days after an acquisition feeding period of 3–4 days. Surviving insects were transferred to another seedling for 3 days.

The presence of RYMV was checked serologically with the agar gel diffusion test.

In one test with ten beetles transferred daily, five insects transmitted RYMV for 1–5 successive transfers. The symptoms appeared after 9–16 days.

In two experiments with groups of insects, 5 out of 7 and 12 out of 15 groups of beetles transmitted RYMV during one of the two transfers. The symptoms appeared after 8–20 days.

Other possible ways of transmission

The virus was recovered from sap obtained from the roots and also from the guttation fluid of diseased rice plants, as well as from irrigation water from heavily infected fields at Otonglo. No seed transmission was observed in experiments with a limited number of seeds from infected rice plants, nor was transmission obtained by growing rice in soil collected around diseased plants in the field.

Purification

The purification scheme was a modification of a method used by Proll and Schmidt (1964) for ryegrass streak virus.

16.5–20 g of young 'Sindano' leaves were cut, ground in a mortar and squeezed through muslin cloth. The last two steps were done twice. 20 ml 0.1 M phosphate buffer pH 5.0 per gram of leaf blade was used in the grinding. The resulting liquid was then mixed with chloroform (2:1) and the mixture gently shaken in a separating funnel for 5 min. The emulsion was centrifuged for 10 min at 1000 g. Thereupon ammonium sulphate was added to the clear aqueous phase at the rate of 25 g per 100 ml liquid while stirring with a magnetic stirrer. The precipitate was removed by centrifuging for 15 min at 2000 g and to the supernatant (S_1), 40 g ammonium sulphate was added per 100 ml liquid. After standing for at least 20 min the solution was centrifuged for 20 min at 4750 g and the precipitate (N_2) resuspended in 0.1 M phosphate buffer pH 5.0 (3–6) ml). This suspension was dialysed for 12–18 h against 0.1 M phosphate buffer pH 5.0. The insoluble components were removed by centrifuging for 20 min at 3000 g, leaving a strongly opalescent suspension (S_3) which was fairly pure. The whole procedure was carried out at room temperature.

Further purification was done with a Beckman L_{II} or with an M.S.E. "Super Speed 40" ultracentrifuge. The virus was precipitated by centrifuging for 90 min at 130,000 g and resuspended in 1 ml 0.1 M phosphate buffer pH 5.0. After standing for 30 min at 4 °C, the insoluble components were removed by slow speed centrifugation (3 min – 6000 rpm). The supernatant (S_5) was strongly opalescent and contained highly pure virus while preparations made from healthy plants showed no light scattering. The virus suspension was used for electron microscopy and for preparing an antiserum.

Occasionally for electron microscopy and for analytical ultracentrifugation the virus was further purified by means of a density gradient column. This had been kept at 4 °C for 24 h and was made of 4, 7, 7, 7 and 3 ml 0.01 M phosphate buffer pH 6.7, which

contained 40, 30, 20, 10 and 0 g sucrose per 100 ml, respectively. Supernatant S_5 was layered on top of it and then centrifuged for 100 min at 23,000 rpm (Beckman SW 25.1 rotor). A very clear band resulted at 9–13 mm under the meniscus. This band was removed with an injection needle. The collected suspensions were diluted 5 times with 0.01 M phosphate buffer pH 6.7. After centrifuging for 90 min at 133,000 g the precipitate (N_6) was resuspended in a small amount of 0.1 M phosphate buffer pH 5.0 (S_7).

The virus suspensions S_5 and S_7 proved infective when inoculated mechanically on 'Sindano' seedlings.

Electron microscopy

RYMV preparations sent by air to the Laboratorium voor Virologie, Agricultural University, Wageningen, The Netherlands, were examined in a Siemens Elmiskop-I electron microscope after negative staining with 1 % sodium phosphotungstate (PTA) pH 5.5 Fractions S_5 and S_7 did not differ in purity. The preparations contained many polyhedral particles about 32 mμ in diameter (Fig. 7). A certain amount of damage to

Fig. 7. Electron micrograph of RYMV, fraction S_5 negatively stained with PTA. The empty particles are possibly damaged by PTA.

