5. Rice stripe necrosis virus: a soil-borne rod-shaped virus

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SUMMARY

A disease of upland rice known locally as 'crinkling rice disease' has been recorded for many years in a number of West African countries. This disease was previously thought to be either bacterial in origin or a physiological disorder. Recently in the Ivory Coast, this disease has been found to be associated with a rod-shaped virus called rice stripe necrosis virus (RSNV). The symptoms of the disease are: bright yellow stripes along the leaves, necrosis of the leaves or of the whole plant, dwarfing, crinkling and reduced tillering. The percentage of infection varies greatly according to the year and to the variety. Crop loss was estimated, for different varieties, to vary between 14% and 100%. The agent is soil-borne and a soil fungus vector of the order Plasmodiophorales, Polymyxa graminis, is always associated with the diseased plants, both in the field and in laboratory transmission. The causal agent of the disease is not mechanically transmissible from rice to rice (Oryza sativa), but only to other local lesion hosts, and back transmission from local lesion hosts to rice has never been obtained. The rod-shaped virus is 20 nm in width and we have observed particles of three different lengths: 110-160, 270 and 380 nm. An antiserum with a titre of 1/250 has been prepared and no serological relationship was obtained with the several rod-shaped viruses tested. Infected rice cells show virus-like aggregates and cytoplasmic inclusion bodies, typical of rod-shaped viruses. RSNV has many properties of the proposed furovirus group including hollow rod-shaped particles with several components and transmission by a soil fungus. Furthermore, the amino acid composition of the coat protein of RSNV particles is very different from tobamoviruses and may be typical of 'furoviruses'.

INTRODUCTION

Until 1978, only one virus had been isolated from rice in Africa, rice yellow mosaic virus (RYMV; Fauquet & Thouvenel, 1977), a typical African...
sobemovirus transmitted by chrysomelid beetles. For many years in West Africa, another disease was known to appear on upland rice (Oryza sativa) cultures. This disease which develops in young seedlings was called, because of its symptoms, 'rice crinkling disease'. This disease was first described from the Ivory Coast by Louvel & Bidaux (1977) and several hypotheses as to the nature of the causal agent have been proposed: a physiological disorder, a nematode (Vuong, 1968), or a bacterial agent (Buddenhagen, pers. comm.). However, most of the symptoms in infected rice seedlings resemble those caused by rice stripe virus, a virus isolated in South East Asia (Ling, 1972; Ou, 1972). Indeed, Fauquet & Thouvenel (1983) later demonstrated that this 'rice crinkling disease' was associated with viroids that were rod-shaped, and soil-borne. The results obtained showed that the virus had not previously been described from rice or from other graminaceous plants. As a result, because of the typical symptoms induced (Plate 3), we proposed the name: rice stripe necrosis virus (RSNV). The natural vector of the disease is the soil fungus, Polymyxa graminis, which colonizes the roots of rice in the infected areas. The modes of transmission of the agent were identified and a number of biochemical and biological parameters were studied. As far as we can tell, this virus shares most of the properties of the newly proposed furovirus group (Shirako & Brakke, 1984). The objective of the present paper is, with the addition of new information, to summarize the current state of knowledge of this disease.

THE DISEASE

Symptomatology. Louvel & Bidaux (1977) gave an accurate description of the symptoms of this disease, which we repeat briefly here. In its symptomatology we have to distinguish between three levels of description: the precocity of the infection, the different types of symptoms and the intensity of the symptoms. The first characteristic of the disease is its very early infection of the crop, i.e. some three weeks after sowing severe symptoms may be seen on seedlings. This suggests that the vector, whatever it is, is active soon after germination of the rice. The chronological order of appearance of the symptoms is as follows: chlorotic or yellow stripes on leaves (Fig. 5-1(2)), then a general stunting of the plant (Fig. 5-1(1)), followed by necrotic stripes along the limb, reduction of tillering (very often one tiller only; Fig. 5-1(3)), and crinkling distortion of young leaves (Fig. 5-1(3)).