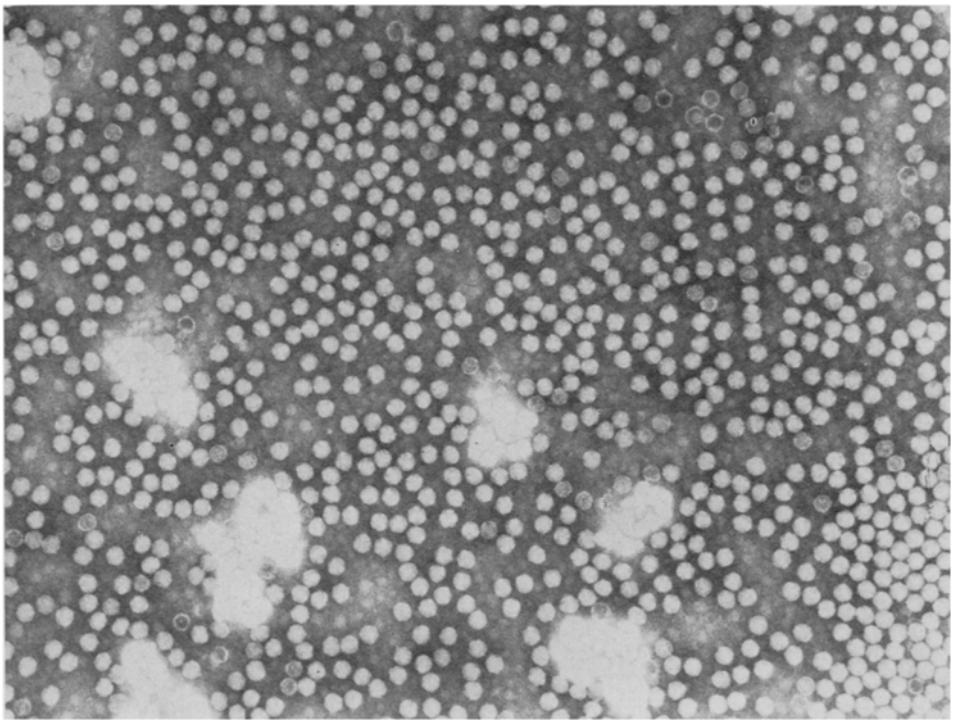

Fig. 7. Elektronenmicroscopische foto van RYMV, fraktie S_5 negatief gekleurd met fosforwolfraamzuur. De lege deeltjes zijn waarschijnlijk beschadigd door fosforwolfraamzuur.

Fig. 8. Ultraviolet absorption picture of RYMV (fraction S_7) in the analytical ultracentrifuge.

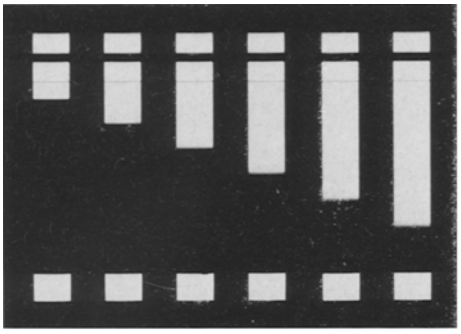

Fig. 8. Ultraviolet-absorptiebeelden van RYMV (fraktie S_7) in de analytische ultracentrifuge.

Fig. 9. Schlieren diagram of RYMV (fraktion S_5).

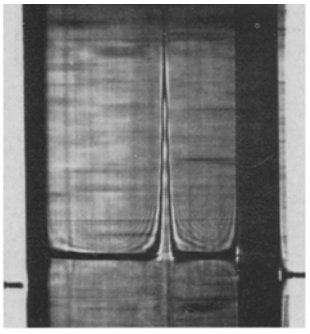

Fig. 9. Schlierendiagram van RYMV (fraktie S_5).

the particles was noticed in places. Treatment with 0.1 % formalin for 10 and 45 min prior to the staining did not reduce this damage, which was thought to be caused by the PTA (Serjeant, 1967).