The intensity of symptoms varies greatly, depending upon date of infection and variety. Often, there is a necrosis of the whole plant about 30 days after infection and when the percentage of infection in the field is high, the infected patch looks like an area of low stunted rice (Fig. 5-1(1)).
Many years in West Africa rice (Oryza sativa) seedlings was called, this disease was first described (1977) and several hypotheses have been proposed: a virus or a bacterial agent. The symptoms in infected plants include typical stripe necrosis and a fungus, Polymyxa grisea or a virus isolated by Auzuet and Thouvenel. The results obtained led from rice or from wild rice plants and from rice cultivated areas. The modes of biochemical transmission can tell, this virus is a furovirus. The results obtained in this paper are, with the state of knowledge available description of the disease is its very severe disease after sowing severe attack that the vector, the typical symptoms that follow: chlorotic mottling of the plant leaf, reduction of tillering distortion of the panicle. Depending upon date of sowing, the plant about 30 days after sowing the infection in the field is visible (Fig. 5-1(1)).

Fig. 5-1(1) Field of upland rice contaminated with rice stripe necrosis virus.
Fig. 5-1(2) Yellow stripe symptoms on rice leaves of cv. IRAT 10, infected with rice stripe necrosis virus. Healthy rice leaf at bottom.
Fig. 5-1(3) Rice plant, cv. IRAT 10, infected with rice stripe necrosis virus, showing little tillering and poor development of the tillers 85 days after sowing.
Fig. 5-1(4) Malformation of a panicle of cv. IRAT 10, induced by rice stripe necrosis virus.
If infection occurs later the plant does not die, but tillering will be considerably reduced and will most often give only one very distorted and almost sterile panicle (Fig. 5-1(4)). If infection is incomplete, the plant will grow one or more tillers which compete with the infected tillers and will eventually produce healthy panicles.

Incidence of the disease. Levels of disease incidence and their effects on growth and yield of infected rice have only been estimated in the Ivory Coast (Louvel & Bidaux, 1977; Fauquet, unpubl.). Assessment of disease incidence in a crop must take into account its impact on individual plants and the frequency with which it occurs. The impact of the RSNV is dependent on the age of infection of the plants, the number of infected tillers on each plant, on the intensity of the symptoms on each tiller and on the variety considered. In a study made with three rice varieties, three different stages in the development of the disease were distinguished: from 21 days after sowing (DAS) to 39 DAS when the number of diseased plants in the field increases, from 39 DAS to 45 DAS when there is a decrease in the number of infected plants (this stage corresponds to the end of tillerage of the rice), and finally from 45 DAS to 85 DAS when the number of infected plants remains constant. This apparent reversion of symptoms is a result of competition from healthy tillers. Within a collection of 30 varieties tested in the Ivory Coast in 1977 and 73 tested in 1981 and 1983, the percentage of infected plants ranged from 0 to 48% in 1977 (Louvel & Bidaux, 1977) and from 0 to 100% in 1981 and 1983 (Fauquet, unpubl.). For each set of observations, the most susceptible varieties were Lung Sheng 1 and its hybrids, including IR8. Crop losses with the three susceptible varieties, estimated from the number of panicles, varied between 76 and 92% for infected plants, and from 13 to 16% over the whole plot.

Epidemiology of the disease. The epidemiology of the disease has not received much attention from research workers, therefore relevant data are almost non-existent. The disease is not a severe problem in West Africa; it recurs erratically over the same areas from season to season. Nevertheless, field observations indicate that epidemics occur when short periods of rain alternate with long periods of drought, such as often occur at the beginning of the rainy season, which is also the time for planting upland rice. The disease appears in patches of variable size in the fields, but there is no correlation between the low growth areas and the infected areas within the rice fields. It has been noted that the percentage of infection is greater when one rice crop succeeds another. The disease has been observed in many different parts of Ivory Coast (Fauquet, unpubl.), Nigeria (Rossel, pers. comm.), Liberia and Sierra Leone (Buddenhagen, pers. comm.) with apparently the same pattern and importance of spread.
Ice and their effects on estimated in the Ivory. Assessment of disease act on individual plants impact of the RSIVV is the number of infected plants on each tiller and on rice varieties, three were distinguished: from the number of diseased plants there is a decrease in the end of tillering when the number of dissemination of symptoms is a selection of 30 varieties in 1981 and 1983, the 19% in 1977 (Louvel & Fauquet, unpubl.). varieties were Lung with the three panicles, varied 13 to 16% over the season to season. occur when short, such as often occur-5 the time for planting the size in the fields, ease and the infected the percentage of dollar. The disease has (Fauquet, unpubl.), one (Buddenhagen, importance of spread.