Analytical ultracentrifugation

Fraction S_7 was examined after a 1:1 dilution with 0.01 M phosphate buffer pH 7 in a Spinco model E analytical ultracentrifuge by applying the ultraviolet absorption technique (Fig. 8 and 9). The preparations revealed one component with a S_{20} value of 116S.

Serology

An antiserum was prepared by injecting a rabbit with fraction S_5 (N_4 resuspended in 0.85 % NaCl). The highest dilution still giving a reaction against crude sap and purified virus when determined with the agar gel diffusion test was 1/256.

Discussion

Although viruses of rice have been known for a long time, it is only recently that these pathogens have attracted more widespread attention.

Most viruses affecting rice in the field are transmitted by leaf- or planthoppers (Walker, 1969). Only rice mosaic in the Philippines is transmissible mechanically to maize, while no information is available about transmission from rice to rice (Martinez et al., 1960).

A number of viruses affecting other Gramineae can be transmitted mechanically to rice, such as those of sugarcane mosaic (Anzalone, 1963), ryegrass mosaic (Mulligan, 1960), maize dwarf mosaic (Brambl and Dale, 1967), brome mosaic, and barley stripe (Kahn and Dickerson, 1957). Differences in particle size and/or host range (Slykhuis,

1967) already indicate that these virus diseases as well as others not tested on rice differ from RYM in Kenya. Cocksfoot mottle virus (CFMV) and phleum mottle virus (PMV), both transmitted by the beetles *Lema melanopa* L. and *Lema lichenis* Weiss., have the same shape as RYMV. CFMV differs in properties in expressed sap and in host range, while insufficient information is available on these properties of PMV (Serjeant, 1964 and 1967; Catherall, 1966, 1967, 1968a and 1968b). CFMV and RYMV both have only one component in the ultracentrifuge.

The ease of sap transmission and the high dilution end point of RYMV suggest that mechanical transmission by farmers in the field is quite possible, especially when the leaves and roots are cut at transplanting.

In contrast to the feeding damage caused by caged beetles, damage to rice plants in the field due to the vector was never severe, notwithstanding that high numbers of these insects were caught on ratoon rice. This suggests that the insects feed for short periods only. On damaged plant parts, especially on stems, a fluid exudate often appears and this may be one of the ways in which irrigation water becomes contaminated.

Poor cultural practices by the peasant farmers are largely responsible for the spread of the disease at Otonglo. In this area, which has two growing seasons in the year, rice is to be found in all stages of growth throughout the year, at least on small acreages. There is inadequate water control and a lack of tillage after harvest. Most of the ratoon rice and germinated dropped seeds are infected with RYMV, thus furnishing many sources of infection.

To prevent a rapid extension of the area affected by the disease a strict discipline in the cultivation of rice is necessary as a primary measure. Overlapping of the growing seasons should be avoided by using varieties with short vegetative periods, and no ratoon rice should be allowed. As all the tested rice varieties are susceptible to RYMV, breeding for resistance must be incorporated in future rice breeding programmes in East Africa.

Acknowledgments

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Samenvatting

Rice yellow mottle, een mechanisch over te brengen virusziekte van rijst in Kenya

In Kenya wordt rijst, voornamelijk 'Sindano', verbouwd op familiebedrijfjes aan de kust van de Indische Oceaan en langs het Victoria Meer, en door pachters van het meer naar het binnenland gelegen 'Mwea Irrigation Settlement'. In de omgeving van Kisumu (Nyanza Province), waar rijst geteeld wordt volgens slechte cultuurmethoden, wordt sinds 1966 een tot dusver onbekende ziekte, hier rice yellow mottle genoemd, waargenomen.

In het veld vallen de zieke planten onmiddellijk op door de gelige verkleuring van de bladeren. De jongste bladeren zijn gevlekt of geel-groen gestreept (Fig. 1). De uitstoeling is gering en de plant gedrongen (Fig. 2). De zaadopbrengst wordt sterk verlaagd door het optreden van steriliteit.

Ongeveer 7 dagen na sap-inoculatie van 'Sindano'-zaailingen verschijnen de eerste symptomen. Na inoculatie in een jong stadium van de plant krullen de eerstgevormde bladeren spiraalvormig (Fig. 3). Later worden de bladeren geel en necrotisch. Jong geïnfecteerde planten kunnen zelfs afsterven. De pluimen komen onvoldoende uit de bladschede, zijn misvormd en dragen veelal kleine en misvormde bloembakjes, die meestal loos zijn (Fig. 4). 'Sindano' rijst, 3 weken voor het in pluim schieten geïnoculeerd, liet een zeer sterke opbrengstvermindering zien.

In sap, verkregen van wortels van zieke rijstplanten, in de guttatievloeistof en in irrigatiewater van ernstig zieke rijstvelden kon het virus gemakkelijk worden aangetoond. Overdracht van RYMV door zaad en grond werd niet waargenomen.

RYMV was nog infectieus na een verhitting gedurende 10 min bij 80 °C. De verdunningsgrens hing af van het tijdstip waarop het bladmateriaal van de zieke planten getoetst werd en liep uiteen van 10^{-10} (2–3 weken na inoculatie) tot 10^{-6} (4–5 weken na inoculatie). Sap bewaard bij kamertemperatuur was nog infectieus na 33 dagen, maar had deze eigenschap verloren na 51 dagen. Bewaard in een koelkast bleef het sap zeker 71 dagen infectieus.

Al de getoetste rijstrassen werden na sap-inoculatie door het virus geïnfecteerd, evenals *Oryza barthii* en *Oryza punctata* (Fig. 5). Niet vatbaar waren: *Oryza eichingeri*, baardtarwe, eleusine gierst, gerst ('Proctor'), haver ('M.F.C.15/67' en 'Lampton'), mais ('Hybrid 611B', 'Hybrid 612' en Hybrid 613B'), parelgierst, rogge, sorghum ('H726' en 'H6060'), suikerriet ('NCo 310' en 'Q 45'), tarwe ('Kenya Kudu' en 'Wisconsin'), evenals 20 andere monocotyle en 9 dicotyle plantesoorten, waaronder de doorgaans bij plantevirusonderzoek gebruikte toetsplanten.

Het kevertje *Sesselia pusilla* Gerstaecker (fam. Chrysomelidae, Galerucinae; zie Fig. 6) bracht het virus over. Een enkel insekt was in staat het virus gedurende tenminste 5 dagen over te brengen.

RYMV was gemakkelijk te zuiveren. Hierbij werd uitgegaan van 16,5–20 g jonge duidelijk zieke bladschijven van jonge 2–3 weken tevoren geïnoculeerde rijstplanten ('Sindano') door uitschudden met chloroform en uitzouten met ammoniumsulfaat, iets gewijzigd naar Proll en Schmidt (1964). De elektronenmicroscopische beelden van de gezuiverde virussuspensie laten één type veelkantige deeltjes zien met een diameter van ongeveer 32 mμ (Fig. 7). De S_{20}-waarde is 116S (Fig. 8 en 9).

Ter bestrijding van de ziekte moet op korte termijn allereerst de cultuurmethode

van de rijst op de familiebedrijfjes verbeterd worden. Het gehele jaar in verschillende groeistadia voorkomen van rijst op een klein gebied en veelal zieke opslag op juist geoogste velden, dragen belangrijk bij tot de verspreiding van de ziekte. Het gebruik van kortgroeiende rijst-varieteiten wordt aanbevolen om overlapping van de groeiseizoenen te voorkomen.

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