Soil transmission of the disease agent. The field spread of the disease in discrete, progressively expanding patches, indicated soil transmission, which was tested and confirmed by laboratory experiments. The disease was always associated, in the root cells of infected plants, with the presence of a soil fungus, *P. graminis*. All infected plants obtained by soil transmission experiments developed the symptoms typical of the disease in the field (Fig. 5-2(1)). In laboratory soil transmission experiments, up to 45% of rice seedlings were infected when the soil used was extracted from a depth of 0 to 20 cm from around infected plants in the field, as compared

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Fig. 5-2(1) Soil transmission experiment with rice stripe necrosis virus and cv. IRAT 10. Two diseased plants are shown in the foreground with three healthy plants at the rear.

Fig. 5-2(2) Resting spores of *Polymyxa graminis* in the rootlets of rice cv. IRAT 10, naturally infected with rice stripe necrosis virus.

Fig. 5-2(3) Cystosori of *Polymyxa graminis* in the rootlets of rice cv. IRAT 10, naturally infected with rice stripe necrosis virus.

Fig. 5-2(4) Two cystosori of *Polymyxa graminis* in a cell of a rootlet of rice, cv. IRAT 10, infected during a soil transmission experiment with the rice stripe necrosis virus.
with 9% levels of infection in the soil extracted from the same depth around healthy plants. Contaminated soil, dried for 60 days, transmitted the disease to 31% of seedlings tested. Using soil from pots containing plants which showed positive transmission, it was possible to obtain further positive transmissions, sometimes up to 100%. Soil transmission experiments were also positive when sterilized soil was inoculated with the washed roots of infected plants or with fungal zoospores released from infected roots into water and concentrated by low-speed centrifugation.

THE VECTOR

The presence of a soil-borne fungus infecting rice roots was demonstrated, using a lactophenol staining method (Britton & Rogers, 1963) (Fig. 5-2(2)). The size and shape of the cystosori (Fig. 5-2(3)) and plasmodia observed in the root cells of infected rice suggested that the fungus involved might be *P. graminis* (Karling, 1968; Ledingham, 1939; Rao. 1968). This hypothesis was reinforced by the fact that the fungi of this group have specific plant hosts and *P. graminis* invades only graminaceous plants (Karling, 1968; Teakle, 1980). Plants infected by virus from soil, either in natural or artificial conditions, always contained *P. graminis* in the cells of their rootlets.

Transmission experiments were performed with nematode vectors (Fauquet, unpubl.). *Xiphinema spp.* were found in very small quantities in soil samples from contaminated fields but we were unable to correlate the disease transmission with them.

Rice mealybug, *Ripseria oryzae*, which is known to induce crinkling and malformations of the affected plants, were also tested as a vector of RSNV. Rice mealybugs, bred on rice seedlings infected by RSNV, were transferred to healthy rice seedlings. The symptoms that developed were not those of RSNV (Fauquet & Thouvenel, 1983).

THE VIRUS

Mechanical inoculation. The virus is not mechanically transmissible from rice to rice or perhaps only with an extremely low rate of transmission. One positive plant was obtained from more than ten thousand plants tested (Fauquet & Thouvenel, 1983). Many different inoculation buffers, conditions, techniques and inoculum sources (young leaves and rootlets from field and laboratory infected plants) were tried without success. Nevertheless, it is possible to obtain local lesions in leaves of *Chenopodium amaranticolor* or *Nicotiana benthamiana* inoculated with infected crude rice sap. The local lesions produced on *C. amaranticolor* are very similar to those induced by peanut clump virus (Thouvenel & Fauquet, 1981). Infection is limited to which enlarge and et symptoms. Symptom appeared also to be r to achieve back trans possible to obtain loc *N. benthamiana* with inoculum.

Seed transmission, Ti using several thoua field. They were fro IRAT 10, IRAT 1 percentage of germir occurred (Fauquet &

The causal agent. R roots of infected ric virus purification t alternate low and butanol clarification purification was pe opalescent bands we isolated bands disp viruses with 5% of yield of purified vin infectious on the lo bands observed in su lengths: 110–160, 2 rod-shaped, with a width of about 20 n

Electron microscopy virus induces cytop rod-shaped viruses Thouvenel & Fauq number of cells co paracrystalline cytc inclusions were pre experimentally infe

Serology. The virus prepared with the observed when the tomato mosaic tob
Infection is limited to the inoculated leaf. Symptoms appear as yellow spots which enlarge and invade the whole leaf, which shows typical oak-leaf symptoms. Symptoms in *N. benthamiana* took the form of yellow spots and appeared also to be restricted to the inoculated leaves. It was not possible to achieve back transmission to rice from local lesion hosts, but it was possible to obtain local lesions in inoculated leaves of *C. amaranthicolor* or *N. benthamiana* when the same plant species was used as a source of inoculum.

Seed transmission. The possibility of seed transmission has been examined using several thousand rice seeds, collected from infected plants in the field. They were from the most susceptible varieties of rice, Lung Sheng 1, IRAT 10, IRAT 13, 63 × 83, LS 104 and Dourado précoce. The percentage of germination was very low, but no transmission of the disease occurred (Fauquet & Thouvenel, 1983).

The causal agent. RSNV could be purified both from the leaves and the roots of infected rice seedlings. The purification method was a classical virus purification technique using potassium phosphate buffer with alternate low and high speed centrifugations, following chloroform-butanol clarification (Fauquet & Thouvenel, 1983). The final step of purification was performed in a sucrose density gradient where three opalescent bands were differentiated (RF = 0.40, 0.51 and 0.56). The three isolated bands displayed biophysical properties typical of rod-shaped viruses with 5% of nucleic acid (Fauquet & Thouvenel, 1983). The total yield of purified virus was on average 10–15 mg/kg. The purified virus is infectious on the local lesion hosts cited above, but not rice. The three bands observed in sucrose gradients correspond to three different particle lengths: 110–160, 270 and 380 nm (Fig. 5-3). The virus particles are rod-shaped, with a hollow centre of different lengths but with the same width of about 20 nm (Fig. 5-4).

**Electron microscopy.** In the parenchyma cells of infected rice seedlings, the virus induces cytoplasmic inclusions that resemble the inclusions of other rod-shaped viruses (Christie & Edwardson, 1977; Jackson & Lane, 1981; Thouvenel & Fauquet, 1981; Francki, Milne & Hatta, 1985). A large number of cells contains virus-like particles (Fig. 5-4(1), (2), (3)) and paracrystalline cytoplasmic inclusions (Fig. 5-4(4)). These cytoplasmic inclusions were present both in the infected plants from the field and in experimentally infected seedlings.

**Serology.** The virus is poorly immunogenic but using the antiserum prepared with the purified virus having a titre of 1/250 no reaction was observed when the following viruses were tested: tobacco mosaic and tomato mosaic tobamoviruses; the tobraviruses tobacco rattle and pea
early browning; barley stripe mosaic hordeivrus; soil-borne wheat mosaic virus (SBWMV), *Nicotiana velutina* mosaic virus, beet necrotic yellow vein virus (BNYVV) and peanut clump virus (Fauquet & Thouvenel, 1983).

Properties of the coat protein. The molecular weight of the coat protein of RSNV, determined by polyacrylamide gel electrophoresis, is 24,000 d (Fauquet, 1985). The number of amino acids per molecule is as follows: ASP (29), THR (14), SER (22), GLU (20), PRO (9), GLY (20), ALA (20), CYS (2), VAL (16), HIS (6), LYS (10). This amino acid compn is typical of viruses trar Dejardin & Thouvenel.
Electron micrograph showing virus-like particles (V) scattered in the cytoplasm of a rice cell infected with rice stripe necrosis virus.

Electron micrograph showing virus-like particles (V) aggregated in the cytoplasm of a rice cell infected with rice stripe necrosis virus.

Electron micrograph showing virus-like particles (V) aggregated in the cytoplasm of a rice cell infected with rice stripe necrosis virus.

Electron micrograph showing paracrystal inclusion (PI) in the cytoplasm of a rice cell infected with rice stripe necrosis virus.

The amino acid composition of the coat protein of RSV is as follows: CYS (2), VAL (15), MET (2), ILE (9), LEU (16), TYR (7), PHE (6), HIS (6), LYS (10), ARG (13), TRP (2) (Fauquet & Thouvenel, 1987). This amino acid composition is characteristic of rod-shaped particles and typical of viruses transmitted by soil-fungi (Fauquet, 1985; Fauquet, Dejardin & Thouvenel, 1986a; 1986b).
A rod-shaped virus (RSNV) is always associated with rice crinkling disease which occurs occasionally in West Africa (Louvel & Bidaux, 1977; Fauquet & Thouvenel, 1983). This virus, although it could not be artificially transmitted to healthy rice, is nonetheless the most probable causal agent of the disease. All the results obtained point to it being transmitted by a soil fungus; no other likely agent could be identified. The symptoms of RSNV are typical of those caused by other rod-shaped viruses in cereals (Atabekov & Novikov, 1971; Brakke, 1971; Inouye & Fujii, 1977; Ling, 1972) and the results of soil transmission experiments with the disease agent are indicative of fungus transmission: *P. graminis* is the fungus probably involved.

There are at least two viruses that infect rice and are transmitted by *P. graminis* (Ling, 1972). Furthermore, rice necrosis mosaic virus (RNMV) induces symptoms in rice leaves similar to those associated with RSNV. However, RNMV has rigid filamentous particles with predominant lengths of 275 and 550 nm and induces pinwheels in the infected cells (Inouye & Fujii, 1977) thereby differing from RSNV.

Among other viruses infecting graminaceous plants, only soil-borne wheat mosaic (SBWMV) (Brakke, 1971) and oat golden stripe virus (Plumb, Catherall, Chamberlain & Macfarlane, 1977) are fungus-transmitted and have similar rod-shaped particles. SBWMV and OGSV are easily mechanically transmitted to different hosts and SBWMV does not infect rice. By contrast RSNV naturally infects rice but cannot be transmitted mechanically. Furthermore, SBWMV is not serologically related to RSNV.

Amongst soil-transmitted rod-shaped viruses, beet necrotic yellow vein virus (BNYVV) resembles RSNV in appearance and modal length, but BNYVV does not infect graminaceous plants and the two are not serologically related.

The rod-shaped virus isolated from infected rice plants is composed of particles of at least three different lengths: 110-160, 270 and 380 nm. However, it is not known whether all particles are essential for infection. Although neither the type of nucleic acid nor the number of genomic components are known, RSNV has affinities with the furoviruses as judged by amino acid composition of coat proteins (Fauquet, Desboise, Fargette & Vidal, p. 19, this volume).

At present, the disease does not seem to be economically important for upland rice culture in Africa, although the impact of the disease on individual infected plants can be very severe or even lethal. Conditions necessary for the development of the disease are imperfectly known but seem to reflect the epidemiology of the fungal vector. Apparently, this disease is present over wide areas of West Africa but it does not appear to be spreading and occurs at a relatively low frequency. The range of varietal susceptibility is not yet known. Consequently, the disease might be best controlled by the use of resistant varieties. Consequently, the disease might be best controlled by the use of resistant varieties.
susceptibility is very wide and there are resistance genes in certain varieties. Consequently, selection/breeding programmes would seem to offer an appropriate strategy for controlling RSNV.

REFERENCES


Fauquet, C., Dejardin, J. & Thouvenel, J.-C. (1986b) Evidence that the amino acid composition of the particle proteins of plant viruses is characteristic of the virus group. II. Discriminant analysis according to structural, biological and classification properties of plant viruses. Intervirology 25, 190–200.


VIRUSES WITH FUNGAL VECTORS


6. Physical and ye

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Beet necrotic yellow sugar-beet rhizomania Polymyxa betae, has decade. BNYVV has four different lengths by means of electron processing methods. A single-stranded right-I of 4 turns, involving possesses a segmente ately. The four RNA been cloned and sequ determined; RNA-3 play an important rol BNYVV isolates f have closely similar and the method of choice are not unambiguou means of diagnosis in

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*Abteilung Mikrobi